The Role of Grasslands in a Green Future

Threats and Perspectives in Less Favoured Areas

Edited by

Á. Helgadóttir
A. Hopkins

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The Role of Grasslands in a Green Future

Threats and Perspectives in Less Favoured Areas

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Edited by
Áslaug Helgadóttir
Alan Hopkins

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Foreword

One of the most important tasks ahead for agriculture worldwide is to secure sufficient food for a growing population without further straining our environmental resources. The challenge is to produce more food with less external input. At European level grassland is a valuable resource for meeting increasing demands for meat and milk from livestock systems yet at the same time competition between feed production from grassland and food production from arable land needs to be minimized. Grasslands also have to be maintained in their own right as they have an important role to play in preserving biodiversity and procuring ecosystem services at all levels. The 17th Symposium of the European Grassland Federation therefore appropriately focuses on the role of grasslands in shaping a green future and will hopefully provide valuable solutions to the challenges facing us ahead.

Grasslands play a particularly important role in marginal regions of Europe where livestock production has been the traditional form of agriculture. With the expected climate change and increased emphasis on sustainable agriculture it can be expected that these areas will become more important for food production. The question is how agricultural production can be maintained in these areas and even improved while emphasising efficient use of local resources and minimising environmental impact. How important are grasslands for carbon sequestration in the more marginal areas and can they serve as a natural gene bank by maintaining biodiversity? Can grasslands become a valuable resource, also in these areas, as attempts to replace fossil fuel with bio-energy gain more weight? Threats to the exploitation of marginal grasslands by severe environmental fluctuations, soil degradation through loss of organic material and nutrients, acidification and soil compaction are a real concern in this context. Iceland provides an ideal setting for addressing all these questions as it certainly is a marginal environment situated just below the Arctic Circle and its agriculture is primarily grassland-based livestock production.

Many people have contributed to the making of this conference. We would like to thank all authors for their papers and presentations, numerous reviewers for their valuable remarks and Dr. Alan Hopkins in particular for revision of the English language and careful checking of all written contributions. Finally, we are grateful for the generous support of the Ministry of Industries and Innovation and we express our gratitude to all other sponsors.

We hope that the local environment will stimulate fruitful discussions during the course of the three days of the Symposium and that all participants will take back with them vivid memories from their stay in Iceland.

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Chairman of the Organising Committee

Thórunn Reykdal
Secretary of the Organising Committee

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Opening Session

Potential of legume-based grassland-livestock systems in Europe

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Abstract

European grassland-based livestock production systems are challenged to produce more milk and meat to meet increasing world demand and to achieve this by using fewer resources. Legumes offer great potential for coping with such requests. They have numerous features that can act together at different stages in the soil-plant-animal-atmosphere system and these are most effective in mixed swards with a legume abundance of 30-50%. The resulting benefits are a reduced dependency on fossil energy and industrial N fertilizer, lower quantities of harmful emissions to the environment (greenhouse gases and nitrate), lower production costs, higher productivity and increased protein self-sufficiency. Some legume species offer opportunities for improving animal health with less medication due to bioactive secondary metabolites. In addition, legumes may offer an option for adapting to higher atmospheric CO_2 concentrations and to climate change. Legumes generate these benefits at the level of the managed land area unit and also at the level of the final product unit. However, legumes suffer from some limitations, and suggestions are made for future research in order to exploit more fully the opportunities that legumes can offer. In conclusion, the development of legume-based grassland-livestock systems undoubtedly constitutes one of the pillars for more sustainable and competitive ruminant production systems, and it can only be expected that legumes will become more important in the future.

Keywords: yield, symbiotic N_2 fixation, forage quality, nutritive value, voluntary intake, animal performance, greenhouse gas emission, nitrate leaching, condensed tannins, animal health, clover, climate change, energy, plant secondary metabolites, management

Introduction

European grassland-based livestock production systems have changed considerably over the last two decades and will continue to evolve in response to societal and environmental pressures. Grassland production will need to keep pace with requirements for higher meat and milk production from ruminant systems and with a changing climate. At the same time, grassland production needs to minimize competition from arable land and between food and feed production, and preserve biodiversity and ecosystem services (Thornton, 2010). Legumes offer important opportunities for sustainable grassland-based animal production because they can contribute to important key challenges by (i) increasing forage yield, (ii) substituting inorganic N fertilizer inputs with symbiotic N_2 fixation, (iii) mitigating and supporting adaptation to climate change, as elevated atmospheric CO_2, warmer temperatures and drought-stress periods increase, and (iv) increasing the nutritive value of herbage and raising the efficiency of conversion of herbage to animal protein.
A concerted programme of research supported by the European Commission is devoted to improving our understanding of the roles played by legumes in grassland systems. Of

particular interest are four current initiatives: MultiSward (www.multisward.eu), Legume Futures (www.legumefutures.eu), AnimalChange (www.animalchange.eu) and LegumePlus (www.legumeplus.eu). The objective of MultiSward is to support developments and innovations for grassland production and management in different European farming systems, and under different pedoclimatic and socio-economic conditions. They focus (i) on enhancing the role of grasslands at farm and landscape levels in terms of environmental services and biodiversity, and (ii) on optimizing their economic, agronomic and nutritional contributions for innovative and sustainable ruminant production systems. An important aspect is the investigation of the effects of multispecies mixtures, where grasses and forbs are combined with shallow and deep rooting clovers, on forage production, grazing systems and ecosystem services. The Legume Futures project is designed to optimize the use of legumes in European agriculture. It takes account of the agronomic, economic and environmental impacts of legume cultivation and uses experimentation and modelling to develop novel legume-based cropping systems. AnimalChange will provide options for the livestock sector to cope with climate change in the future: (i) by reducing uncertainties concerning greenhouse gas (GHG) emissions from livestock systems, (ii) by developing cutting-edge technologies for mitigation and adaptation to climate change, (iii) by assessing the vulnerability of livestock to climate change and feedbacks on GHG emissions, and (iv) by providing direct support to set up policies for the livestock sector in order to mitigate and adapt to climate change. Legumes can offer important options and these will be examined in this project. The key objective of LegumePlus is to investigate how bioactive forage legumes, in particular sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.), can improve protein utilization in ruminant livestock farming. Of equal importance is the potential that these legumes offer for combatting parasitic nematodes in ruminants. This project will study the combined effects of nitrogen and methane emissions plus their impact on food quality, e.g. milk, cheese and meat quality.

This paper, by authors involved in these four European research programmes, aims to review the literature for opportunities that forage legumes can offer in order to meet key challenges, which will be faced by future grassland-animal husbandry systems. The authors also seek to highlight research that is needed to enable increased utilization of legumes in Europe. It is, however, not the aim to give detailed profiles of individual forage legume species as this information was compiled by Frame (2005) for more than 30 legume species.

Political and socio-economic background

Over many years, the European Union's Common Agricultural Policy (CAP) has encouraged large increases in agricultural production and the intensification of agricultural systems. In addition, low energy prices during the second half of the 20th century resulted in an abundant supply of cheap synthetic nitrogen (N) fertilizer, which further reduced the demand for production from legume-based grasslands (Rochon *et al*., 2004). These changes have had adverse environmental impacts by increasing greenhouse gas emissions and by lowering biodiversity, which has been linked strongly to the use of synthetic N fertilizer (Schulze *et al*., 2009; Stoate *et al*., 2009). There is no specific requirement under current EU agricultural policy to support legume-based cropping systems or to develop home-grown protein crops, but revisions to the policy are envisaged by the European Commission by 2020, which are likely to encourage synergies between crop and livestock farming in order to make better use of protein sources (European Commission, 2010).

World-trade agreements have promoted imports of grain legumes into Europe and have led to lower European production despite increased consumption. This dependence of Europe's livestock industry on imports of grain legumes has raised questions about the sustainability and security of such a production model against a background of increasing demands for food and concerns about the environmental impact of livestock production systems (Godfray *et al.*, 2010). Galloway *et al.* (2008) estimated that South America exported a net amount of 2.3 Mt of N in grain legumes to Europe in 2004. As well as creating an imbalance in global N cycles, this export of grain legumes to Europe has also led to a large-scale change in land use in South America, as forests have been cleared for soya production (Weightman *et al.*, 2011). Governments of European countries are becoming increasingly concerned about the security of their protein supplies, and the UK, Germany and the European Parliament have recently discussed the development of new policies to support national protein security (Aigner, 2009; European Parliament, 2011).

The magnitude of benefits offered by the use of legumes in European farming systems is informed by scientific studies in a number of areas. This paper summarizes some of the key issues, and highlights remaining areas of uncertainty. Such uncertainties, however, need not act as a barrier to developing policy where the balance of evidence supports change. The large amounts of public funding (in the Common Agricultural Policy) that are used to support European Agriculture can be expected to deliver public benefits (such as those associated with reduced environmental impact) as well as a sustainable food production sector.

Legume-grass swards a key to increased yield

Under fertile agricultural conditions monocultures of selected, highly productive grass species give high forage yields with high inputs of fertilizer nitrogen (N) (Frame, 1991; Daepp *et al.*, 2001). The need to increase not only productivity but also resource efficiency (sustainable intensification) poses new challenges for agriculture. Plant communities with higher species number (richness) are expected to (i) utilize available resources better due to species niche complementarity, (ii) have a higher probability of showing positive interspecific interactions, and (iii) may contain highly productive species that dominate the community (selection effect) (Tilman, 1999; Loreau and Hector, 2001; Loreau *et al.*, 2001). Thus, cropping mixtures could be a promising strategy for sustainable intensification.

Indeed, many experiments in nutrient-poor grasslands have shown that biomass production was enhanced in species-rich swards, compared with the average monoculture yield (Spehn *et al.*, 2002; Hille Ris Lambers *et al.*, 2004; Hooper and Dukes, 2004; Hooper *et al.*, 2005; Roscher *et al.*, 2005; Marquard *et al.*, 2009; Mommer *et al.*, 2010). In a meta-analysis of 44 biodiversity experiments that manipulated plant species richness, Cardinale *et al.* (2007) found that the most diverse mixtures, on average, achieved a yield benefit of +77% compared to the average monoculture. However, compared to the most productive monoculture, these mixtures showed a yield disadvantage of -12%. Transgressive overyielding (mixtures outperform the best monoculture; Trenbath, 1974; Schmid *et al.*, 2008) occurred in only 12% of the experiments and it took, on average, about five years to become evident. However, in an agronomic context, mixtures with transgressive overyielding are clearly preferred, as stakeholders can select the highest yielding species for monoculture cultivation, and any mixture performance has to compete against this high benchmark.

A pan-European experiment carried out across 31 sites in 17 countries under the auspices of COST Action 852 'Quality legume-based forage systems for contrasting environments' (www.cost.eu/domains_actions/fa/Actions/852) tested whether higher yields, compared with monocultures, can be achieved under typical agricultural conditions with grass-legume mixtures containing four species (Kirwan *et al.*, 2007; Lüscher *et al.*, 2008; Nyfeler *et al.*, 2009). These four species represented four functional groups of plants: a fast-establishing grass, a fast-establishing legume, a slow-establishing grass and a slow-establishing legume. These functional groups of plant species were chosen to maximize beneficial interspecific interactions: legumes enable symbiotic fixation of atmospheric nitrogen, and fast/slow combinations were intended to maximize sward cover by species with known different temporal patterns of development. The legume species examined were *Trifolium pratense* L. (red clover, 29 sites), *Trifolium repens* L. (white clover, 26), *Medicago sativa* L. (lucerne, 3), *Medicago polymorpha* L. (burr medic, 2), and *Trifolium ambiguum* M. Bieb. (Caucasian clover, 2) (Finn *et al*., 2013).

Across the three years, the yield of sown species (total yield excluding weed biomass) was higher in the mixture than the average monoculture yield for 99.7% of the mixture plots, with a yield advantage of 77% of the mixtures above the average monoculture. It was most remarkable that transgressive overyielding was achieved in 79% of the mixture plots and the yield advantage of the average mixture was 18%, compared with the highest yielding monoculture (Finn *et al*., 2013). At the Swiss site, which tested red and white clover, a comparison across N fertilizer input levels revealed a high potential for N fertilizer replacement: grass-clover mixtures containing 40-60% clover and receiving 50 or 150 kg ha^{-1} yr^{-1} fertilizer N achieved the same yield as grass monocultures fertilized with 450 kg N ha^{-1} yr^{-1} (Nyfeler *et al*., 2009). Over the whole pan-European experiment advantages of grass-legume mixtures were surprisingly robust: they persisted over the three experimental years, over the different legume species tested, and over the large climatic gradient covered by the experimental sites, spanning a latitudinal range from 40°44'N (Sardinia, Italy) to 69°40'N (Tromsø, Norway) (Finn *et al*., 2013; Sturludóttir *et al*., 2013).

Evenness, the degree of even species abundance in swards, had a highly significant effect on the yield and diversity effect (excess of mixture performance over that expected from average monoculture performances) (Kirwan *et al*., 2007; Finn *et al*., 2013; Sturludóttir *et al*., 2013). Increase in mixture evenness at low values resulted in a steep increase of the diversity effect (Connolly *et al*., 2013). However, these benefits of raising evenness showed a fast saturation and the diversity effect remained relatively constant across a wide range of medium to high evenness values (Kirwan *et al*., 2007; Connolly *et al*., 2013; Finn *et al*., 2013; Sturludóttir *et al*., 2013), indicating a high robustness of the diversity effect to changes in relative abundance of different species. Because high evenness in these mixtures was jointly linked to legume percentages of 35-65%, results of Finn *et al*. (2013) are in agreement with the recent finding of significant transgressive overyielding in mixtures over a wide range of about 30 to 80% clover percentage in the sward (Nyfeler *et al*., 2009).

These findings suggest that grass-legume mixtures offer a great potential for increased production even at relatively low species richness. In the pan-European experiment of Finn *et al*. (2013), grass-legume mixtures outperformed both grass and legume monocultures. Because symbiotic N_2 fixation cannot explain the highly significant yield advantage of mixtures over legume monocultures (detailed results in Nyfeler *et al*., 2009), access to atmospheric N_2 could not have been the only factor causing increased mixture yields. In diversity experiments, the positive interactions between N_2-fixing legumes and non-N_2-fixing plant species often contributed to a significantly larger extent to mixing effects in biomass yield than the interactions between other functional groups (Spehn *et al*., 2002; Li *et al*., 2007; Temperton *et al*., 2007; Kirwan *et al*., 2009; Nyfeler *et al*., 2009). However, other trait combinations can yield important diversity effects also (Van Ruijven and Berendse, 2003; Roscher *et al*., 2008). In the pan-European experiment, mixtures strongly benefitted from the combination of fast-establishing with slow-developing, but temporally persistent species (Kirwan *et al*., 2007; Finn *et al*., 2013). Further research is needed to quantify the mechanisms of the complementarity for a variety of functional traits and their contributions to the effects on mixture yield. This knowledge would allow the design of mixtures that combine species with high complementarity of different traits, which in turn will result in optimized resource exploitation through niche differentiation (Hill, 1990; Lüscher and Jacquard, 1991). Such research should also include legumes other than *Trifolium pratense* and *T. repens*, on which most evidence is based so far. In particular, legumes known to perform well under cold and/or dry conditions should be tested. Extension of designs will also reveal whether a further increase in species richness can lead to an additional gain in diversity effects and mixture yields (Connolly *et al.*, 2013).

Legume yield is a main driver for large N inputs by symbiotic N_2 fixation

The massive acceleration of the global N cycle by N fertilizer from the industrial Haber-Bosch process and by N emissions from the combustion of fossil fuels, has enabled humankind to increase greatly food production. This, however, has also led to a host of environmental problems, ranging from eutrophication of terrestrial and aquatic ecosystems to global acidification and climate change (Gruber and Galloway, 2008; Rockström *et al.*, 2009; Vörösmarty *et al.*, 2010; Hooper *et al.*, 2012). Anthropogenic N released to the environment is greater than 160 teragrams (Tg) N yr^{-1}, which clearly exceeds the amount supplied by biological N_2 fixation in natural systems (110 Tg N yr^{-1}) (Gruber and Galloway, 2008; Herridge *et al.*, 2008). Substitution of industrial N fertilizer by improved exploitation of N_2 fixation from symbiosis of legumes with *Rhizobium* bacteria would thus be an important contribution to environmental-friendly and resource-efficient agricultural systems.

In grasslands, symbiotically fixed N_2 by legumes can range from 100 to 380 kg of N ha^{-1} yr^{-1} and exceptionally large amounts of > 500 kg of N ha^{-1} yr^{-1} have also been reported (Boller and Nösberger, 1987; Ledgard and Steele, 1992; Zanetti *et al.*, 1997; Carlsson and Huss-Danell, 2003). In mixed grass-legume systems, additional amounts of 10-75 kg of N ha^{-1} yr^{-1} are transferred from legumes to grasses, while the amount transferred depends on the donor and the receiver plant species (Pirhofer-Walzl *et al.*, 2012). The control of symbiotic N_2 fixation operates through a series of ecophysiological triggers (Hartwig, 1998; Soussana *et al.*, 2002), with the amount of symbiotically fixed N_2 being tightly coupled to the gap between N demand (sink) and N availability (source) from mineral-N sources at several scales from plant physiology to the whole ecosystem (Soussana and Hartwig, 1996; Hartwig, 1998; Soussana and Tallec, 2010).

In grass-clover mixtures containing red and white clover, Nyfeler *et al.* (2011) observed stimulatory effects of the accompanying grasses on the symbiotic N_2 fixation activity of clover (% N derived from symbiosis). This effect was so strong that: (i) the amount of N from symbiosis was maximized not in pure clover stands but in mixtures with 60-80% of clovers, and (ii) 40-60% of clovers in the mixture were sufficient to attain the same amount of N from symbiosis as in pure clover stands. This stimulatory effect fits well into the sink/source model of the regulation of symbiotic N_2 fixation. The activity of symbiotic N_2 fixation of clover plants was very high in grass-dominated swards, where the availability of mineral N to clovers was very low; this was evident from the fact that most of the N from mineral sources was taken up by the competitive grass component (Nyfeler *et al.*, 2011). However, in clover-dominated swards (> 60% of clovers), the activity of symbiotic N_2 fixation was down-regulated. This was due to the clovers' adequate access to mineral N sources because of low grass abundance (Nyfeler *et al.*, 2011) as well as due to a significantly reduced N demand of the whole sward in clover-dominated stands, as these were less productive than well balanced mixtures (Nyfeler *et al.*, 2009). The sensitivity of legume plants to down-regulate their symbiotic N_2 fixation (percentage of N derived from symbiosis) seems to show interspecific differences (Rasmussen *et al.*, 2012). In general, forage legumes grown in mixtures (with a reasonable abundance of grasses) receive most of their N (> 80%) from symbiotic N_2 fixation (Boller and Nösberger, 1987; Heichel and Henjum, 1991; Carlsson *et al.*, 2009; Oberson *et al.*, 2013), which implies that the amount of N derived from symbiosis depends on the forage legume dry matter production (Unkovich *et al.*, 2010; Lüscher *et al.*, 2011).

Legumes have often been studied in intensively managed grasslands under productive soil and climatic conditions. In extensively managed non- or low-fertilized grassland experiments, high values of the proportion of N derived from symbiosis have also been measured in a range of legume species (Carlsson *et al*., 2009; Roscher *et al*., 2011). However, low values of the amount of N derived from symbiotic N_2 fixation were observed at low temperatures in two growth chamber experiments on nutrient solution (Kessler *et al*., 1990; Nesheim and Boller, 1991). This was not only related to low growth and total nitrogen accumulation of white clover, but also to a marked reduction of the proportion of N derived from symbioses. Thus, the authors concluded that the negative effect of low temperature on processes of nitrogen fixation-nodulation (e.g., Roughley and Dart, 1970) and nitrogenase activity (e.g., Cralle and Heichel, 1982) were primarily responsible for the small contribution of nitrogen fixation to nitrogen nutrition of white clover at low temperatures. Only few field studies have been published on symbiotic N_2 fixation under marginal conditions at high altitude (e.g. Bowman *et al*., 1996) or high latitude (e.g. Henry and Svoboda, 1986; Sparrow *et al*., 1995). Jacot *et al.* (2000a; 2000b) studied the significance of symbiotic N_2 fixation for the legumes and for the N balance of the whole grassland ecosystem of species-rich semi-natural pastures in the Alps. The legume species examined were *Lotus corniculatus* L., *L. alpinus* Schleicher, *Vicia sativa* L., *Trifolium pratense* L., *T. repens* L., *T. nivale* Sieber, *T. thalii* Vil., *T. badium* Schreber, and *T. alpinum* L.. Along an altitudinal gradient from 900 m a.s.l. to the altitudinal limit of legume occurrence at 2600 m a.s.l., all the legume species met most of their N requirements from symbiosis (59 to 90%). This suggests that symbiotic N_2 fixation is well adapted both to the climatic and acidic soil conditions (pH 5.6 to 4.1) of the sites investigated. Nevertheless, the amount of N derived from symbiosis decreased significantly with increasing altitude (18 to 1 kg N ha^{-1} yr^{-1}) due to a strong decrease in the total productivity of the plant community and to a decrease from 15 to 4% of legumes in the sward.

These findings and the sink/source model of regulation of symbiotic N_2 fixation at the ecosystem level have several critical implications for the exploitation of atmospheric N_2 in grassland systems. First, grass-clover mixtures can potentially fix more N_2 from the atmosphere than clover monocultures. Second, there is no trade-off between high productivity and high gains of symbiotically fixed N_2 because they are positively linked through N demand (sink). Consequently, sufficient availability of other nutrients, such as P, K, and S, is crucial for stimulating demand of N from symbiosis (Hartwig, 1998; Brown *et al*., 2000; Tallec *et al*., 2009). Third, very high inputs of N into the ecosystem through symbiosis can cause a risk for N losses into the environment. However, such unwanted N losses can be prevented by sufficiently great abundance of grasses in the sward to ensure a highly competitive uptake of mineral N from the soil.

Efficiency of conversion of forage into animal products

Legumes increase nutritive value and voluntary intake

Livestock production is influenced by both the nutritive value and the voluntary intake of forages. The chemical composition and nutritive value of forages have been summarized by INRA (2007). Compared to perennial ryegrass (*Lolium perenne* L.), white clover, red clover and lucerne have high concentrations of crude protein (CP) and minerals, such as calcium, but contain relatively low concentrations of water-soluble carbohydrates (WSC). The nutritional advantage of white clover over grasses is well established (Beever *et al*., 1986; Peyraud, 1993). Organic matter digestibility and net energy concentration, as well as the supply of metabolizable protein, are generally higher for white clover than for grasses (INRA, 2007). These results reflect a lower proportion of structural cell wall components, which are less digestible than cell contents. Red clover and lucerne are less digestible and their net energy concentration is lower than that of white clover at a similar growth stage, the difference being greater for lucerne (5.54, 6.10 and 7.17 MJ kg DM^{-1} for lucerne, red clover and white clover, respectively (INRA, 2007)). These values are further reduced in silage and hay. Lucerne, and to a lesser extent red clover, should be cut at an early growth stage in order to maximize the net energy concentration of the conserved forage. In contrast, their net energy and metabolizable protein content is high when fed as fresh forage, and is almost at the recommended level for optimal feeding of dairy cows (INRA, 2007) and higher than that recommended for low-producing cows. Hay-making maintains the high metabolizable protein content but ensiling reduces its content.

Voluntary intake of dry matter (DM) of legumes is 10 to 15% greater than that of grasses of similar digestibility and this is true whether forage legumes are fed as silage, hay or fresh (INRA, 2007). These differences are attributed to a lower resistance of legumes to chewing, a faster rate of digestion and a faster rate of particle breakdown and clearance from the rumen (Waghorn *et al*., 1989; Jamot and Grenet, 1991; Steg *et al*., 1994; Dewhurst *et al*., 2009), which in turn reduce rumen fill. Dewhurst *et al*. (2003) reported that DM intake of silage is increased by 2 to 3 kg when cows are fed red clover or white clover silages, compared with perennial ryegrass silage. White clover is often used in a mixture with perennial ryegrass and this raises the question of the optimal proportion of white clover. Harris *et al*. (1998) showed that the DM intake of housed dairy cows was at its maximum when white clover reached 60% in the forage.

Herbage intake by grazing livestock is generally constrained by herbage allowance or pasture structure. At the same herbage allowance, Alder and Minson (1963) found that herbage intake was 15 to 20% higher with pure lucerne relative to pure cocksfoot pastures. The beneficial effects of white clover on herbage intake and performance by livestock grazing a white clover-grass pasture have also been demonstrated (Wilkins *et al*., 1994; Ribeiro-Filho *et al*., 2003; 2005). The difference in daily herbage intake increased with increasing percentage of clover in the diet and reached 1.5 kg on average in these latter studies. In addition to the positive effect of legumes on voluntary intake, it is also probable that leaves of legumes are more favourable for intake than stems and sheaths of grasses, particularly during the spring-heading period. Thus, Ribeiro-Filho *et al*. (2003) have reported a higher rate of intake on mixed white clover/perennial ryegrass pastures compared with pure perennial ryegrass pastures.

An additional benefit of white clover is that the rate of decline in nutritive value throughout the plant-ageing process is much lower than for grasses. This has been known for many years (Ulyatt, 1970). Digestibility and voluntary DM intake decreased by 20 g kg^{-1} and 0.2 kg day^{-1} per week during the first growth of grass herbage, whereas these decreases were half that for white clover herbage (INRA, 2007; Peyraud *et al*., 2009). Peyraud (1993) and Delaby and Peccatte (2003) reported the digestibility of DM to be >75% after 7 weeks of regrowth or at the flowering stage during the first growth in spring. Ribeiro-Filho *et al*. (2003) showed that the DM intake of herbage declined by 2.0 kg day^{-1} on a predominantly grass pasture compared to 0.8 kg day^{-1} on mixed pastures. This makes mixed pastures much easier to manage than pure grass pastures for maintaining their nutritive value and makes mixed pastures particularly attractive on farm because of their greater flexibility. For example, it allows intervals between two successive grazing periods of more than 4 to 6 weeks in the summer. For lucerne and red clover, the decline in nutritive value with advancing maturity is intermediate between that of white clover and grasses (INRA, 2007). Sturludóttir *et al*. (2013) observed that the yield increase of legume-grass mixtures compared to monocultures was not accompanied by a reduction in herbage digestibility and crude protein concentration that is usually observed with increased DM yield. Low-lignin lucerne cultivars could be another opportunity to further reduce the decline of nutritive value with advancing maturity (Undersander *et al*., 2009).

Legumes increase performance of livestock

Several experiments have shown that pure legume silages and legume-dominated silages can increase milk production compared to pure grass silages (Castle *et al*., 1983; Dewhurst *et al*., 2003). Chenais (1993) summarized the results of ten French experiments, which had studied the effect of a mixed diet, based on maize (*Zea mays* L.) silage and red clover, or lucerne silages compared with pure maize silage-based diets. The mixed diets led to similar levels of performance by dairy cows when the legume silages were of a high nutritive value and in particular when their DM content was >300 g kg^{-1}. The same applied to beef production, where red clover silage made it possible to obtain growth rates that were identical to maize silage as long as it was well-preserved (Weiss and Raymond, 1993). However, it should be pointed out that legumes can be difficult to conserve; special care must be taken to ensure good silage quality and to minimize leaf losses during hay-making (Arnaud *et al*., 1993). Increasing the concentration of total non-structural carbohydrates (TNC) of legumes will undoubtedly facilitate the production of high-quality silages and increase animal performance. This can be achieved by cutting the plot during the afternoon when sugar content raised its maximum (Brito *et al*., 2008; Pelletier *et al*., 2010; Morin *et al*., 2011). Plant breeding and gene manipulation might also be an option to increase TNC in legume plants (Tremblay *et al*., 2011).

A higher content of white clover in the pasture increased the daily milk yield of cows by 1 to 3 kg in several short-term experiments when the same DM herbage allowance was offered to dairy cows grazing pure perennial ryegrass pasture and mixed pastures (Philips and James, 1998; Ribeiro-Filho *et al*., 2003). In a study with housed dairy cows, milk yield increased with increasing white clover content in the diet and reached a maximum when the percentage of white clover averaged 50 to 60% (Harris *et al*., 1998). Conversely, milk yield is reduced when the abundance of clover is low (<20%, Gately 1981). As a consequence of higher energy intake, milk protein concentration tends to increase on mixed pastures. However, growth rates of growing cattle are relatively similar with these types of pasture. Nevertheless, on set-stocked swards, which were maintained at a similar height, mixed grass-legume pastures supported a slightly higher growth rate of lambs than fertilized grass pastures (Orr *et al*., 1990; Speijers *et al*., 2004).

As mixed pastures are managed with very low N fertilization, the biomass ha^{-1} might be lower than from highly fertilized grass pastures at a same age of regrowth. Therefore, mixed pastures often have lower milk yields and liveweight gains ha^{-1} than pure perennial ryegrass pastures as stocking rates are generally slightly lower in order to maintain similar herbage allowances (Institut de l'Elevage, 2004; Humphreys *et al*., 2009). Difficulties in maintaining well balanced grass-legume mixtures and their tendency to lose key species (Guckert and Hay, 2001) may also be a reason for the preference of pure grass swards by farmers. Alternatively, good flexibility in utilization of mixed pastures allows the use of intervals between two successive grazings of more than 4-6 weeks in summer, thereby compensating for lower productivity without penalizing the performances of the cows.

Legumes show a low efficiency of N digestion in the rumen

Losses of ruminal N in legume-fed ruminants are always high due to an imbalance between degradable N and fermentable energy in the forage. The rumen degradability of protein is higher for forage legumes in comparison with perennial ryegrass (Beever *et al*., 1986). This leads to an inefficient utilization of forage N in the rumen and high urinary-N excretion (Peyraud, 1993). White clover increases N excretion relative to perennial ryegrass from 20.1 to 29.8 g kg^{-1} DM intake and the amount of N that enters the duodenum is always below N intake, averaging 75% of N intake for white clover compared to 93% for ryegrass. From the data of Ribeiro-Filho *et al*. (2005) it can be calculated that N excretion increased from 17.0 to 20.7 g kg^{-1} milk on mixed white clover-perennial ryegrass pastures compared with perennial ryegrass pastures.

The WSC contained within the forage should be such as to balance the crude protein (CP) concentration of herbage in order to maximize microbial protein synthesis. However, the WSC content in temperate swards is variable and normally low. Promising results have been obtained by plant breeding and gene manipulation to increase WSC concentration in perennial ryegrass (Miller *et al*., 2001) and this led to a slight increase in digestibility and a reduction in urinary-N losses (Miller *et al*., 2001; Lee *et al*., 2002). However, legume forages typically have high CP and low WSC concentrations. Increasing WSC concentrations might improve ruminal-N utilization and plant digestibility in the case of lucerne and red clover. Combining grasses with high WSC concentrations and legumes with low CP concentrations in mixed swards should allow a reduction in N fertilizer inputs and a reduction in the risk of high N excretion from livestock grazing swards with high CP concentrations. Significant variation within white clover and associated materials for lower CP and higher WSC concentrations has been identified (N.D. Scolan, pers. comm.). An experiment using mixed pastures based on perennial ryegrass with enhanced levels of WSC and white clover with variation in CP concentrations is being undertaken in the Multisward project (http://www.multisward.eu).

The extensive degradation of CP, which occurs during ensilage, worsens the imbalance between degradable protein and energy in legume silages and leads to inefficient N utilization and high urinary N excretion (Dewhurst *et al*., 2003; Cohen *et al*., 2006; Dewhurst *et al*., 2009). Supplementation with cereal grains can overcome the relatively low energy concentration of legume silages and, hence, reduce urinary N losses per unit of forage intake (Cohen *et al*., 2006). Legume silages or hays complement maize silage in mixed diets well (Chenais, 1993; Rouillé *et al*., 2010) as they can provide sources of both degradable and undegradable protein. They also offer some potential to substitute imported soybean meal with home-grown protein, which will contribute towards protein self-sufficiency of livestock production on farm (Peyraud *et al*., 2012).

Plant secondary metabolites are a key feature of legumes

This section of the paper will focus on a few legumes that possess additional features which offer promise for ruminant nutrition and health, and for reducing greenhouse gas emissions. These features include tannins, polyphenol oxidase and protease enzymes (Mueller-Harvey, 2006; Kingston-Smith *et al*., 2010). Table 1 lists key (dis)advantages of some current legume species with such features and are being investigated in the current EU LegumePlus project (http://legumeplus.eu). Sainfoin (*Onobrychis viciifolia* Scop.) holds particular promise for alkaline and drought-prone soils, which cover much of central and southern Europe (Sölter *et al*., 2007). All legumes improve soil fertility and thus contribute to sustainability; but sainfoin contributes 16,200 kg of dry matter from fine roots ha^{-1} compared to 4,200 kg ha^{-1} from lucerne (Sergeeva, 1955). Together these legume species cover soil pH from 4 to 8.5 and temperature tolerance from southern to northern Europe. Red clover varieties have been improved for over 50 years, but few sainfoin or birdsfoot trefoil (*Lotus corniculatus* L.) cultivars are available. Plant breeding goals and achievements were recently reviewed for the main legume species red clover (Boller *et al*., 2010), white clover (Abberton and Marshall, 2010) and lucerne (Veronesi *et al*., 2010) but also for minor legume species (Piano and Pecetti, 2010) including birdsfoot trefoil and sainfoin.

Condensed tannins are oligomers and polymers of flavanols and have been found in a few forage legumes, such as birdsfoot trefoil, sainfoin, sulla (*Hedysarum coronarium* L.) and the flowers of *Trifolium* species (Waghorn *et al*., 1998; Waghorn, 2008). Total concentrations and compositions depend on accession/variety (Häring *et al*., 2007; Azuhnwi *et al*., 2011;

Stringano *et al*., 2012), season (Theodoridou *et al*., 2011), plant organ (Häring *et al*., 2007) and processing method (Hoste *et al*., 2006). Varieties with relatively stable concentrations and compositions will be needed to ensure that farmers can obtain reliable benefits from legumes containing condensed tannins. Whilst the biosynthesis of monomeric polyphenols and flavanols is now known, the search is still on for the genes and enzymes involved in the synthesis of condensed tannins (Dixon *et al*., 2012). Although it is likely that the quantitative and qualitative traits (i.e. concentrations and structures) of condensed tannins are under genetic control (Scioneaux *et al*., 2011), the question remains how amenable these traits are to improvement by plant breeding. Research will also be needed in order to develop screening tools that are suitable for breeding new legume varieties with optimized composition of condensed tannins.

Table 1. Advantages and disadvantages of bioactive legumes, which are suitable for covering a range of European environments and soil conditions.

Characteristics	Legume species		
	Trifolium pratense Red clover	*Lotus corniculatus* Birdsfoot trefoil	*Onobrychis viciifolia* Sainfoin
EU latitudes	35° to 64°	35° to 56°	35° to 54°
Soil pH:			
Tolerance	4.5 – 8.5	5.5 – 7.5	6.0 – 8.9
Optimum	6.0 – 7.5	6.0 – 6.5	6.5 – 8.0
Yield	Good	Fair	Medium
Establishment	Easy	Moderate	Difficult
Persistence	Poor	Medium	Very good
Tolerance to:			
Water logging	Fair	Good	Poor
Drought	Medium-poor	Medium-poor	Good
Anthelmintic	No	Low	Yes
Anti-bloating	No	Yes	Yes
Oestrogenic	Yes	No	No
Bioactive constituents	Polyphenol oxidase	Tannins	Tannins; oxidase

Plant secondary metabolites for increasing the efficiency of ruminal protein digestion

The role of condensed tannins in reducing ruminal protein degradation has been well documented (Jones and Mangan, 1977; Waghorn, 2008). A meta-analysis by Min *et al.* (2003) showed that increasing the concentration of condensed tannins progressively increased the amount of undegraded feed protein flowing into the duodenum without affecting microbial flow. By forming complexes with dietary proteins, condensed tannins generally slow the rate of protein degradation during fermentation in the rumen and during ensiling (Mueller-Harvey, 2006). Moreover, most plant proteases are located in the vacuole - just as condensed tannins are. Therefore, it is likely that, during the initial stages of digestion, condensed tannins may also reduce autolysis simply by complexing these enzymes (Kingston-Smith *et al*., 2010). However, what is not yet fully understood is which types of condensed tannins (or plant features) create optimal degradation rates. For example, high concentrations of condensed tannins in trefoils (*Lotus pedunculatus* Cav., var. Maku) may be too 'potent' as ruminants cannot utilize its dietary protein fully as evidenced by high faecal-N contents (Waghorn *et al*., 1998). However, in a few cases (birdsfoot trefoil, var. Goldi, and some sainfoin accessions), dietary protein appears to be appropriately protected by condensed tannins from ruminal degradation and available for post-ruminal digestion (Waghorn, 2008).

Previous research indicated that this protective effect could not be transferred from sainfoin plants containing condensed tannins to red clover, which is free of condensed tannins (Beever and Siddons, 1984). Subsequent research found, however, that red clover contains polyphenol oxidase (PPO), which can generate covalent bonds between protein and polyphenols when cells disintegrate, and this probably precluded any additional benefits from the condensed tannins in sainfoin. Promising results have been obtained by co-ensiling sainfoin and lucerne. This improved not only fermentation in laboratory silos, but more importantly it increased digestibility in sheep (Wang *et al*., 2007). Synergistic effects have also been observed during *in vitro* fermentation of sainfoin and cocksfoot (*Dactylis glomerata* L.) (Niderkorn *et al*., 2013).

The increased amount of duodenal N flow, associated with the presence of condensed tannins, is rarely matched by a greater utilization of amino acids in the intestine (Egan and Ulyatt, 1980; Aufrère *et al*., 2008). When ruminants eat tanniniferous legumes, they excrete less urinary nitrogen and slightly more faecal nitrogen compared to other iso-nitrogenous diets. This is important since urinary urea is quickly converted to ammonia and nitrous oxide (N_2O) and faecal nitrogen is more likely to contribute to soil organic matter (Mueller-Harvey, 2006; Woodward *et al*., 2009).

Few studies have investigated the effects of legumes containing condensed tannins on milk yield under European conditions; however, a study from New Zealand found higher milk yields in dairy cows when feeding increasing proportions of birdsfoot trefoil in perennial ryegrass diets (Woodward *et al*., 2009). In contrast to the USA, Canada and New Zealand, hardly any plant breeding programmes in Europe have involved legumes containing condensed tannins and it is, therefore, not surprising that Western Europe has only a few isolated areas where they are still grown.

Relatively little attention has also been paid to plant proteases, which appear to be active during the early stages of ruminal digestion (Kingston-Smith *et al*., 2010). Concentrations of plant proteases differ two-fold among legumes and, together with other features, contributed to twenty-fold differences in protein half-lives in a simulated, but microorganism-free, rumen environment (a half-life of 19 h in sainfoin *versus* 1 h in white clover) (Kingston-Smith *et al*., 2003). A few legumes also contain other features that are worth exploring. Red clover contains polyphenol oxidase, which can lead to covalently linked polyphenols and proteins. The resulting complex protects protein from rapid ruminal degradation, which may generate nutritional benefits for ruminants (Kingston-Smith *et al*., 2010). Polyphenol oxidase potentially reduces ruminal proteolysis (Jones *et al*., 1995) but *in vivo* experimental evidence for the positive effect of polyphenol oxidase on ruminal digestion is still lacking.

Plant secondary metabolites for improving animal health while reducing medication

Polyphenols and condensed tannins offer several opportunities to farmers for managing the health of their herds and flocks (Wang *et al*., 2012). For instance, bloat is a serious digestive disorder which causes painful suffering or death to animals plus financial losses to farmers. It generally occurs when readily digestible plants degrade too fast in the rumen; this produces a stable proteinaceous foam that traps fermentation gases, which can no longer be eructed by animals (Wang *et al*., 2012). However, plants containing condensed tannins, such as sainfoin, birdsfoot trefoil, crownvetch (*Coronilla varia* L.) and cicer milkvetch (*Astragalus cicer* L.), on their own or in mixtures with potentially bloat-forming forages never cause bloat (Mueller-Harvey, 2006). McMahon *et al*. (2000) showed that using fresh sainfoin as a complement to grazed lucerne helps to prevent bloat in cattle.

Coccidia cause diarrhoea and can result in serious economic losses. Recent research has obtained promising results from *in vitro* and *in vivo* studies with sainfoin in sheep (Saratsis *et al*., 2012). In addition, condensed tannins are effective against fly-strike in sheep, which occurs when sheep are affected by wet faeces. Fly-strike can be controlled with forages containing condensed tannins, as these yield drier faeces, which in turn prevent flies from depositing their eggs on sheep (Waghorn, 2008).

An area that is currently receiving much attention concerns the use of secondary plant metabolites against parasitic worms, which are now a worldwide threat to animal welfare and production. Nematode resistance against all three classes of broad-spectrum anthelmintic drugs is challenging conventional treatments (Molento, 2009). Some farmers in the USA already rely on the legume, *Lespedeza cuneata* G.Don, rather than on veterinary drugs to control the *Haemonchus contortus* worm (Burke *et al*., 2012). Condensed tannins represent a relatively untapped natural resource and can modulate nematode biology at key life-cycle stages (Hoste *et al*., 2006). It is of particular interest that the anthelmintic bioactivity was still present or even enhanced after sainfoin was conserved as hay and silage (Hoste *et al*., 2006; Häring *et al*., 2008). Thus, sainfoin can be fed when it is needed most, before and after parturition when host immunity of the mother and new-born is low. Numerous studies have shown that flavanol monomers and condensed tannins are effective *in vitro* against parasitic nematodes from sheep, goats, cattle, deer and other species (Molan *et al*., 2003; Novobilský *et al*., 2011). Positive results have also been obtained *in vivo* (Min *et al*., 2003; Häring *et al*., 2008; Burke *et al*., 2012; Azuhnwi *et al*., 2013). Condensed tannins are thought to act directly against the parasites because of their ability to form strong complexes with proline-rich proteins, which are present on nematode surfaces (Mueller-Harvey, 2006). Recent studies have also shown the potential for indirect effects because condensed tannins can stimulate the immune response in T-cells (Provenza and Villalba, 2010; Tibe *et al*., 2012). This is particularly important as helminths are inherently immune-suppressive and down-regulate or inappropriately skew the host immune response (Maizels and Yazdanbakhsh, 2003).

Forage legume-based systems have potential for reducing the negative effects of livestock systems on the environment

The great opportunities of legumes for environmentally friendly yet productive grassland-livestock systems derive from the different features reviewed above: (i) increased yield, (ii) replacement of industrial-N fertilizer by symbiotically-fixed N_2, (iii) higher nutritive value and voluntary intake of forage and (iv) greater livestock performance. Taken together, all of these effects create important environmental advantages of legume-based grassland-husbandry systems. These advantages are evident not only at the sward level, but also at the whole-farm level. In addition, the benefits apply also to the functional unit of managed land area and to the functional unit of the final product.

Forage legumes can reduce nitrate leaching

At the sward level, the sometimes very high N inputs to the ecosystem from symbiosis can result in a risk of nitrate leaching (Hooper and Vitousek, 1997; 1998; Scherer-Lorenzen *et al*., 2003; Palmborg *et al*., 2005). Loiseau *et al*. (2001) reported higher annual leaching losses of N from lysimeters when swards were sown with pure white clover (28 to 140 kg N ha^{-1}) than with pure perennial ryegrass (1 to 10 kg N ha^{-1}). Much higher values are reported for bare soils (84 to 149 kg N ha^{-1}). However, as long as the percentage of grass in mixed grass-legume swards is sufficient to take up mineral-N from the soil, it can be expected that this will prevent N losses by leaching. Under a mowing regime, Nyfeler (2009) found an increased risk of leaching of nitrate only if the percentage of legumes in the mixture was above 60-80% and this was combined with 150 kg N fertilizer input ha^{-1} yr^{-1}. Nevertheless, few studies have assessed the changes in nitrate content in the soil under mixtures containing legumes during periods of more than a few years. In the longer term, soil nitrate content could rise due to the mineralization of nitrogen-rich legume residues. In a five-year experiment, Oelmann *et al*. (2011) observed a positive effect of the presence of legumes on the NO_3-N content in the soil, but this effect did not increase with time, and therefore did not indicate an increasing risk of N leaching over the 5 years.

It has been suggested that a higher proportion of white clover in perennial ryegrass pastures, at the expense of mineral-N fertilizer, is an important component of low-input sustainable systems for livestock production (Thomas, 1992; Pflimlin *et al*., 2003). Evidence comes not only from cut plots (above) but leaching of nitrate was also lower under grazing of mixed white clover-grass swards compared with highly fertilized pure grass swards (Hooda *et al*., 1998; Ledgard *et al*., 2009; Peyraud *et al*., 2009). These results are explained mainly because mixed pastures do not support as high stocking rates as fertilized grass pastures and, to a lesser extent, by the down-regulation of symbiotic N_2 fixation under high mineral-N availability. At a similar stocking rate (3.3 cows ha^{-1}) and milk yield per hectare (13,200 kg milk ha^{-1}), Ledgard *et al*. (2009) reported similar leaching of N (30 kg N ha^{-1}) under mixed grass-clover pastures and pure grass fertilized with 160 kg N ha^{-1}, whereas N leaching increased to 60 kg N ha^{-1} for a more intensively fertilized grass pasture (207 kg N fertilizer ha^{-1}, 15,500 kg milk ha^{-1}). Also, Vertès *et al*. (1997) found a 5 to 10% reduction of NO_3 leaching under grass-clover, compared to fertilized pure-grass pastures. Losses of nitrate under grazed grass-clover swards can rise with increasing proportions of clover (Schils, 1994; Ledgard *et al*., 1999). There is less information available for other legumes. Losses through leaching were lower under pastures with a lucerne-grass mixture than from a white clover-grass mixture (Russelle *et al*., 2001) for a similar yield.

At the level of the whole-farm system, despite an apparently negative effect on N excretion by ruminants, legumes actually provide opportunities for reducing N losses. For example, N-use efficiency decreases with the application of increasing amounts of mineral-N fertilizer (Scholefield *et al*., 1991) and legumes overcome the need for a precise and timely supply of mineral-N fertilizer and hence reduce the amount of available ammonia N in the soil (Jarvis and Barraclough, 1991).

Forage legumes can contribute to reduced greenhouse gas emissions

<u>Methane</u>

Methane produced in the rumen is a large contributor to the greenhouse gas (GHG) emissions by livestock systems (Tamminga *et al*., 2007; Waghorn and Hegarty, 2011). Legumes can contribute to reducing ruminal methane production per unit of intake. Ruminants fed legume forages generally emitted less methane than grass-fed animals, per unit of feed intake (McCaughey *et al*., 1999; Waghorn *et al*., 2006) although not in all cases (Van Dorland *et al*., 2007). This may be due to a modification of the ruminal fermentation pattern toward propionate, which in turn is a hydrogen carrier and thus reduces the amount of methane produced. Inconsistency of results between experiments can arise from difference in forage composition (stage of maturity, presence of condensed tannins) and animal genotypes.

Condensed tannins may also be useful for reducing greenhouse gases (Kingston-Smith *et al*., 2010) as several studies have shown that condensed tannins reduced methane production *in vitro*. A recent meta-analysis revealed a general anti-methanogenic effect of condensed tannins above 20 g kg^{-1} DM in feeds (Jayanegara *et al*., 2012). Some effects of condensed tannins were also reported from *in vivo* studies with sainfoin (Waghorn, 2008), birdsfoot trefoil (Woodward *et al*., 2004) and sulla (Woodward *et al*., 2002). It would appear that the anti-methanogenic properties of condensed tannins stem either from direct effects against methanogens and/or from indirect effects on protozoa. Interestingly, there were marked differences in the selectivities of different tannins (Pellikaan *et al*., 2011). Preliminary results suggest that polymer size of condensed tannins is an important structural feature for anti-methanogenic activity (Tavendale *et al*., 2005).

Nitrous oxide

Each kg of N as ammonium nitrate produced in the industrial Haber-Bosch process consumes large amounts of energy (58 MJ) and also emits significant amounts of greenhouse gases (8.6 kg CO_2 equivalents) thereof 19 g N_2O (ecoinvent, 2010). In addition, the IPCC (2006) suggested that for every 100 kg of N fertilizer added to the soil, on average 1.0 kg of N is emitted as N_2O, which is a greenhouse gas that is *ca.* 300 times more active than CO_2 (Kingston-Smith *et al.*, 2010). The process of denitrification is the most important source of N_2O from pasture systems (Soussana *et al.*, 2010). Denitrification occurs when the soil is wet, oxygen availability is restricted and nitrate concentration is high. Nitrification is favoured by a supply of ammonium-N in well-drained soils. Thus, large peaks of N_2O emissions are measured in grasslands immediately after N-fertilizer applications (Ineson *et al.*, 1998; Klumpp *et al.*, 2011). There are three reasons why N_2O emissions from legume-based grassland systems should be lower than from fertilized grass systems: (i) nitrogen is fixed symbiotically within the legume nodules and thus is not freely available in the soil in a reactive form, (ii) symbiotic N_2 fixation activity is down-regulated if the sink of N for plant growth is small and (iii) in optimized grass-legume mixtures the grass roots take up N derived from legume roots and from mineralization of soil organic matter. Indeed, a compilation by Jensen *et al.* (2012) showed that annual N_2O emissions were largest in N fertilized grass swards (19 site-years; 4.49 kg N_2O-N ha^{-1}) followed by pure legume stands (17 site-years; 0.79 and 1.99 kg N_2O-N ha^{-1} for white clover and lucerne, respectively) and mixed grass-clover swards (8 site-years; 0.54 kg N_2O-N ha^{-1}). Within the revised greenhouse gas guidelines (IPCC, 2006), symbiotic N_2 fixation has actually been removed as a direct source of N_2O because of a lack of evidence of significant emissions arising from the fixation process itself (Rochette and Janzen, 2005). These authors concluded that the N_2O emissions induced by the growth of legume crops/forages may be estimated solely as a function of the above-ground and below-ground nitrogen inputs from crop/forage residue during pasture renewal. Emissions of N_2O from legumes do occur as a result of the decomposition of residues from leguminous plants but the magnitude of such emissions remains uncertain (Baggs *et al.*, 2000).

At the level of the whole livestock system, Ledgard *et al.* (2009) and Basset-Mens *et al.* (2009) showed by using life-cycle analysis, that greenhouse gas emissions decreased by 1.15 to 1.00 kg eq-CO_2 kg^{-1} milk with mixed grass-clover pastures compared to pure grass pastures because of the reduction of N_2O emissions in New Zealand dairy farms. Basset-Mens *et al.* (2005) have compared greenhouse gas emissions from dairy farm systems in Sweden, southern Germany and New Zealand using life-cycle analysis and emission coefficients. The New Zealand system relies essentially on permanent grass-white clover pastures, which are grazed all year round with an annual N fertilizer input of 100 kg ha^{-1} and less than 10% of the feed requirement of cows is provided by feed supplements. They showed that the total emission per 1 kg milk is 30 to 80% lower from the New Zealand system. Greenhouse gas emissions are high from intensive European dairy farms based on predominant grass pastures; the contribution of methane is reduced in proportion and CO_2 emissions were much higher in proportion (i.e. 3.7 times higher than from the New Zealand system), because of the production and transport of feed concentrates and mineral-N fertilizer and because of effluent management. Schils *et al.* (2005) compared the total emissions from dairy systems in the Netherlands, which were either fertilized ryegrass or grass-clover pastures (i.e. inputs of 208 and 17 kg mineral-N ha^{-1} yr^{-1}): greenhouse gas emissions kg^{-1} of milk were 20% lower for grass-clover pasture-based systems.

Carbon dioxide

Industrial production of each kg of inorganic N emits 2.25 kg of CO_2. Legumes offer a big advantage because the entire C needed for symbiotic N_2 fixation comes directly from the atmosphere via photosynthesis and, thus, are 'greenhouse-gas neutral'.

A further option to mitigate climate change is C sequestration into the soil. New C can only be introduced into the soil via photosynthesis by plants and the C:N ratio of soil organic matter is fairly constant in almost all soils (Kirkby *et al.*, 2011). Consequently, C sequestration into soil organic matter ultimately means sequestration of N into soil organic matter (80 kg N t^{-1} of C). Current evidence suggests that humus formation is particularly limited by the availability of N (Christopher and Lal, 2007). This again points to the importance of legumes and their symbiotic N_2 fixation for coupling C and N cycles and for delivering the N needed to sequester C into soil organic matter. Data from a large survey of soil organic matter in France (Arrouays *et al.*, 2001), and models (Soussana *et al.*, 2004), show that the conversion of short-term N-fertilized grass leys into grass-legume mixtures could sequester C into soil organic matter. Indeed, several studies found higher soil organic matter contents under grass-legume mixed swards than under pure grass swards (Ruz-Jerez *et al.*, 1994; Mortensen *et al.*, 2004).

Forage legumes reduce consumption of non-renewable energy

The introduction of legumes reduces non-renewable energy consumption in livestock farms since they use atmospheric N and since no direct financial or energetic cost is linked to this N input. In comparison, each kg of inorganic N produced in the industrial Haber-Bosch process consumes large amounts of energy. The estimations are highly variable and range from 44 MJ (Kaltschmitt and Reinhardt, 1997) to 78 MJ (Kitani, 1999). Studies estimated that under French conditions 0.17 MJ of energy are required to produce 1 MJ of net energy with ryegrass fertilized at 150 kg N ha^{-1}, but only 0.06 MJ with a ryegrass-white clover mixture, and 0.13 MJ for maize silage planted after wheat (Besnard *et al.*, 2006). Similarly, energy consumption decreased from 5.0 MJ kg^{-1} milk for intensive dairy farms in the Netherlands to 4.0 MJ kg^{-1} milk for French farms using maize silage and fertilized grasses and to 3.1 and 1.4 MJ kg^{-1} for systems based on grazing in Ireland and New Zealand, respectively (Le Gall *et al.*, 2009; Peyraud *et al.*, 2009). The higher energy consumption in Irish grassland-based systems appears to be linked to the utilization of high amounts of N fertilizer on pure ryegrass pastures, in comparison with the lower use of N in New Zealand systems.

Legumes offer an option for adapting to atmospheric change

Legumes – again through their coupling of the C and N cycles – provide a useful option for adapting to atmospheric change. Elevated atmospheric CO_2 concentrations stimulate photosynthesis and this leads to a one-sided increase in C availability within the ecosystem. Research has shown that, under controlled conditions and ample nutrient supply, the yield response of plants follows the increased rate of photosynthesis. However, under field conditions, N is the major limiting factor in the yield response of grasslands to elevated CO_2. Thus, elevated atmospheric CO_2 concentrations resulted in a decrease in the index of N nutrition of grasses (Soussana and Hartwig, 1996; Zanetti *et al.*, 1997), which indicates an increased N limitation of growth. Legumes, with their access to the unlimited N source of the atmosphere, have the potential to close such an increased gap between N demand and N availability of the ecosystem. Indeed, in fertile grasslands, legumes benefit more from elevated atmospheric CO_2 concentrations than non-fixing species (Hebeisen *et al.*, 1997; Lüscher *et al.*, 1998; 2000; Campbell *et al.*, 2000) and this results in a significant increase in symbiotic N_2 fixation due to higher proportions of legumes in the sward and due to a higher proportion of N derived from symbiosis in the legume plant (Soussana and Hartwig, 1996; Zanetti *et al*., 1997). In fact, the additional N harvested under high atmospheric CO_2 concentrations was derived solely from increased activity of symbiotic N_2 fixation (reviewed in Lüscher *et al*., 2004; Soussana and Lüscher, 2007).

There are other reasons why legumes can be suggested to be well adapted to future climatic conditions. Legumes have higher temperature requirements for growth than their companion grasses (Mitchell, 1956; Davies and Young, 1967). Warmer temperatures should, thus, result in a competitive advantage for the legumes as indicated by the seasonal cycle of the white clover proportion in mixed swards, which is high in the summer and low in spring and autumn (Lüscher *et al*., 2005). Especially in temperature-limited environments of high altitudes and high latitudes, the projected increase in temperature could result in an advantage for legumes. The projected increase in the frequency and severity of drought-stress periods may increase interest in the use of deep-rooting species such as red clover, lucerne, birdsfoot trefoil and sainfoin, as they allow the use of water reserves in deeper soil layers. In addition, niche theories not only predict higher yields of mixed swards compared to monocultures, but also that they can better deal with climatic variability and stress and that they show higher resilience after cessation of stress (insurance hypothesis; Naeem and Li, 1997; Yachi and Loreau, 1999). Accordingly, drought-stress vulnerability and resilience of deep (red clover, sainfoin) and shallow rooting (white clover) legumes and of grass-legume mixtures are investigated in the projects AnimalChange (www.animalchange.eu) and LegumePlus (www.legumeplus.eu).

Technology needs for keeping a more stable abundance of legume in the sward

In conclusion, grass-clover mixtures with 30 to 50% of legumes seem to be an optimal system: they yield high amounts of N from symbiosis, generate high forage yields of high nutritive value, which generates high voluntary intakes and livestock performances and, at the same time, they minimize the risk of N losses to the environment. The big challenge for legume-based grassland-husbandry systems, however, will be to maintain the proportion of legumes within this optimal range.

Legumes have a distinct competitive advantage in N-limited systems (Hartwig, 1998). When competing with non-fixers, legumes avoid N deprivation by supplementing mineral-N uptake with symbiotic N_2 fixation, thereby retaining a relatively high growth rate even in a low soil-N environment (Woledge, 1988). In contrast, where mineral-N is abundant, N_2 fixation is energetically costly and N_2 fixers tend to be competitively excluded by non-fixing species (Faurie *et al*., 1996; Soussana and Tallec, 2010). There was a strong decrease in the proportion of legumes in the swards of the pan-European experiment in its third and final year (Nyfeler *et al*., 2009; Finn *et al*., 2013) and this further confirms the difficulties of maintaining the desired abundance of legumes in mixtures (Frame, 1986; Guckert and Hay, 2001). Sward management strategies with reduced N fertilizer input and/or increased cutting frequencies can increase the proportion of white clover (Schwank *et al*., 1986). The effectiveness of such management treatments to regulate the proportion of white clover is evident from the Swiss Free-Air CO_2 Enrichment (FACE) experiment (Hebeisen *et al*., 1997; Zanetti *et al*., 1997). Averaged over the first three years, the contribution of white clover was 14% at infrequent defoliation combined with high N fertilization, whereas it was 57% at frequent defoliation combined with low levels of N fertilizer. However, due to large seasonal variations, there were also periods with unsustainably high clover percentages of above 80% (Lüscher *et al*., 2005).

Another option to optimize and stabilize legume abundance in mixtures is an optimized composition of seed mixtures. This will require decisions on how many and which species to include, and which proportions of the species and which cultivar of the species to choose.

This option is evident from the Swiss site of the pan-European experiment, where, besides the experimental four-species mixtures (Kirwan *et al*., 2007; Nyfeler *et al*., 2009), Swiss Standard Mixtures (Suter *et al*., 2012) were also examined. These mixtures contain more species (up to eight) and their composition (relative and absolute abundance of species) was continuously ameliorated during the last few decades based on experiments and observations on farms. Over the three years of the experiment, the decline in clover abundance in the Swiss Standard Mixtures was much smaller than that in the four-species mixtures of the pan-European experiment (Suter *et al*., 2010). Development of seed mixtures containing species with comparable competitive abilities could result in more balanced and stable mixtures (Lüscher *et al*., 1992). Moreover, not only do species differ in their competitive abilities but also cultivars within species. Suter *et al*. (2007) found that the realized species composition of the established sward differed tremendously depending on which cultivars were chosen for the seed mixture. All these results demonstrate that the composition of the seed mixture offers a multi-factorial opportunity for optimization.

Conclusions

As a component of mixed grass-legume swards, forage legumes offer important opportunities for tackling future agricultural challenges. The great potential of legumes for sustainable intensification is related not just to one specific feature; their strength stems from the fact that several of their features can act together on different 'sites' in the soil-plant-animal-atmosphere system. It is interesting to note that their advantages are most pronounced in mixed swards with 30-50% of legumes. These advantages are: (i) increased forage production; (ii) 'greenhouse gas-neutral' and 'energy-neutral' N input into grasslands via symbiotic N_2 fixation; (iii) support of non-N_2-fixing plants in the grassland through transfer of symbiotically fixed N; (iv) higher nutritive value and voluntary intake of the forage with a less marked decline of quality with advancing maturity than grasses, which lead to (v) higher livestock performance; in addition, bioactive plant secondary metabolites of legumes can enhance (vi) efficiency of protein digestion by ruminants and (vii) benefit animal health through lower medication. These multiple advantages benefit the whole grassland-husbandry system through reduced dependency on fossil energy and industrial N fertilizer, lower nitrate and greenhouse gas emissions into the environment, lower production costs, higher productivity and protein self-sufficiency. In addition, legumes may offer an option for adapting to higher atmospheric CO_2 concentrations and to climate change. Legumes generate these benefits at the level of the land management unit and also at the level of the final product unit. However, legumes suffer from some limitations and future research is needed to fully exploit the opportunities they offer. The most important areas for research are: (i) more predictable and controllable proportions of legumes within mixed plant communities, which, most probably, is achievable through innovative management strategies, optimized seed mixtures and breeding for increased competitive ability and/or niche complementarity; (ii) improved nutritive value of fresh forage and, especially, silage, which can be addressed by optimizing the energy/protein balance within the plants (e.g. by increasing water-soluble carbohydrate concentration); (iii) better exploitation of the multiple opportunities offered by plant secondary metabolites, which requires knowledge of optimum structures and concentrations of these compounds, and development of cultivars and cultivation techniques that enable farmers to produce these optimized plant secondary metabolites reliably. The development of legume-based systems of grassland husbandry undoubtedly constitutes one of the pillars for more sustainable and competitive ruminant production systems and it can only be expected that legumes will become more important in the future.

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Agriculture in Iceland – A grassland based production

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Abstract

Iceland is located just below the Arctic Circle, yet it enjoys a mild maritime climate. The mean temperature in Reykjavík is -0.5 and 10.6°C for January and July respectively. The growing season is cool and generally fairly wet and extends over four months from May to September. Such conditions are ideal for grass growth and therefore agriculture is primarily grassland-based livestock production. Winter fodder for the livestock is mostly harvested from permanent grass fields, almost half of which are on drained organic soils. Arable land is now approximately one tenth of the cultivated area, divided equally between barley production and green fodder. Climatic conditions have been favourable for barley cultivation in the last two decades and the annual production provides around a quarter of the current concentrate requirements. This could be doubled in the next few years if climatic conditions remain favourable. Iceland has only a single breed for each of dairy cows, sheep and horses. These are all unique to the country and have remained almost completely isolated since the settlement around 1100 years ago. Import of exotic genetic material is strictly controlled. The country is self-sufficient in both meat and dairy products. The dairy cow is rather small and produces on average 5,600 kg milk yr^{-1}. Milk yields have been steadily increasing over the last 40 years thanks to improved fodder and genetic improvements in the local breed. The Icelandic sheep has been bred for good ewe productivity and superior meat quality. The sheep population is around 460 thousand heads and this outnumbers all 27 EU countries on the basis of animal numbers per 1000 inhabitants. The Icelandic horse is characterized by its many coat colours and five different gaits. It is estimated that two thirds of the horse population are currently found abroad. Projected climate change indicates that conditions for crop plants will improve and the cultivation of arable crops will become more secure in the near future.

Keywords: grassland agriculture, marginal environment, barley production, local breeds

The geography of Iceland

Iceland is an isolated island of about 103,000 km^2 surrounded by the North Atlantic Ocean and is extends between the latitudes 63.2°N and 66.3°N (Figure 1). The shortest distance to neighbouring countries is 278 km to Greenland, 420 km to the Faroe Islands and 708 km to Scotland. Approximately one quarter of the country is below 200 m elevation, where all major settlements as well as most of the agricultural areas are located. The population of Iceland is approximately 320,000. The population density is only 3.1 inhabitants km^{-2}, compared to 112 inhabitants km^{-2} in EU-27. Even the Scandinavian countries Norway, Finland and Sweden are several magnitudes above Iceland with 15-22 inhabitants km^{-2}. Almost 80% of the population lives in the south-west corner of the country, in or close to the capital. For most of the agricultural areas population density is therefore well below the country average.

The unique demographic conditions in Iceland result in vulnerable and non-competitive agricultural industry. Firstly, the local market is small and the processing industry produces a wide range in very small quantities making it difficult to benefit from scale. Secondly, transport costs are high, both for live animals and raw milk from farms to the processing plants, and of processed food from the plants to consumers. Thirdly, centralized services such as technical support, farm extension and health inspection are inevitably scattered in small, often inefficient units. Fourthly, the retail market is dominated by few companies, giving the farming industry a weak position against the retail industry.

Figure 1. The Arctic Region showing the Arctic Circle (broken line) and the 10°C isotherm for July (solid line).

The natural environment

Despite its northern location the country enjoys a mild maritime climate, thanks to a branch of the Gulf Stream, which brings warm sea from the Gulf of Mexico to the southern and the western coast of Iceland. The mild Atlantic air meets colder Arctic air over the country and this causes both frequent changes in weather and storminess, with more rainfall in the southern and western part than in the northern part of the island (Einarsson, 1984). The mean temperature (1961-1990) in Reykjavík is –0.5° and 10.6°C for January and July, respectively, and the annual precipitation is 799 mm. The 10°C isotherm for July stretches further south over Iceland, compared to Northern Scandinavia (Figure 1). Iceland is the only country in the world where agricultural production can be found north of this line. Accumulated Day Degrees (Tsum >5°C) in Reykjavík are currently only around 700 compared to 1300 in south Finland (Stoddard *et al.*, 2009), and 1100 at Umeå in Sweden and Trondheim in Norway, which are at comparable latitudes to Reykjavík. Summer temperatures (mean for May-September) have, however, varied in recent decades reaching only 8.5°C on average in the years around 1980 compared to the years around 1930 and the present value of approximately 10°C (Figure 2). This can primarily be explained by the North Atlantic Oscillation (NAO), a climatic phenomenon in the North Atlantic Ocean, which results from fluctuations in the difference of atmospheric pressure at sea level between the Icelandic low and the Azores high (Hurrell, 1995).

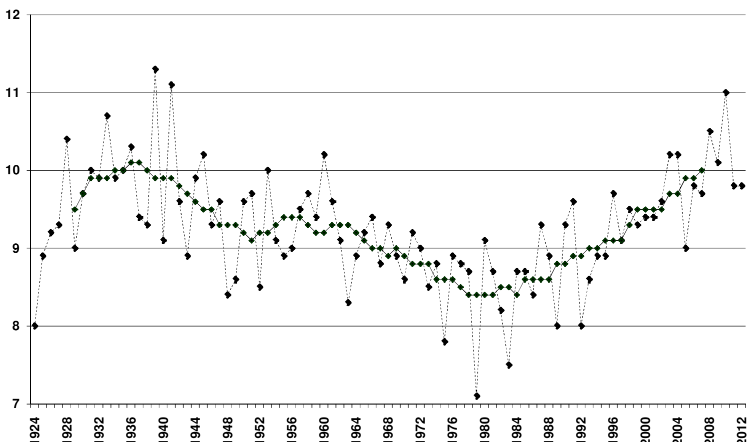

Figure 2. Mean summer temperature in Reykjavík (May-September) from 1924 to 2012 (-----actual mean values; ──── 11 year moving averages).

Iceland lies on the Mid-Atlantic Ridge. The parent materials are of recent volcanic origin and consist mostly of basaltic tephra. Soils which form under such conditions have unique characteristics and are classified as Andosols (Arnalds, 2004). The soils in Iceland have been divided into two main groups: soils of deserts and soils under vegetation (Arnalds, 2008). The soils of the former group are dominated by poorly weathered volcanic tephra and are termed Vitrisols. They are infertile, poor in organic carbon (<1%) and exposed to intense cryoturbation and erosion. Soils under vegetation are andic (Andosol properties) and/or histic (organic) in nature depending on drainage and the dominant influence of eolian and tephra input. The organic carbon can thus range from <12%C in Brown Andosols (freely drained) to >20% in Histosols in some wetland areas. The Andosols generally have high porosity with a range of pore sizes that can retain large amounts of water. They have high organic C and N content, and a strong tendency to fix phosphorus. All these characteristics provide an excellent environment for root growth (Nanzyo *et al.* 1993). Andosols lack cohesion and are therefore open to erosion by water or wind, especially if the vegetation is weakened, for example, by overgrazing.

The native flora is species poor compared to areas of comparable latitude, and it consists of fewer than 500 species. It mostly lacks nitrogen-fixing plants, possibly further adding to the vulnerability of the Icelandic vegetation. It has been postulated that when Iceland was settled in the late 9th century, approximately 60% of the country was covered with vegetation of which some 15-20% were forests (Thorsteinsson, Olafsson and van Dyne, 1971). Today, however, natural birch woodlands cover only about 1% of the total area (Aradottir *et al.* 2001), and according to a recent survey of soil erosion 42% of the island is 'considerably', 'severely' or 'extremely' eroded (Arnalds, 2011).

A brief history of agriculture in Iceland

Iceland was settled in the middle of the Viking Era between 850 and 1000 AD. At that time there was a need for more space for a growing population in countries that were already overpopulated and degraded from excessive exploitation of their natural resources. The unspoiled nature of this remote island tempted the Iron Age farmers and they became the true settlers in Iceland rather than the expansionist Vikings. Most of them sailed from the western shores of Norway and they brought with them knowledge on traditional Scandinavian farming of cereal production, animal husbandry and fishing, as well as forestry, iron making and hunting, depending on their exact origin. In the new country they found ample space, good soil for cultivation, and extensive grazing areas free from predatory animals; in fact, everything that a farmer wants to base his existence on. They soon realized, however, that the country was ill suited to arable production and the farm animals, which they brought with them, became the foundation on which the inhabitants built their livelihood.

For centuries sheep husbandry was the main farming activity in Iceland and productivity was very low. Haymaking relied on indigenous species obtained from wild pastures and wetland areas grown with *Carex* species. It has been postulated that in the first centuries after the settlement, vegetated areas available for fodder production, either for haymaking or grazing, were around 4 million ha. At that time (ca. 1200) the Icelandic population was probably around 80,000 people whereas the population of Norway was 250,000, or just over three Norwegians to one Icelander (Snævarr, 1993). The vegetation slowly deteriorated and by 1700 around 1.5 million ha had already eroded. The natural resources of the country, without any external inputs, could thus support around 360,000 sheep by utilizing grazing all year round and hay obtained from wetlands in more difficult years. This was sufficient to maintain a population of 50-60 thousand (see Guðbergsson, 1996). Today, one million ha more of vegetation have eroded away and presently there are around 15 Norwegians to one Icelander. While conditions improved in our neighbouring countries in the wake of the industrial revolution and a growing middle class in the urban areas, development stagnated in Iceland and even deteriorated. Here, the geographic location and extreme natural conditions played a decisive role (Eldjárn, 1981). Thanks to scientific innovation, technical advancement and new approaches in social development, which emerged during the 19th century, the Icelandic nation has managed to keep pace with its neighbours in modern times.

The growing urban population in the late 19th century created a market for agricultural products, and food security for the whole nation became the major political driver for agriculture. Farmers slowly adopted new but primitive technology in hay production and for improving their hay fields. Artificial fertilizers arrived on the scene. In the years between the world wars, and particularly after the end of World War II, the rural population decreased rapidly and various public support and subsidy systems were set up to reward for increased production. Advanced machinery was imported to convert wetland areas to new agricultural land. Agriculture was driven towards extensive cultivation of grassland seeded with introduced non-adapted grass cultivars and greater intensification with the use of artificial fertilizer and concentrates. Unfavourable climatic conditions in the 1960s caused severe winter kill in cultivated grasslands in many parts of the country. Overproduction in the 1970s, particularly in the sheep sector, called for revision of the extensive subsidy system. A complete revision of the legal framework for agricultural policies was carried out in 1980-1985. The main objectives were to promote structural adjustment and increase efficiency in agricultural production and processing for the benefit of producers and consumers and to adjust the level of production to domestic demand and secure sufficient supply of agricultural products as far as practicable at all times. A quota system was introduced and farmers had to adapt to production limitations.

From 1995 the emphasis has been on improved efficiency and creating conditions for greater multifunctionality. Food habits have been rapidly changing and the proportion of local agricultural products in the total food budget becomes progressively lower. The drive is now towards maintaining margins by reducing inputs as well as by increasing outputs. Dairy and sheep production is steady but the number of “traditional” farms is declining, especially in the dairy sector. Increasing urban demand for rural estates is causing a significant rise in farmland prices. Farmers and other landowners are looking to alternative land uses in addition to food production and agriculture is becoming progressively more multifunctional.

Icelandic agriculture in a European context

Bearing in mind the size of the Icelandic population, agricultural production is minute compared to most other countries in Europe (Table 1). Iceland accounts for only 0.5% of the total sheep population in the EU, or 462,000 animals, and 0.1% of the total number of dairy cows, or 25,400 animals. Only Malta has fewer dairy cows than Iceland but many countries have fewer sheep, even though Iceland lies well below the average. However, when animal numbers per 1000 inhabitants are compared Iceland ranks 6th among all 27 EU countries for dairy cows. For sheep it ranks first with almost twice as many sheep per 1000 inhabitants as Greece and Ireland. This is even more striking in the light of the fact that sheep numbers have been more than halved in Iceland over the last 30 years.

Farms in Iceland are much larger in hectares than in other European countries (Table 1). The mean farmland size per farm is well over 600 ha compared to, for example, around 60 ha in Denmark. However, most of the agricultural land can be defined as extensive grazing areas, often stretching well into the central highlands, and not suitable for cultivation. Currently, only around three hectares on average per farm are used for arable crops even though a part of the grass fields are renovated on a regular basis. Iceland is therefore unique in this respect. Only a quarter of the country, or around 2.5 million ha, is below 200 m, of which only 600 thousand ha at the most are suitable for arable cultivation according to current estimates.

Assuming a minimum patch size of 30 ha, a realistic requirement for large scale production, good quality agricultural land would only be around 160,000 ha (Sveinsson and Hermannsson, 2010). There are efforts under way to define the quality of arable land more accurately with respect to soil and climatic conditions. The classification will be used by community planners for protection of the most valuable agricultural land for future use in accordance with provisions in the Planning Act recently approved by the Icelandic Parliament.

Table 1. Number of dairy cows and sheep (total no. in thousands and per thousand inhabitants) in 2008, and average farm size in hectares divided into arable land and other utilisable agricultural area (UAA) in selected EU countries and Iceland (Eurostat, 2013).

Country	Dairy cows		Sheep		Arable land	Other UAA
	Total ×1000	Per 1000 inhabitants	Total ×1000	Per 1000 inhabitants		
Iceland	**26**	**83**	**462**	**1,502**	**3**	**612.8**
Austria	530	64	333	40	9	10.3
Denmark	566	104	90	17	57	7.1
Estonia	100	75	62	46	33	15.0
Finland	288	55	94	18	35	0.6
France	3,794	60	7,715	121	36	17.0
Germany	4,229	51	1,920	23	40	16.2
Greece	154	14	8,994	805	NA	NA
Ireland	1,105	266	1,575	794	NA	NA
Malta	7	18	13	31	1	0.2
Netherlands	1,587	97	1,545	94	14	11.9
Poland	2,697	71	270	7	7	2.4
Slovenia	113	56	139	69	2	4.1
Spain	888	20	19,952	449	11	12.6
Sweden	366	40	521	57	37	6.8
United Kingdom	1,903	31	21,856	360	26	52.2

Fodder production and conversion of wetlands to hay fields

In former times winter fodder for the animals was obtained from extensive grasslands and wetland areas. At the turn of the last century the demand for agricultural produce increased with a growing population. Drainage of wetland to enable cultivation of hay fields in order to increase agricultural production was initiated through legislation set by the Icelandic Parliament in 1923, which provided support to farmers for the cultivation of undisturbed land or drained bogs (Figure 3). The development was slow initially but advanced rapidly during and after World War II when modern machinery arrived on the scene. These activities peaked around 1964 but began to slow down until public support ceased around 1990. In all, 31,600 km of open drainage ditches were excavated during this period and in the years 1962-1993 an additional 61,600 km of sub-surface drainage was placed in soil. It has been estimated that the total area drained is in the range of 340,000-389,000 ha (Gudmundsson *et al*., 2013). This means that a large part of virgin wetland in the lowlands has already been drained. The total area of cultivated land established reached around 170,000 ha, but currently this figure is 129,000 ha, of which approximately 45% is on drained wetland (Hallsdóttir *et al*., 2012).

Figure 3. Annual conversion of undisturbed land to cultivated hay fields (ha) and construction of drainage ditches (km) 1941–1990.

Around 90% of the cultivated area is currently used for permanent grass fields, most of which are older than five years. In the early days all grass seed sown was imported, primarily from Denmark and Norway (Helgadóttir, 1996). Limited information is available on the exact species and cultivars sown but initially it was commonly recommended to sow a mixture of several species, including timothy (*Phleum pratense),* meadow foxtail (*Alopecurus pratensis),* smooth meadow grass (*Poa pratensis),* fescues (*Festuca rubra, F. pratensis),* perennial ryegrass (*Lolium perenne)* and even floating foxtail *(Alopecurus geniculatus)*. Timothy slowly became the dominant grass species and records of actual seed imports show that it made up 40-60% and 75-85% of the total area sown, 1971-1990 and 1995-2005, respectively (Helgadóttir and Sveinsson, 2006). Timothy is the only forage species where cultivars are available which have been bred specifically for Icelandic conditions. Two of these (Korpa and Adda) were based on survivors collected from farmers' fields in Iceland but the third (Snorri) is the outcome of a joint breeding programme for Iceland and the northern areas of Scandinavia, where the primary breeding aims were sufficient winter hardiness, superior forage yield and good regrowth potential (Helgadóttir and Björnsson, 1994).

Species composition of Icelandic hay fields, surveyed in 1990-1993 over the whole country, demonstrated that timothy was the third most common species at the time, judging either from the proportion of fields where it occurred or from the average ground cover (Thorvaldsson, 1994). The actual contribution depended though on geographic location, moisture content, degree of winter damage, elevation and not least the age of the sward. Timothy made up 60% of the ground cover in first-year leys, 34% in fields between 2-5 years and less than 10% in fields over 10 years. Indigenous species, such as smooth meadow grass, red fescue (*Festuca rubra*), tufted hairgrass (*Deschampsia caespitosa)* and bentgrass (*Agrostis capillaris)*, thus gradually replace sown species and usually dominate fields that are older than 30 years.

Forage legumes have played a minor role in grassland agriculture in Iceland. They were introduced in the early part of the 20th century but disappeared from the market with the advent of artificial fertilizers after World War II. Extensive experiments with grass-legume mixtures have been carried out over the last three decades giving promising results but the main limiting factor has been the availability of adapted cultivars. The cultivation has gradually become more secure thanks to warmer summer temperatures in the last 10 years and positive outcome of increased breeding efforts of both white and red clover (Helgadóttir *et al.*, 2008; Marum, 2010). Recent experiments have shown that grass-legume mixtures are more productive and show greater yield stability over time than their individual components in monoculture irrespective of fertilizer treatment (Helgadóttir *et al.*, 2013; Sturludóttir *et al.*, 2013). Farmers are beginning to realize the potential of forage legumes as reflected in increased sales of seed of both white and red clover (MAST, 2013).

Most of the fodder produced for winter feeding is conserved in silage bales, which replaced the traditional field dried hay around 1990. Even though a negative relationship between nutritive value and forage dry matter yield for timothy is well documented (Bélanger *et al.*, 2001), it has a clear quality advantage over other grass species that are currently available for forage production in Iceland in spite of being higher yielding. For example, results from experiments carried out at Korpa Experimental Station in Iceland over a 20-year period have shown that fodder quality of timothy is superior to indigenous grass species found in Icelandic hay fields, with a mean dry matter digestibility (DMD) of 726 and 679 g kg^{-1} DM, respectively (Helgadóttir and Hermannsson, 2001). Studies on the palatability of forage species for dairy cows have also revealed the superiority of timothy if harvested before mid-heading, recording daily voluntary DM intake of timothy-based diets of 2.9-4.6% of live weight depending on the lactation stage and age of the cows. No other grass species tested could match this intake (Sveinsson and Bjarnadóttir, 2006).

Arable land is approximately one tenth of the cultivated area in Iceland. This is divided equally between barley production and green fodder such as annual ryegrass, oats and various *Brassica* species. It is known that Icelandic farmers grew barley for grain until the 14th century probably using landraces that settlers brought with them from Norway. It was not until 1923 that barley was successfully cultivated again for a few years in succession, but local production never became widespread or of any significance in agriculture. Climatic conditions have been favourable for barley cultivation in the last two decades (see Figure 2) and a barley breeding programme has been carried out from 1990 at the Agricultural University of Iceland resulting in four registered cultivars. It has mostly been based on high yielding Nordic cultivars with the aim of producing cultivars adapted to local conditions by improving earliness, lodging and wind resistance. Barley is currently cultivated on around 5,000 ha, giving an annual production of 16,000 tons. The mean yield is thus 3.2 t ha^{-1}, which is approximately 26% below the EU-average (FAO, 2013). Production has increased more than 30-fold since 1991 (Figure 4) and it now supplies a quarter of all cereals required for livestock. The annual production could be doubled in the next few years if climatic conditions remain favourable and could thus replace all imported barley. However, the cultivation has currently stagnated mainly because of undeveloped markets in the country for barley grain.

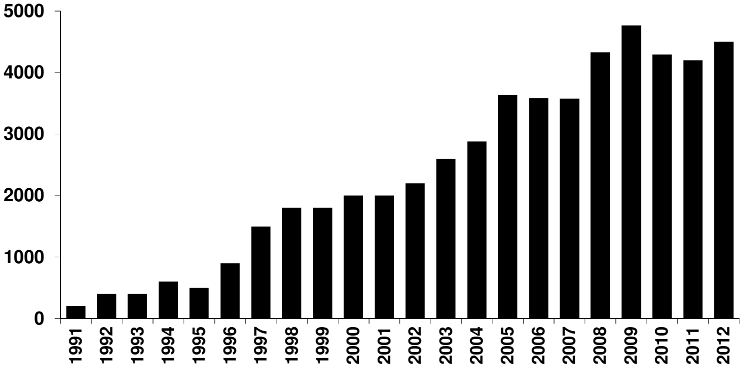

Figure 4. Annual cultivation of barley in hectares in Iceland from 1991 to 2012.

Livestock production

Iceland has only a single breed of dairy cows, a single breed of sheep (and goats) and a single breed of horses. All these breeds are unique to the country and have remained almost completely isolated since the settlement approximately 1100 years ago (Adalsteinsson, 1981). Import of exotic genetic material is strictly controlled in order to minimize the potential risk of disease distribution but also to protect the Icelandic breeds and their unique genetic traits. The country remains free of many common animal diseases and out of a total of 121 screened diseases in Europe (WAHID, 2012) only 14% have been found in Iceland, of which Johne‘s disease (Paratuberculosis) is the only clinical disease.

Dairy and beef production

Genetic studies have shown that the Icelandic dairy breed is related to North Scandinavian cattle breeds but the divergence seems to have happened some 1000 years ago (Kantanen *et al.*, 2000). It is rather small (live wt. 450-500 kg) and is generally considered to be well adapted to the prevailing harsh climate, rough fodder and rough terrain; and is similar to other northern cattle breeds, even though this has not been scientifically proven. Coat colour combinations found in the breed are diverse and in many ways unique, as the breed has never been subject to selection on the basis of colour. Similarly, the milk has unique combinations of beta-casein proteins and it has been suggested that there is a link between this trait and the risk for diabetes-I in children (Birgisdottir *et al.*, 2006) as well as improving the efficiency of cheese production from the milk (Ólafsson *et al.*, 2003).

A combination of long winters and cool summers, but perhaps more importantly unstable weather conditions, severely strains the dairy production resulting in low production security. The grazing period normally extends from late May to early September. The average milk yield was 5,600 kg cow^{-1} in 2012 (Association of Icelandic Dairy and Beef Cattle Farmers, 2013). This is considerably less than in most common dairy breeds in Europe and is, for example, only around 80, 65 and 60% of the annual milk production of the Norwegian Dairy Cattle (NRF), Swedish Red Cattle (SRB) and Swedish Friesian Cattle (SLB), respectively (Kristófersson *et al.*, 2007). However, the milk yield has been improving steadily over the last few decades (Figure 5). There was a distinct increase from 1997 to 2007 followed by a drop for two years, probably reflecting the economic recession, but it seems that milk yield is again increasing. There may be several explanations for the increased milk yield per cow. Firstly, the use of concentrates (in terms of kg cow^{-1}) grew rapidly in the period 1995 to 2007 (Figure 5). This can be attributed to the increased local barley production by dairy farmers (see Figure 4), as well as to increased import of concentrates. Secondly, extensive barley cultivation calls for systematic crop rotation which means more frequent renovation of grass fields. This in itself ensures better quality fodder (see above). Thirdly, digestibility of conserved feed has been steadily improving over the last 30 years. Farmers have been harvesting the primary grass growth progressively earlier, a change made possible by wilted round-bale ensiling introduced in the 1980s. Fourthly, increased milk yield per cow can also be associated with improved genetic gains in the local dairy herd (+727 kg cow^{-1} in the period 1970-2009) (Sigurdsson and Jonmundsson, 2011). Still, genetic improvement in such a small breed is bound to be rather slow, because of the limited selection intensity, risk of inbreeding accumulation and less-accurately estimated breeding values.

The dairy breed has not been bred for meat production and is therefore not well suited for beef production. Many dairy farmers, however, raise male calves as a side production. The growth potential is low, or 321 g carcass weight day^{-1} according to a recent study (Sveinsdóttir, 2010). Sporadic imports of semen from three beef breeds, Galloway, Aberdeen Angus and Limousin, have improved the situation somewhat. Strict import regulations and limited stock of genetic material have led to slow progress of producing purebred herds and increased the risk of inbreeding depression.

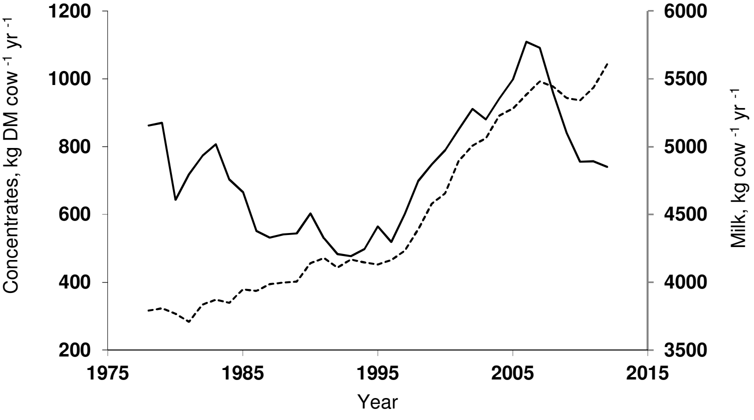

Figure 5. Mean milk yield, kg cow^{-1} yr^{-1} (-----) and the total use of concentrates, kg DM cow^{-1} yr^{-1} (———) from 1978–2012 (Association of Icelandic Dairy and Beef Cattle Farmers, 2013).

Sheep production

The Icelandic sheep breed belongs to the short-tailed North-European sheep and originates from the Settlement Era. The breed is the largest population of short-tailed sheep still used for production (Dýrmundsson and Niżnikowski, 2010) and genetic studies show clear but distant relation to breeds in Norway and the Faroe Islands (Tapio *et al*., 2005). Some 1000 animals belong to a distinct strain within the breed, the so called 'leader sheep' (Dýrmundsson, 2002). The Icelandic sheep is primarily a meat producing breed. Organized breeding (including AI) over the last 70 years has combined the national flock into one herd book which has resulted in a homogenous breed. The primary breeding aims have been good ewe productivity and production of lamb meat of excellent quality. The average lambing rate is 1.8 lambs per ewe giving roughly 26 kg of meat per ewe per year. This has been stable for the last 10-15 years. For meat quality the main breeding emphasis has been on carcass conformation in recent years resulting in improvement of 1.5 points on the 15 point EUROP scale for conformation since the implementation of the scale in 1998 (Sigurðsson, 2012).
Only a handful of farms use the sheep for milk production, and wool production is of limited economic importance for farmers. Icelandic wool is made of two types of fibres, fine bulky underwool and coarse outer hair. The wool is processed locally and the products are known as lightweight garments with excellent insulation (Gudmundsson, 1988). Pelts from lambs are mostly exported raw and are especially well suited for processing into double-face woolskins used for winter clothing (Näsholm and Eythorsdottir, 2011).
Sheep production is well suited to the prevailing environmental conditions. The maritime climate, with cool summers and relatively mild autumns, encourage semi-extensive production methods although most farmers house their flocks from November to May. The lambing season is in May, and after 3-5 weeks on lowland fields the flocks are moved to the highlands for 8-10 weeks. The slaughtering season is in September and October. Large common grazing areas in the central highlands ensure good growth conditions for the lambs at low cost.

The Icelandic tradition of grazing on common land is very old and written into several laws and regulations, the oldest dating as far back as the 12th century. Most farmers own their land but they only use a fraction of it for crop production (see Table 1). The majority of the farm land is exclusively used for extensive grazing. This may or may not be fenced and is sometimes even undivided between two or more farms. In addition to the common use of farm land, most sheep range freely in the central highland during summer. Most of this land is by now owned by the Icelandic state, but farmers have the intrinsic right to continue this grazing tradition. Relating this practice to EU-terminology, the term *Pastoralism* would probably cover the summer grazing in the common areas of the highlands. *Transhumance*, on the other hand, is not common in modern times although it was frequently practised before the 20th century.

Although the Icelandic sheep breed is free from a large range of common diseases, attempts to import live animals for breeding purposes have repeatedly resulted in outbreaks of severe epidemics. In the 18th and 19th century, the sheep scab mite (*Psoroptes ovis*) arrived with imported English and Spanish sheep causing severe losses. It still occurs sporadically although it may now possibly have been eliminated completely (Richter and Eydal, 1997). *Scrapie*, a prion-based disease, appeared after the import of a single English ram, imported from Denmark in 1878. The disease became a serious epidemic after 1950, probably because of more intensive production methods, longer housing period and increased herd size. From 1982 all outbreaks have been met with immediate whole-herd culling and strict control measures. The final attempt to import sheep in the 1930s resulted in three epidemics; Jaagsiekte (*Ovine Pulmonary Adenomatosis*), Maedi and Johne's disease. The Maedi virus, described by Icelandic scientists in 1952, was the first lentivirus to be isolated (Sigurdsson *et al.*, 1952). These epidemics were fought by massive slaughtering of sheep in the period 1941 to 1959 and restrictions on sheep transport within the country, many of which are still in place. Neither Jaagsiekte nor Maedi have been detected in Iceland since 1960 but Johne's disease is still found on rare occasions. No further attempts have been made to import genetic material for the Icelandic sheep breed.

These disease epidemics affected the size of the sheep population, together with serious volcanic outbreaks such as the Laki eruption in 1783 (Figure 6) (Statistics Iceland, 2013). The sheep production peaked in the late 1970s when the number of winter-fed sheep reached almost 900,000, but since then the numbers have reduced significantly and during the last 15 years the total number has been relatively stable at around 460,000 heads.

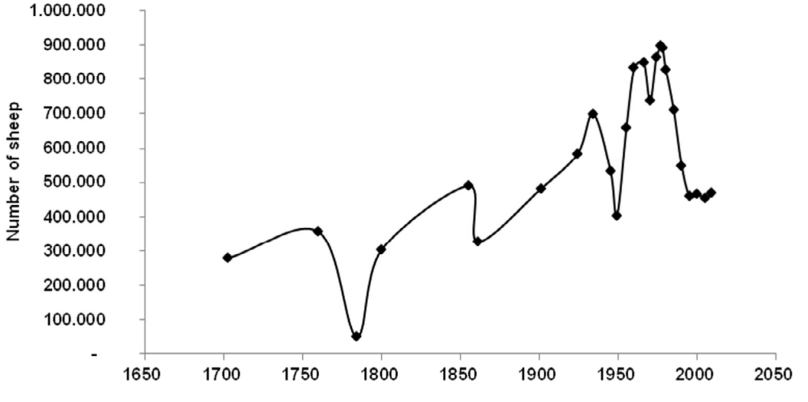

Figure 6. The size of the sheep population in Iceland from 1703–2011 (Statistics Iceland, 2013).

The Icelandic horse

The Icelandic horse is a unique breed that has been bred in isolation for centuries like the cattle and the sheep. There has been no documented import of horses since the time of settlement and today import is practically unthinkable on ethical grounds, although legally not impossible. Special characteristics of the breed are its many coat colours and five different gaits. The Icelandic horse is valuable for both leisure and sport. Presently there are approximately 77,000 horses in Iceland and the number has remained relatively stable since the early 1990s. The breed has become popular in many countries and it is estimated that two thirds of the total Icelandic horse population are located abroad. Every year around 2000 riding horses are exported, mostly to EU-countries. A world-wide herdbook, Worldfengur (http://www.worldfengur.com/), collects information on phenotypes and pedigree of all registered Icelandic horses in 35 countries, and breeding values are estimated for the combined population across countries. A small part of the horse population in Iceland is kept entirely for meat production but most horse breeders aim for the production of high quality riding horses. Horse breeding produces important externalities with regard to horse rentals, horse shows and large-scale exhibitions, and riding tours often coupled to farm tourism.

Other livestock

The local production of pork, chicken and eggs is sufficient to meet the domestic consumption. Most of the feed is imported but some pig farmers grow their own barley for feed. Production costs are generally high and the small market does not allow for any significant economy of scale. Genetic material for pig improvement is imported regularly from Norway and the production is based on the same crossbreeding system as in the Nordic countries. Production systems are also comparable with Norway as Iceland maintains strict limitations on the use of hormones, growth enhancers and feed-added medicines.

Currently there are 22 mink farms in the country and the yearly production is around 150,000 skins. The mink production has reached international standards and skins have been fetching good prices at Kopenhagen Fur. The feed is produced locally using raw materials (by-products) from both the fish industry and from slaughterhouses.

The Icelandic goat is yet another old-settlement breed and the only animal breed in Iceland that is defined as endangered according to FAO standards. The population is very small and has only on few occasions exceeded 1000 animals. Goats in Iceland are almost entirely held to conserve the breed and as hobby farming.

Wild terrestrial mammals

The terrestrial mammalian fauna of Iceland is composed of just four species. Only the Arctic fox (*Alopex lagopus*) is indigenous whereas the others were brought to the country, either inadvertently or on purpose (Ministry for the Environment, 2001). The wood mouse (*Apodemus sylvaticus*) was probably the first wild mammal to be brought to Iceland. It thrives in most regions of the country and is protected by law in the wild. The reindeer (*Rangifer tarandus*) in Iceland is of Norwegian domestic stock and was imported during the period 1771 to 1787. Only one herd remains in the north-east and east, consisting of 2,000-2,500 animals. The population is kept stable at that level by annual hunting quotas. The American mink (*Mustela vison*) was imported in the early 1930s for fur-farming. Some animals escaped and in spite of considerable efforts to stop its spread in the wild, mink had spread throughout the country by 1975. The population size is probably now around 10,000 animals in autumn.

Future perspectives

Climate change scenarios project that temperature in Iceland will have increased by 1°C by the middle of the 21st century and by further 1.4 to 2.4°C towards the end of the century. Most probably temperature will increase more during winter than summer (Björnsson *et al*., 2008). However, the short term natural oscillations of 25-40 years discussed earlier may play a crucial role when considering the effects of climate change on agriculture in the coming decades. These natural variations in past climate have made it possible to estimate their effects on agricultural production and such estimates can subsequently be used to project future effects. It has been demonstrated that herbage yields increase by 735 kg DM ha^{-1} for each degree that temperature rises during winter and spring (Björnsson and Helgadóttir, 1988). Mild winters are accompanied by less frost in the ground and, hence, longer growing season. Similarly, as long as barley is being cultivated under such climatic conditions, where it does not reach full maturity, grain yields increase by 970 kg DM ha^{-1} for each degree (Hermannsson, 1993).

Projected increase in temperature, together with increased CO_2 levels in the atmosphere, therefore indicate yield increases in all agricultural crops that are now being cultivated in Iceland. Cultivation of perennial crops that are currently at their margin, such as forage legumes and perennial ryegrass will become more secure and new arable crops will enter the scene such as oats, wheat and canola. Many of the globally important grain-producing regions are expected to suffer from severe drought and heat stress as a result of climate change, which most likely will have a negative impact on the yielding capacity in these regions (Zhang and Cai, 2011). Hence, grain production needs to be expanded to other regions in order to keep up with the increasing demand, and it has been postulated that areas at comparable latitudes to Iceland will open up as potential grain production regions in the near future, but at the same time being challenged by increased threats from pests and diseases (e.g. Hakala *et al*., 2011).

Potential new agricultural land is no limitation to the expansion of agricultural production in Iceland. The future role of the country as a food producer for its inhabitants and a contributor to world food security, however, rests in the hands of politicians and policy makers.

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Session 1

Improving Eco-Efficiency in Mixed Farming Systems

Influence of ley-arable systems on soil carbon stocks in Northern Europe and Eastern Canada

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Abstract

Grassland is one of the dominant forms of land use in Europe and is considered to have a considerable potential for sequestration of soil carbon. Forage-based crop rotations under Nordic conditions often include both ley and arable crops. These systems, having components of both grassland and cropland, are expected to have C stocks intermediate between those in permanent grasslands and croplands. The effect of ley-arable rotations *versus* continuous annual cropping systems on stocks of soil organic carbon was quantified by analysing data from long-term field experiments in Nordic countries. Unpublished data from four sites in Sweden are presented and results from 11 published studies are summarized. In the different studies, depending on the species composition, management, experimental period and soil depth, on average 0.52 Mg ha^{-1} yr^{-1} (range 0.3 to 1.1; median 0.42 Mg ha^{-1} yr^{-1}) more carbon was retained in soils in ley-arable compared to exclusively annual cropping systems.

Keywords: carbon sequestration, greenhouse gases, grassland, land use change, long-term field experiments, mitigation

Introduction

The net removal of C from the atmosphere through photosynthesis into pools in soils with long turnover times is termed C sequestration, and is a strategy promoted for mitigating climate change. Because soil organic carbon (SOC) stocks are generally higher in grassland and forest ecosystems, land-use conversion into cropland results in a net increase of CO_2 emissions from soils (Poeplau *et al*., 2011). The historic expansion of agricultural land has led to a SOC loss of 78 ± 12 Gt (10^9 tons) (Lal and Follett, 2009). According to recent estimates, the net loss of C from tropical vegetation and soils caused by land-use change is 1.3 ± 0.7 Gt C yr^{-1}, corresponding to approximately 17% of the CO_2 emissions caused by fossil fuels and cement production (Pan *et al*., 2011).

Most grasslands in Europe are managed for feeding livestock through grazing or the production of forage. It represents one of the dominant forms of land use, covering 22% of the European Union's (25 countries) land area (European Environment Agency, 2005) and grassland has a considerable potential for C sequestration. At the global scale, this potential has been estimated to vary between 0.01 and 0.3 Gt C yr^{-1} (Lal, 2004). Measures for achieving SOC sequestration are also likely to have positive trade-offs for soil quality. However, practices need to be developed and evaluated for individual agricultural ecosystems according to prevailing climatic, edaphic and social conditions and land-use history (Smith, 2012).

A detailed 2-year study of net ecosystem C fluxes in nine grazed grasslands in Europe revealed on average 1.04 ± 0.073 Mg C ha^{-1} yr^{-1} was sequestered (Sousanna *et al.*, 2007). Increasing production of biomass by plants is the major determinant of C sequestration in agro-ecosystems and will result in higher SOC stocks in soils. Management practices that increase plant production include use of mineral fertilizers (Ammann *et al.*, 2009), biological-N fixation (De Deyn *et al.*, 2009) and improved grazing regimes (Conant *et al.*, 2003). This is supported by results from long-term experiments on grassland under cold Atlantic conditions in Iceland. Three different N fertilizers were compared at three grassland sites at Akureyri, Sámsstaðir and Skriðuklaustur. The experiments lasted 61, 60 and 42 years, respectively (Gudmundsson *et al.*, 2004; 2008). On average, C stocks to a depth of 10 cm increased by approximately 1 kg C (0.4-1.9) for each kg N applied annually as ammonium nitrate or calcium nitrate. On a very sandy soil in Iceland, the effect of applied N was higher; C stocks to a depth of 20 cm increased by 3.4 kg C ha^{-1} yr^{-1} on average during 50 years for each kg ammonium nitrate applied annually (Gudmundsson *et al.*, 2011). Reclamation of Icelandic degraded soils has resulted in an average sequestration rate of 0.6 Mg C ha^{-1} yr^{-1} over more than 50 years (Arnalds *et al.*, 2000).

Under management with generally no or low fertilizer inputs, Swedish grassland soils are probably sequestering less than 0.1 Mg C ha^{-1} yr^{-1}. According to a recent investigation of data from the Swedish soil inventory (http://www-markinventeringen.slu.se), which is based on more than 20,000 permanent plots positioned in a regular grid pattern over Sweden resampled at 10-year intervals, the sequestration rate of SOC in grasslands is about 30 kg C ha^{-1} yr^{-1} (Karltun *et al.*, 2010). In a Swedish case study, where natural unfertilized grassland was sampled in 1937, 1971 and 2002 in a regular grid pattern, average changes in SOC were slightly positive (0.1 Mg C ha^{-1} yr^{-1}) but not statistically different from zero (Kätterer *et al.*, 2008). However, in an adjacent field that had a history of cropping but was converted to grassland in 1971, the average SOC sequestration rate was significantly higher by a factor of four (0.4 Mg C ha^{-1} yr^{-1}). This illustrates the importance of previous land use and land-use change on soil C stocks (Conant *et al.*, 2005), which needs to be taken into account when quantifying C sequestration. Natural grasslands that have been used in the same way for centuries are expected to have reached a steady state if other factors influencing the C balance do not change, such as N deposition, drainage and the type of management (Venteris *et al.*, 2004; Lettens *et al.*, 2005; Murphy *et al.*, 2006).

The reasons why carbon inputs to grassland soils are generally higher than those in croplands are because of a higher production potential and higher root:shoot ratios. Thus, land-use change from grassland to cropland is expected to result in lower C inputs and, consequently, in lower stocks of SOC and *vice versa* (Poeplau *et al.*, 2011). Several studies in the UK (reviewed by Johnston *et al.*, 2009) have shown that intensively managed grassland that is fertilized and harvested frequently, seems to accumulate more C than extensively managed grassland. According to Johnston *et al.* (2009), it took approximately 25 years to increase SOC after conversion of cropland to grassland to a level half-way between that of an old arable field and permanent grassland.

Forage-based crop rotations under Nordic conditions often include perennial forage crops, which, especially at high latitudes, are kept for more than three years. These systems have components of both grassland and cropland and are, therefore, expected to have C stocks somewhere between those of permanent grassland and cropland. The objectives of this paper are (i) to present data from a series of long-term experiments conducted at four sites in Sweden focusing on the effect of perennial leys in rotations *versus* continuous cereal cropping on SOC, and (ii) to review and summarize the main findings from published studies of long-term field experiments conducted in countries with a Nordic climate (Northern Europe and eastern Canada) in order to quantify the effect of rotations on stocks of SOC. The leys in these experiments were not grazed but were cut, usually 2-3 times per year.

The Swedish humus balance experiments

A series of field experiments, the 'Swedish humus balance experiments', were started between 1970 and 1980 at four sites in Sweden (latitudes between 56° and 64°N). They were designed to investigate the effect of N fertilization on SOC in a forage-based rotation (one year of spring barley undersown with grasses and clovers followed by three years of a grass-clover ley) and a cereal-based rotation (mainly spring-sown barley, oats and wheat) where crop residues were either removed or incorporated into the soil. Ley and cereal rotations were compared on adjacent fields at the experimental stations, Lanna, Lönnstorp, Röbäcksdalen (Röbäck II) and Säby, and the study is still on-going. In the ley rotation, four N-fertilization levels were randomized within each out of four blocks. In total, there were 16 experimental plots and each plot covered an area of 105 m^2 at Röbäcksdalen and 90 m^2 at the other sites. The layout of the cereal rotations was a split plot design with residue handling on main plots randomized within each of four blocks and N-fertilization levels as a split-plot factor randomized within each main plot.

Top soil (0-20 cm) C concentrations were determined in archived soil samples taken at the start of the experiments and in samples taken in autumn 2008. Data on bulk density needed for calculating SOC stocks were taken from a Swedish database including the soils of the four experimental stations (Kätterer *et al.*, 2006).

Soil organic C increased significantly with the rate of N fertilization in the cereal rotation. Site and N-fertilization rate explained 85% of the total variance according to analysis of covariance. Differences in C stocks between N-fertilized and unfertilized treatment revealed that, on average, 1.1 and 1.7 kg C were accumulated per kg^{-1} fertilizer-N applied annually when straw was exported or left in the field, respectively. However, this is probably an underestimate because only the amount of C down to a depth of 20 cm was considered and the impact on deeper soil layers was neglected. Straw removal resulted in lower SOC stocks (2.2 Mg ha^{-1}) but the effect of straw handling was not statistically significant ($P = 0.09$).

Soil organic C stocks were higher in ley rotations by 0.34-0.56 Mg C ha^{-1} yr^{-1} and 0.42-0.60 Mg C ha^{-1} yr^{-1} compared with the cereal rotations with or without or residue removal, respectively (Table 1). However, in contrast to the cereal rotation, N fertilization had no significant impact on SOC stocks in the ley rotations. This may be surprising but is explained by an increasing proportion of clover in the leys at low N-fertilization rates.

Analysis of covariance revealed that 86% of the total variation in changes in SOC, averaged over sites and N levels, could be explained by rotation, residue handling and the initial SOC at the four sites. This means that changes in SOC are more likely to be negative when initial SOC stocks are high (Figure 1). Thus, site history before the start of the experiment determines whether a certain rotation or management will lead to increasing or decreasing SOC stocks in absolute terms. This means that, although leys will accumulate more C in soils than cereal-dominated rotations, even ley-dominated systems may result in decreasing SOC stocks when SOC stocks are high due to previous land use and management.

The effect of ley and cereal rotations on SOC stocks in this study is similar to those reported from other studies (Table 1). Also, the effect on SOC of how residues were handled is within the range of results reported for other European studies. Evaluating a number of long-term experiments (7-35 years) with no-straw vs. straw treatments from Scandinavia, Germany, France, Belgium and the UK, Smith *et al.* (1997) estimated that the incorporation of about 5 Mg of straw ha^{-1} yr^{-1} would increase the SOC content by approximately 0.75% yr^{-1}. Thomsen and Christensen (2004) reported that after 18 years, compared to no-straw, the treatments incorporating 4, 8 and 12 Mg ha^{-1} yr^{-1} of straw contained 12, 21 and 30% more SOC, respectively. In two long-term experiments of around 20 years in Norway, Singh *et al.* (1997) estimated that retaining all straw increased the SOC content by about 100 kg C ha^{-1} yr^{-1}.

Table 1. Mean annual increases in SOC stocks (ΔC) in ley-arable rotations compared with continuous annual cereal cropping in long-term experiments (≥ 10 years) in Northern Europe and Eastern Canada. When data for different management options within rotations were available, the most extreme are reported, e.g., removal of crop residues in the annual system (Sweden, unpublished data) and grass-legume leys rather than pure lucerne leys.

Site	Country	Duration (years)	Depth (cm)	ΔC [a] (Mg ha^{-1} yr^{-1})	Reference
Saint-Lambert	Canada	10	20	0.80	Quenum *et al.* (2004)
Elora	Canada	20	40	0.33	Yang and Kay (2001)
Woodslee	Canada	35	70	1.10	VandenBygaart *et al.* (2003)
Erika I	Estonia	40	60	0.27	Reintam (2007)
Erika II [b]	Estonia	28	20	0.66	Viiralt (1998)
Ås	Norway	30	20	0.40	Uhlen (1991)
Röbäck I [c]	Sweden	30	25	0.40	Bolinder *et al.* (2010)
Offer [c]	Sweden	52	25	0.36	Bolinder *et al.* (2010)
Ås [c]	Sweden	30	25	0.87	Bolinder *et al.* (2010)
Röbäck II	Sweden	27	20	0.54	Unpublished data
Lanna	Sweden	27	20	0.42	Unpublished data
Lönnstorp	Sweden	27	20	0.60	Unpublished data
Säby	Sweden	37	20	0.45	Unpublished data
Rothamsted	UK	36	23	0.30	Johnston *et al.* (2009)
Woburn	UK	58	25	0.30	Johnston *et al.* (2009)

[a] Only one or two figures are quoted indicating the uncertainty of the calculations
[b] Difference between barley receiving no N fertilizer and grass-clover + faeces + green manure
[c] Farmyard manure was applied in ley rotations but not in arable cropping system

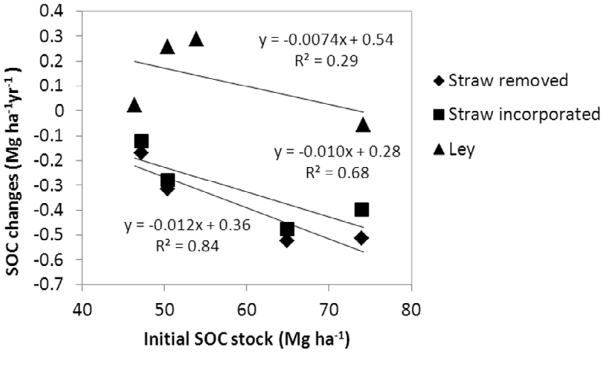

Figure 1. Changes in SOC as a function of initial C stocks at the four sites, Lanna, Lönnstorp, Röbäcksdalen (Röbäck II) and Säby (see also Table 1). Mean values for all N levels and all sites for grass-clover ley (Ley) and continuous cereals with straw incorporated or removed.

Ley-arable rotations in Northern Sweden

These long-term experiments were initiated in 1957 at three sites in Northern Sweden (60° to 65°N), Offer, Ås and Röbäcksdalen (Röbäck I). Four 6-year rotations differing in proportions of annual and perennial crops were investigated (Bolinder *et al.*, 2010). The rotations start with undersown barley, followed by either 1, 2, 3 or 5 years of leys (i.e., a mixture of red clover, timothy and meadow fescue). The other crops in these rotations are mainly winter rye, fodder rape, green fodder and some root crops. The rotations with three and five years of ley received about 4.5 Mg C ha^{-1} through farmyard manure, and the rotation with two years of leys received about 3 Mg farmyard manure-C ha^{-1} over a complete 6-year period. Data on top soils (0-25 cm) after 30 years indicated that SOC changes were proportional to the frequency of leys in the rotation (Figure 2). Furthermore, the initial concentrations of SOC at the sites determined the direction of subsequent changes in SOC concentrations. In the silt loam soil at Röbäcksdalen, which had a high initial SOC concentration, there was an overall decline in SOC concentration. In the gravelly loam at Ås with an intermediate initial SOC concentration, SOC concentration was maintained only in the 5-year ley rotation but in the silty clay loam at Offer with the lowest initial SOC concentration, only the 1- and 2- year ley rotation showed a net loss.

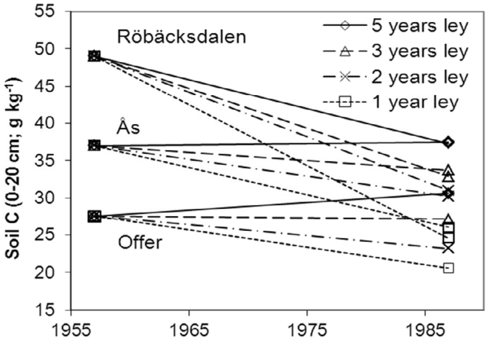

Figure 2. Changes in SOC concentrations (0-25 cm) at three long-term experimental sites in Northern Sweden in four 6-year rotations using different proportions of perennial forage and annual arable crops (1, 2, 3 or 5 years leys) and receiving different amounts of farmyard manure. Data from Ericson (1994). For a detailed description see Bolinder *et al.* (2010).

The Offer site was resampled in 2008 and data represent a longer temporal change (52 years) compared with the 30-year changes for the Ås and Röbäcksdalen sites (Bolinder *et al.*, 2010). The linear mean sequestration rate of SOC at Offer was +0.12 Mg C ha^{-1} yr^{-1} for the 5-year rotation of leys. The rotation of 3-years ley remained more or less at equilibrium (-0.04 Mg C ha^{-1} yr^{-1}), whereas the rotations with only two or one years of leys showed decreasing trends (-0.18 and -0.24 kg C ha^{-1} yr^{-1}, respectively). The changes in SOC concentration at Röbäcksdalen did not follow the 'general' pattern found at the other two sites. This, however, could probably be related to poor drainage prior to the initiation of the experiment. The site was tile-drained only four years before the experiment started and it is likely that better aeration induced important changes in the soil physical properties also causing a higher rate of SOC decomposition (Bolinder *et al.*, 2010). The rotation with only 1 year of leys and dominated by annual arable crops can be used in comparison with the rotation having 5 years of leys to estimate the SOC increase when moving from an annual arable to a perennial ley system. This increase in SOC concentration for the three sites ranged from 0.36 to 0.87 Mg C ha^{-1} yr^{-1} (Table 1).

Ley-arable rotations in Norway

The Norwegian site at Ås had a 6-year cereal rotation (barley, oats and spring wheat) and a 6-year rotation of barley followed by undersown spring wheat, and 4 years of leys (timothy and red clover). The ley rotation at this site received farmyard manure but not the cereal rotation. The soil, a clay loam, had an intermediate initial SOC content (about 3.8% C). Similarly to the Swedish sites, SOC content in the top soil (in this case 0-20 cm) had slightly increased after 30-years for the rotation with 4 years of leys (to about 4% C), and slightly decreased (to about 3.5% C) for the continuous cereal rotation (Table 1; data from Uhlen, 1991). Differences between rotations corresponded to an approximate difference in SOC of 0.4 Mg C ha^{-1} yr^{-1} (Table 1).

Ley-arable rotations in Eastern Canada

Ley-based cropping systems in central parts of the Province of Quebec (46°N) commonly involve 1-2 years with small-grain cereals, followed by 3-8 years of leys. In a 10-year soil quality monitoring programme in Saint-Lambert de Lauzon, a 4-year continuous ley-based rotation (undersown barley followed by three years of ley) was compared with a rotation of continuously grown annual crops (small-grain cereals, oil-seed rape and maize). The results showed declining trends in SOC content for the annual cropping system (about 0.3 Mg C ha^{-1} yr^{-1}), while the continuous ley-based crop rotation gained approximately 0.5 Mg C ha^{-1} yr^{-1} (Quenum *et al*. 2004). Thus, the difference in SOC content between the two systems was about 0.8 Mg C ha^{-1} yr^{-1} (Table 1). A 20-year experiment in the neighbouring Province of Ontario (Elora site, 43°N), that compared a continuous grain-maize cropping system with continuous lucerne (the lucerne was re-seeded every 4 years), yielded an approximate change in SOC between maize and lucerne of 0.3 Mg C ha^{-1} yr^{-1} (Yang and Kay, 2001) (Table 1). Higher rates of change in SOC were observed in an experiment in Ontario (Woodslee site, 35 years, 42°N). The conversion from annual cropland (i.e., continuous maize) to perennial forage (i.e., bluegrass) induced a difference in soil SOC of 1.1 Mg C ha^{-1} yr^{-1} (Gregorich *et al*., 2001; VandenBygaart *et al*. 2003) to a depth of 70 cm (Table 1). The values from these eastern Canadian sites are fairly similar to the rates of change in SOC observed for the ley and cereal rotations in northern Sweden.

Ley-arable rotations in the United Kingdom

Cycles of different ley-arable rotations have been studied in the UK at Woburn since 1938 and at Rothamsted since 1949. In the exclusively annual cropping system at Woburn, SOC contents in the top soil (0-25 cm) decreased from initially 0.98% to 0.74% during 58 years (Johnston *et al*., 2009). On the other hand, in the clover-grass dominated system, SOC content increased by 1.22%. The difference in SOC between the two rotations corresponds to about 0.3 Mg C ha^{-1} yr^{-1}, on average (Table 1). Similar changes were also recorded at Rothamsted in two experiments established on a silty clay loam on an old grassland soil and an old arable soil (Johnston *et al*., 2009). After 36 years, mean annual differences in C stocks of the top soil (0-23 cm) between 3-year ley rotations compared to exclusively arable cropping were about 0.3 Mg C ha^{-1} (Table 1). Lucerne leys were much less favourable for SOC accumulation than grass-clover leys; SOC increases were only about 0.1 Mg ha^{-1} yr^{-1} higher than those in the arable-only system.

Annual crops and perennial swards in Estonia

A small-plot experiment was established in 1963 at Erika (Erika I), Tartu County, Estonia, on till material excavated to a depth of 1.5-3 m. The material was almost free from SOC (0.6 g C kg^{-1}; Reintam, 2007) at the start of the experiment. During the first decade, four treatments were established: (i) grass-clover mixture, (ii) lucerne, (iii) spring barley, and (iv) bare fallow. The plots were not harvested but the spring barley was re-sown every year. Each decade, management of the treatments was changed with respect to removal or retention of the biomass produced. After 40 years, between 28 and 39 Mg C ha^{-1} had been accumulated in the profiles to a depth of 60 cm. Mean annual increases in SOC content to 60 cm during this period ranged from 0.70 to 0.97 Mg C ha^{-1} in the systems originally sown with the spring barley and grass-clover, respectively (Table 1).

In another experiment, started in 1965 at the same site in Estonia (Erika II), the effect of mineral fertilizers and farmyard manure applied to barley and swards with different species composition on SOC content was studied. The soil at this site also originated from excavated till with an initially very low C content. Soil organic matter was measured in 1993, 28 years after the start of the experiment (Viiralt, 1998). Mean annual increase in SOM was measured in 1993 in the top 20 cm of the soil. As methods for organic matter determination were not described (Viiralt, 1998), the van Bemmelen factor (0.58 g C g^{-1} organic matter) was used to convert organic matter to SOC. Reported mean changes in SOC ranged between 0.05 Mg C ha^{-1} yr^{-1} in the fallow treatment and 0.91 Mg C ha^{-1} yr^{-1} in grass-clover swards, receiving faeces and green manure. Spring barley accumulated 0.25 or 0.36 Mg C ha^{-1} yr^{-1}, without or with N fertilization, respectively (Figure 3). Thus, grass-clover swards, receiving faeces and green manure, accumulated 0.66 Mg ha^{-1} yr^{-1} more SOC than barley receiving no N fertilizer (Table 1). Differences in yields and SOC were probably due to species composition and application of different mineral fertilizers, animal faeces and green manure. Yields and changes in SOC in barley and grass rotations were more affected by fertilization than in clover rotations showing that biological N fixation may not affect yields to the same extend (Figure 3).

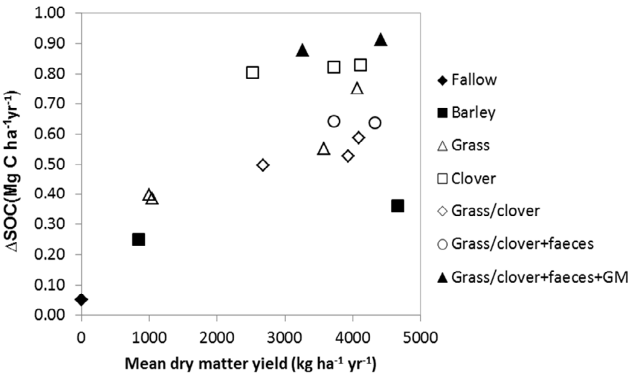

Figure 3. Relationship between mean dry matter yields (1965-1997) and mean annual changes in SOC between 1965 and 1993 in 17 treatments in a small plot experiment established in Estonia (Erika II, Table 1). Data according to Viiralt (1998). GM stands for green manure.

Net primary production and changes in soil organic carbon

Carbon inputs to soil are driven by photosynthesis, i.e., the fixation and transfer of atmospheric CO_2 into plants. Net primary production (NPP) determines the amount of photo-synthetically fixed C that can potentially be sequestered in soil organic matter (Bolinder *et al.*, 2007). Net primary production is defined as the total amount of C converted into the above- and below-ground plant biomass through a given period of time (i.e., excluding the return of C respired by plants that is returned to the atmosphere). The input of extra-root C is included in NPP, where extra-root C is defined as roots dying and decaying during the growing season (plus that during the winter for perennial leys), cell sloughing of epidermal root tissues, and soluble compounds originating from exudation processes (Andrén *et al.*, 1989). Major drivers for the relative difference in SOC stocks between annual cropping systems and perennial ley systems are higher C inputs to soil in the ley systems through plant litter and other sources such as manure. Net primary production and decomposition are governed by climate, plant species and management. Compared to perennial leys, annual crops have often lower NPP because they are grown only during a part of the season. Furthermore, perennial leys typically allocate more C to the below-ground biomass (Bolinder *et al.*, 2007). Higher biomass production also increases transpiration, which usually leads to lower temperatures and moisture contents in soil and, therefore, to decreased decomposition of soil organic matter (e.g., Kätterer and Andrén, 2009).

Bolinder *et al.* (2012) reviewed estimates for root biomass in the literature and found that perennial forage crops had, on average, at least a three times higher root biomass than small-grain cereals (7.85 vs. 2.00 Mg DM ha^{-1}). Furthermore, the amount of above- and below-ground plant biomass that is transformed into long-lived SOC differs between species. Values reported by Uhlen (1991), comparing the no-straw vs. all straw ploughed-in treatments from several experiments in Norway, indicated that around 7% of the small-grain cereal straw C was retained as SOC after 21 to 31 years. The equivalent value for maize-stover from a number of long-term field experiments was about 12% (Bolinder *et al.*, 1999). The proportion of root-derived C remaining in the soil as SOC is generally higher than that derived from above-ground biomass (Hénin and Dupuis, 1945) and this has been found in a number of studies (e.g., Bolinder *et al.*, 1999; Rasse *et al.*, 2005; Johnson *et al.*, 2006). A recent estimation from the Swedish long-term soil organic matter experiment at Ultuna by Kätterer *et al.* (2011) showed that the proportion of total below-ground C input remaining in the top soil after 53 years was 2.3 times higher than that of above-ground crop residues (mostly from small-grain cereals).

In order to illustrate the consequences of the above-mentioned considerations, estimates are presented for annual C inputs to soil from spring barley and leys (Table 2). Annual C inputs to soil were calculated using the approach of Bolinder *et al.* (2007), where above- and below-ground inputs were estimated by multiplying the yield by relevant plant-C allocation coefficients, which has also been used in the evaluation of Swedish long-term experiments (Kätterer *et al.*, 2011; Bolinder *et al.*, 2012). The effect of straw removal on C input was found to be significant. The total annual C input through leys is high compared to that of small-grain cereals. The relative difference between annual and perennial crops is accentuated when it is taken into account that decomposability differs and a larger proportion of C from perennial crops is retained as stable SOC. According to the assumptions presented (Table 2), a 3-year ley with undersown barley would result in a retention of 2.4 Mg C ha^{-1}. The corresponding retention for continuous barley would be 0.6 Mg C ha^{-1} or 1.0 Mg C ha^{-1} with straw exported or incorporated, respectively. Consequently, differences in SOC retention between the ley-arable and continuous barley systems with straw exported or incorporated would be 0.45 or 0.35 Mg C ha^{-1} yr^{-1}, respectively. These figures correspond well to the median change in SOC (i.e., 0.42 Mg C ha^{-1} yr^{-1}) in the studies reviewed in Table 1. This highlights the difference between C inputs to soil between ley-arable systems and exclusively annual species cropping systems.

Table 2. Estimates of annual C input, through above-ground (AG) and below-ground (BG) crop residues to soil, and amounts remaining as stable soil organic carbon (SOC).

	Yield[a]	Annual input of crop residue (C)			Residue C retained as SOC[b]		
		AG	BG	Total	AG	BG	Total
		Mg C ha^{-1} yr^{-1}					
Barley straw retained	1.50	1.33	0.50	1.83	0.13	0.11	0.25
Barley straw removed [c]	1.50	0.33	0.50	0.83	0.03	0.11	0.15
Undersown barley [c]	1.50	0.22	1.27	1.49	0.02	0.29	0.31
Grass ley discontinued	2.70	1.08	4.28	5.36	0.11	0.98	1.09
Grass ley continued [d]	2.70	1.08	1.68	2.76	0.11	0.39	0.50

[a] Yields correspond to national statistics in Sweden (4 Mg ha^{-1} grain yield for spring barley with a moisture content of 14% and 6 Mg DM for the herbage mass of leys)

[b] Assuming 10% for AG and 23% for BG (see text)

[c] When straw was harvested, it was assumed that 25% of the straw was left in the field as stubble

[d] For the leys, total root biomass is only incorporated into soil at the end of last year of the ley. For intermediate years BG occurs only as ER (Bolinder *et al.*, 2012)

Conclusions

Long-term field experiments are indispensable for quantifying the impact of land use and management on SOC stocks and for benchmarking soil C models. The linear approach used here for calculating changes in SOC is a crude simplification which does not considers the dynamics of the soil carbon balance and possible feedbacks on productivity. The evaluation of data from Nordic long-term field experiments, considering species composition, management options, duration and soil depth, showed that (i) on average, 0.5 Mg ha^{-1} yr^{-1} (range 0.3-1.1; median 0.4 Mg ha^{-1} yr^{-1}) more C was retained in soil in ley-arable than annual cropping systems, (ii) straw return had a positive impact on SOC; (iii) the number of years of leys in rotations is linearly correlated to changes in SOC, (iv) clover leys result in similar changes in SOC to those in fertilized grass leys, and (v) initial SOC contents must be considered for predicting the effect of arable-ley rotations on net changes in SOC.

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Herbage nutritive value in less-favoured areas of cool regions

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Abstract

Herbage production is often the only possible agricultural activity in less-favoured areas of cooler regions, including mountainous areas. Growing conditions in those areas are characterized by low temperatures, short growing seasons, harsh winters, and challenging soil conditions. We will address challenges and opportunities for herbage nutritive value in less-favoured areas mostly limited by temperature either because of latitude (Canada, Scandinavia) or altitude (mountainous regions of Europe). The limited choice of species for sown swards, the rapid plant development under long days at high latitude, the autumn harvest and grazing management in areas with severe winters, and the management of species-rich permanent pastures in mountainous areas to optimize yield and nutritive value represent some of the significant challenges for producing herbage of high nutritive value in less-favoured areas. On the other hand, opportunities include improved digestibility under low temperatures at high latitudes and altitudes, and better functional properties (e.g. fatty acid concentration) of some of the species grown in less-favoured areas. The projected climate change for less-favoured areas of cool regions will offer both opportunities (e.g. new herbage species with greater digestibility and an additional harvest due to a longer growing season) and challenges (e.g. decreased digestibility of existing species).

Keywords: digestibility, nitrogen, fiber, climate change, species

Abbreviations: crude proteins (CP), dietary cation-anion difference (DCAD), dry matter (DM), growing degree day (GDD), neutral detergent fiber (NDF), organic matter digestibility (OMD)

Introduction

Herbage production and associated livestock, including dairy, beef, sheep, and horses, is often the only possible agricultural activity in less-favoured areas of cool regions. In the European Union, less-favoured area is a term used to describe land with natural handicaps (lack of water, less favorable climate and/or soil type, short crop season, and tendencies of depopulation), or that is mountainous or hilly, as defined by its altitude and slope. Although mostly used as pastures or conserved feed, perennial herbage grasses, legumes, and woody shrubs grown in these areas also play a role in maintaining the landscape and the biodiversity. Herbage production in less-favoured areas of cool regions is often challenging but it also offers some opportunities. Our objective is to present and discuss both challenges and opportunities for herbage nutritive value in less-favoured areas mostly limited by temperature either because of latitude (Canada, Scandinavia) or altitude (mountainous regions of Europe). Our examples will focus mostly on sown swards of eastern Canada and Finland (Table 1) and permanent semi-natural pastures of the French Pyrenees (Table 2).

Table 1. Climatic and agronomic features of sites limited by low temperatures in eastern Canada and Finland.

		Normandin (Canada)	Kuopio (Finland)
		48°51'N, 72°32'W	62°54'N, 27°41'E
Average temperature (°C)			
	July	17.1	17.2
	January	-18.4	-9.7
Growing degree days (5°C basis)		1350	1200
Length of growing season (days with >5°C)		180	175
Main grass species		*Phleum pratense*	*Phleum pratense* *Festuca pratensis*
Other grass species		*Dactylis glomerata* *Festuca arundinacia* *Bromus inermis* *Bromus riparius* *Phalaris arundinacea*	*Festuca arundinacia* *Lolium perenne* *Dactylis glomerata* *Lolium multiflorum*
Main legume species		*Medicago sativa* *Trifolium pratense*	*Trifolium pratense* *Trifolium repens* *Medicago sativa*

Table 2. Features of two sites and four grasslands of the French Pyrenees.

		Ercé	Portet
		42°51'N, 1°17'E	47°30'N, 2°00'E
Altitude (m a.s.l.)		650	1250
Average temperature (°C) February-June		10.6	7.9
Main grass species	Acquisitive growth strategy	*Lolium perenne* *Holcus lanatus*	*Dactylis glomerata* *Arrhenatherum elatius*
	Conservative growth strategy	*Festuca rubra* *Agrostis capillaris*	*Festuca rubra* *Agrostis capillaris*
Percentage of plants having an acquisitive growth strategy within the four plant communities	Communities dominated by plants with an acquisitive growth strategy	92-96†	68-72
	Communities dominated by plants with a conservative growth strategy	4-8	32-38

† Values for the two grasslands having a close plant community composition

Nutritive value attributes

Herbage digestibility

Herbage digestibility remains one of the most important attributes of nutritive value, particularly in intensive forage-ruminant systems. In terms of ruminant nutrition, the digestibility of herbage dry matter (DM) is mainly a function of the concentration of the cell wall, estimated by the neutral detergent fiber (NDF) concentration, and the digestibility of that cell wall, estimated by the NDF digestibility. In turn, the NDF digestibility is a function of the proportion and linkages between hemicellulose, cellulose, and lignin (van Soest, 1994).

A conceptual model of herbage digestibility based on crop physiology was first proposed in 1997 and clearly presented by Duru (2008). The standing herbage mass (W) can be treated as an assemblage of two main components, metabolic (M) and structural (S); the latter being equivalent to the cell wall or NDF. Because the metabolic component has a digestibility close to one (or 100%), herbage digestibility (D_W) can be expressed as a function of the digestibility of the structural component (D_S) and the ratio of the metabolic component in the standing herbage mass (M/W).

$$D_W = [(1-D_S) \times M/W] + D_S \quad [1] \quad \text{(Duru, 2008)}$$

According to Lemaire *et al.* (1989), the metabolic component (M) decreases in an ontogenic manner according to standing herbage mass as follows:

$$M = \alpha W^{\beta} \quad [2]$$

where α and β are dimensionless constant parameters. This equation can be transposed into the following:

$$M/W = \alpha W^{\beta-1} \quad [3]$$

By substituting Eq. 3 in Eq. 1, the following equation is derived:

$$D_W = [(1-D_S)\, \alpha W^{\beta-1}] + D_S \quad [4] \quad \text{(Duru, 2008)}$$

in which herbage digestibility is expressed as a function of the digestibility of the structural component and the standing herbage mass. Herbage digestibility changes can then be seen to depend on (i) plant growth through the ontogenic decrease in the metabolic/structural tissue ratio and (ii) differentiation of structural tissues. Plant growth and crop yield are affected by the growing conditions while Ds is strongly affected by temperature.

The negative relationship between herbage digestibility and DM yield, described by Eq. 4, has often been reported (Bélanger *et al.*, 2001; Gustavsson and Martinsson, 2004). From this relationship, it can be inferred that agronomic practices and genetic selection increasing herbage DM yield will most likely result in a decrease in herbage digestibility. Conversely, factors decreasing herbage DM yield may result in an increased digestibility.

Other attributes of nutritive value

Nitrogen or crude proteins (CP), non-structural carbohydrates, mineral composition, and functional compounds are other important attributes of nutritive value. The conceptual model described for herbage digestibility can also be used for N concentration (Bélanger *et al.*, 2001). The decrease in N concentration during growth can be attributed to the increasing ratio of structural to metabolic components while the N concentration of the metabolic component is primarily a function of soil N availability.

Water soluble carbohydrates are the main source of fermentable substrates during ensiling and along with buffering capacity influence the ensiling process. Non-structural carbohydrates in herbage also provide a readily fermentable source of energy in the rumen and increased concentration can improve the N use efficiency of dairy cows (Brito *et al.*, 2009). Non-structural carbohydrate concentration in herbage crops is affected by several factors including temperature, herbage species and mixtures, water and N stresses, and time of day when cutting or grazing occurs (Pelletier *et al.*, 2010; Morin *et al.*, 2012; Simili da Silva *et al.*, 2013).

The mineral composition of herbage plays an important role in the development of metabolic disorders in ruminants such as milk fever and grass tetany. Milk fever, a severe hypo-calcaemia, is an economically important metabolic disease (Goff and Horst, 2003) occurring at or near parturition, especially in high producing dairy cows. It affects 5 to 7% of dairy cows in the USA (Sanchez, 1999) and 1.6 to 5.4% of dairy cows in Australia (McNeill *et al.*, 2002). The concept of the dietary cation-anion difference [DCAD = (K^+ + Na^+) - (Cl^- + S^{2-}), Ender *et al.*, 1971] of the ration fed during the transition period (2-3 weeks before calving) has primarily been used in the context of preventing milk fever. Dry dairy cows fed herbage with a high DCAD during this period are more likely to develop hypocalcaemia (Penner *et al.*, 2008). Like Ca for milk fever, cows that do not receive an adequate supply of Mg, especially in early lactation, can develop grass tetany. The risk of grass tetany can be assessed by the grass tetany (GT) index [GT = ($K^+/Ca^{2+} + Mg^{2+}$)] with an increased incidence for cattle grazing forage with a GT index greater than 2.2 (Kemp and ’t Hart, 1957).

Functional compounds in milk and meat have received significant interest in the last decade, mostly because of the positive link between α-linolenic acid (C18:3) and conjugated linoleic acid in milk and dairy, and human nutrition and health. The herbage fatty acid composition has been shown to affect the conjugated linoleic acid found in milk and meat produced (Hebeisen *et al*., 1993; Lourenço *et al*., 2005). The fatty acid composition of herbage is influenced by several factors including species, stages of development at cutting or grazing, N fertilization, and methods of conservation (Boufaïed *et al.*, 2003).

Challenges and opportunities for herbage nutritive value in less-favoured areas of cool regions

Limited choice of species for sown swards

Agriculture at high northern latitudes or in high altitudes is conducted under short growing seasons and low temperatures (Tables 1 and 2). Short growing seasons and harsh winter conditions limit the choice of perennial grasses and legumes for sown pastures and herbage fields, and affect the botanical composition of the permanent pastures found in mountainous areas. Short growing seasons and long cold winters also limit the possibility of grazing to less than six months in many cases and, therefore, increase the dependence on conserved herbage with a resulting requirement for slurry applications, harvesting equipment, and storage structures.

The choice of species in less-favoured areas is also affected by soil conditions, including acidity, excess or lack of water, poor fertility, slope, and stoniness. The reduction of some of those limiting factors is possible through management practices (e.g. liming and fertilization) and soil improvement (e.g. drainage). Direct seeding can also be used in situations when soil tillage is not an option and cultivars tolerant to abiotic stresses (e.g. lucerne cultivars tolerant to soil acidity) can be developed.

The limited choice of herbage species in less-favoured areas might result in lower nutritive value than in areas with more favourable growing conditions. The most widely used herbage species in eastern Canada and the Nordic countries is timothy (*Phleum pretense* L.). In most of those areas, perennial ryegrass (*Lolium perenne* L.) but even tall fescue (*Festuca arundinacea* Schreb.), two herbage grasses widely grown in the United Kingdom and continental Europe, respectively, cannot be grown successfully because of their lack of winter hardiness. In field studies conducted in eastern Canada and Finland, the digestibility of timothy tended to be less than that of tall fescue, except in spring growth at the Canadian site (Table 3). As well, tall fescue tended to have a lower NDF concentration and a greater N concentration than timothy. In a study under controlled conditions with temperatures typical of the Nordic countries, perennial ryegrass had greater DM and NDF digestibilities, and a lower NDF concentration than most other grass species (Thorvaldsson *et al*., 2007).

Herbage species widely used in eastern Canada and the Nordic countries have, however, some positive nutritive value attributes. In several areas of the Nordic countries, lucerne (*Medicago sativa* L.) cannot be grown successfully and the legume species of choice is red clover (*Trifolium pratense* L.; Halling *et al*., 2002). Red clover and lucerne have comparable N concentrations but red clover tends to have a lower NDF concentration, and hence higher DM and NDF digestibilities than lucerne (Table 4). In Wisconsin, Broderick *et al*. (2000) also reported a greater herbage digestibility of red clover compared to lucerne and suggested that the energy and protein in red clover silage may be used more efficiently than in lucerne.

Table 3. Comparison of the nutritive value of timothy and tall fescue in Canada and Finland.

		Normandin (Canada)[1]		Finland[2]	
		Timothy	Tall fescue	Timothy	Tall fescue
IVTD[3] (g kg^{-1} DM)	Spring	867	848		
	Summer	791	855		
IVOMD[3] (g kg^{-1} DM)	Spring			692	727
	Summer			677	684
NDF[3] (g kg^{-1} DM)	Spring	616	600	635	570
	Summer	604	568	587	559
N (g kg^{-1} DM)	Spring	22.3	23.7	16.2	17.4
	Summer	15.5	20.6	18.8	16.6

[1] From Pelletier *et al*. (2010)
[2] From Virkajärvi *et al*. (2012)
[3] IVTD: *in vitro* true digestibility of DM; IVOMD: *in vitro* organic matter digestibility; NDF: neutral detergent fiber

Table 4. Nutritive value attributes of two legume species in spring growth and summer regrowth[1].

	Spring growth		Summer growth	
	Lucerne	Red clover	Lucerne	Red clover
N (g kg^{-1} DM)	33.1	32.8	31.6	32.7
NDF[2] (g kg^{-1} DM)	412	350	393	333
IVTD[2] (g kg^{-1} DM)	809	843	798	855
NDFD[2] (g kg^{-1} NDF)	536	551	486	566

[1] From Pelletier *et al*. (2010)
[2] NDF: neutral detergent fiber; IVTD: *in vitro* true digestibility of DM; NDFD: *in vitro* digestibility of the NDF

Legume species mostly used in northern areas (e.g. red clover) might also have better functional properties. Herbage species differ in their concentration of fatty acids, especially in C18:3, the main fatty acid (Boufaïed *et al*., 2003). In eastern Canada, concentrations of total fatty acids and of C18:3 were in decreasing order of white clover (*Trifolium repens* L), birdsfoot trefoil (*Lotus corniculatus* L.), red clover, and lucerne. In addition, in experiments in Sweden, milk produced from cows fed a red clover-based diet had higher content of C18:2n-6 and C18:3n-3 than milk from cows fed a birdsfoot trefoil and white clover diet (Höjer *et al.*, 2012).

Timothy, the most used herbage grass species in the Nordic countries and eastern Canada, has been identified as a potentially good grass for dry dairy cows (Pelletier *et al.*, 2008). It has a lower DCAD than other cool-season grass species because of its lower K concentration (Tremblay *et al.*, 2006). Timothy is therefore the best suited cool-season grass for producing forages fed to dry cows in the weeks preceding calving. Reed canarygrass (*Phalaris arundinacea* L.), another species well adapted to the less-favoured areas of cool regions, can also be used for feeding dry cows because of its low DCAD value, high yield, and a positive response to Cl fertilization for reducing the DCAD (Tremblay *et al.*, 2013).

Most herbage production from sown swards is from mixtures of two or three species in both eastern Canada and the Nordic countries. Recent results of a pan-European experiment, using two grasses and two forage legumes at 31 sites for three years have demonstrated strong positive mixing effects on herbage DM yield (Finn *et al.*, 2013). A more detailed analysis of the Northern sites (Norway, Sweden, Iceland, and Canada) with their commonly used herbage species (timothy, meadow grass [*Poa pratensis* L.], red clover, and white clover) confirmed the benefits of multi-species for climatically less-favoured areas. In sown swards, this positive effect of mixtures on herbage DM yield is not accompanied by a reduction in herbage digestibility and CP concentration that can often be observed with increased DM yield (Sturludóttir *et al.*, 2013). The intra-year stability of the nutritive value of complex mixtures has often been questioned but a study conducted in the eastern United States showed that complex herbage mixtures were not inherently unstable (Sanderson, 2010).

Species-rich permanent pastures

Permanent pastures are often the only source of herbage in mountainous areas where reseeding is not an option. In those situations, herbage nutritive value is a function of the species present in the sward along with cutting or grazing management, especially the grazing pressure. Species-rich grasslands found in permanent pastures cover much of the less-favoured areas of Europe (Soussana and Duru, 2007). Their management for optimizing yield and digestibility represent specific challenges while they may have interesting nutritive value characteristics such as greater herbage digestibility because of low temperatures and improved functional properties.

The diversity and number of species of permanent pastures make it difficult to analyze, understand, and manage the nutritive value. A new method based on the concept of plant functional types (PFT) was developed by Duru *et al.* (2008a) to rank grass species and grasslands according to the organic matter digestibility (OMD) of the plant material (Table 2). Two extreme PFTs, that is species having a growth strategy based on resource capture (acquisitive) or resource conservation (conservative), were defined based on leaf dry matter content (Duru *et al.*, 2008a). Herbage OMD of grasslands dominated by one of two typical PFTs was analyzed at two locations with differing altitudes in the French Pyrenees (Figure 1). At each location, herbage OMD was higher at an early growth stage for the plant community having an acquisitive growth strategy, but it decreased faster than for the plant community having a conservative growth strategy. When expressed as a function of the standing herbage mass, herbage OMD was higher for grasslands having the greatest proportion of plants with an acquisitive growth strategy, whatever the standing herbage mass.

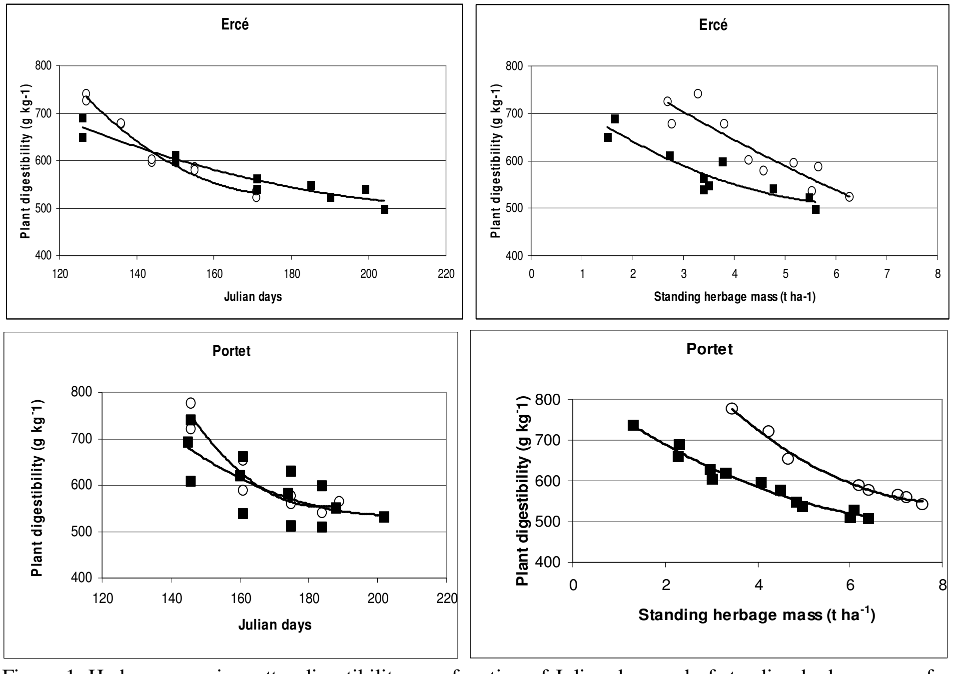

Figure 1. Herbage organic matter digestibility as a function of Julian days and of standing herbage mass for two sites (Ercé and Portet) in the French Pyrenees and two grassland functional compositions: community dominated by plant having an acquisitive (circle) or a conservative (square) growth strategy.

The effect of temperature on herbage digestibility can be assessed through the comparison of the two locations. When expressed as a function of days of the year (Julian days), herbage OMD was higher for grasslands composed of plants having an acquisitive growth strategy than for those composed of plants having a conservative growth strategy. On the other hand, for a same grassland type and standing herbage mass, herbage OMD was greater for the site at a higher altitude and lower temperature (e.g. +20 to 100 g kg^{-1} DM and +30 g kg^{-1} DM for standing herbage yields of 3 and 5 Mg DM ha^{-1}, respectively) than for the site at a lower latitude. These results clearly show a direct negative effect of increased temperature on herbage digestibility due to a decrease of the digestibility of the structural component, and an indirect effect of temperature caused by a higher structural tissue component.
Several studies conducted in the Alps have suggested that the fatty acid composition of milk from mountainous areas differs from that of lowlands (Collomb *et al.*, 2008) with a greater concentration of *n*-3 fatty acids. Several factors (e.g. use of grazing, minimum use of concentrates, botanically-diverse vegetation) could be responsible for this improved milk quality in mountainous areas.

Low temperatures and long days

Low temperatures are known to improve forage digestibility through a reduction of lignification (Deinum, 1981; Thorvaldsson *et al.*, 2007; Bertrand *et al.*, 2008). Consequently, herbage produced in northern latitudes or at higher altitudes should be more digestible. Daily changes in digestibility are also less under cool temperatures. The rate of decline in *in vitro* DM and NDF digestibilities increased with increasing temperatures for seven temperate grass species used in the Nordic countries (Thorvaldsson *et al.*, 2007). The rate of decline in timothy DM digestibility per day was 0.06 percentage units for each degree increase in temperature (Thorvaldsson, 1992).
Long days in northern latitudes, however, result in a rapid development of herbage grasses with rapid changes in digestibility, particularly if combined with high temperatures. Variation in heading dates among perennial ryegrass cultivars is typically 4-6 weeks in UK (Sheldrick, 2000) while it is two weeks in Canada (Bélanger and Richards, 1995) and three days (Kangas *et al.*, 2010) in Finland for timothy. For example, Nissinen *et al.* (2010) reported a 0.11 percentage unit decrease in OMD and a 0.06 percentage unit decrease in CP concentration per GDD in the Northernmost part of Finland (66°N). The window of opportunity for harvesting at the optimal stage of development in several areas of the Nordic countries, primarily the first harvest, is therefore quite narrow with a potential negative impact on nutritive value if the herbage cannot be harvested because of unfavourable weather conditions.
The rapid development rate of grassland also affects grazing management. In Finland, even as small as a five-day difference in turnout date causes major changes in the growth pattern, stem production, sward height, and herbage digestibility of timothy – meadow fescue pastures (Virkajärvi *et al.*, 2003). The challenges to maintain high quality pastures for high yielding dairy cows during mid-summer is one reason why grazing is less and less popular among dairy farmers in Finland, further increasing the importance of silage production.

Autumn harvest or grazing management

In northern areas, grazing opportunities in autumn are limited due to inadequate grass growth because of cool temperatures. This forces agricultural producers to use stored herbage to meet the feed requirements of their livestock. Extending the grazing season is, therefore, a potential way to increase profits. Under the conditions of the mid-north of eastern Canada and the Nordic countries, forage crops are managed in such a way as to minimize risks of winter damage to swards. Harvesting or grazing of most perennial forage species in autumn may affect their persistence and spring regrowth potential if the timing is not adequate. Dates of the autumn harvest can also affect the nutritive value of the herbage. Delaying the autumn harvest of tall fescue from 1 September to 30 October in eastern Canada decreased CP concentration, increased sugar concentration, and had a limited effect on ADF concentration (Drapeau *et al*., 2007). Similarly, in a three-year experiment conducted at two sites in Finland, an autumn harvest in September resulted in a high *in vitro* DM digestibility (on average 720 g kg^{-1} DM; Sairanen *et al*., 2012). Low autumn temperatures are therefore favorable to the production of energy-rich forages.

In Finland, however, the autumn may be wet, which increases the risk of poor ensiling properties (mainly low DM concentration) of harvested grass to be conserved in the form of wilted silage. In addition, the risk of damaging the sward and soil structure during harvesting is substantial. Furthermore, a late grazing in autumn increases the risk of nutrient leaching in general (Saarijärvi, 2008). In all, these reasons may limit the opportunity to utilize the high digestibility of autumn growth.

Will climate change improve herbage nutritive value in cool regions?

Herbage production in less-favoured areas, often limited by soil and air temperature, is most likely to be affected by climate change. Under predicted future climate, risks of winter injury to perennial forage species (e.g. lucerne) in eastern Canada will likely increase because of reduced cold-hardening during autumn and protective snow cover during the cold period (Bélanger *et al*., 2002). In Norway, however, a recent study indicated that climate change is expected to have a minimal impact on the risks of winter injuries to timothy and perennial ryegrass in most regions (Thorsen and Höglind, 2010). The authors concluded that better overwintering conditions might even make it possible to grow perennial ryegrass in areas where it is not grown today. It is therefore likely that climate change in winter will indirectly affect the nutritive value of herbage in northern areas through a change in the species grown.

From a study under controlled conditions with constant temperatures, Bertrand *et al*. (2008) concluded that the forecast increase in air temperature over the next 100 years might result in lower yield of timothy. This effect is likely to be greater in regions where the day/night temperature regime is already close to or above the optimum temperature for timothy growth (22/10°C), mostly in eastern Canada and the southern part of the Nordic countries. However, with conditions closer to those found in several areas of the Nordic countries (e.g. Iceland), increasing growth temperatures from 9 to 17°C increased the DM yield of seven grass species, particularly in early growth stages (Thorvaldsson and Martin, 2004). Changes in herbage yield due to warmer temperatures might affect the herbage nutritive value through the negative relationship between DM yield and digestibility discussed above. Adaptation strategies involving harvest intervals and the grazing pressure might mitigate this impact of climate change.

Increasing air temperatures is also expected to extend the length of the growing season (Izaurralde *et al*., 2011), which at high latitudes can be determined by the period between the time of the last spring frost and the first autumn frost. In eastern Canada, where low temperature is a major limiting factor for growth, the growing season for herbage grasses is expected to increase by an average of approximately 44 days and 778 GDD (0°C basis) by 2040-2069 (Jing *et al.*, 2013b). This should translate into the possibility of an additional harvest. A longer growing season is also expected in Norway with the possibility of one or two extra grass harvests (Thorsen and Höglind, 2010) and in Scotland (Topp and Doyle, 1996) with increases of 25 to 75 days of herbage growth. Simulations conducted in Northern Europe indicated an average increase of 11% in seasonal DM yield with most coming from an increase in the number of cuts rather than an increased yield in individual cuts (Höglind *et al*., 2013). The effects of a longer growing season on herbage nutritive value and crop persistence with growth starting earlier in spring and stopping later in autumn, remain to be determined.

Temperature can also affect herbage nutritive value. Increases above optimal growth temperatures can increase cell wall constituents along with stem tissues, reduce soluble sugar concentration, and decrease herbage nutritive value (Morgan *et al*., 2008). In a study conducted under controlled conditions, Bertrand *et al*. (2008) concluded that increasing day/night air temperature from 17/5 to 22/10°C slightly increased NDF and ADF concentrations of timothy and reduced the digestibilities of DM and NDF. In a study conducted with air temperatures more typical of Iceland, increasing day/night temperatures from 9/5°C to 17/13°C decreased DM and NDF digestibilities of seven grass species (Thorvaldsson *et al*., 2007). The higher atmospheric CO_2 concentration is expected to decrease CP concentration and increase non-structural carbohydrates of herbage (Lilley *et al*., 2001; Körner, 2002; Hopkins and Del Prado, 2007; Bertrand *et al*., 2007). Newman *et al*. (2001) reported that elevated CO_2 decreased lignin and N concentrations of tall fescue, and decreased NDF digestibility but only under high N conditions. High CO_2 or higher temperatures are also expected to increase fibre concentrations (Owensby *et al*., 1996).

Along with affecting the yield and nutritive value of currently grown perennial species in temperature-limited areas, climate change will also impact the distribution of crop species and open the door to new species. Thorsen and Höglind (2010) suggested perennial ryegrass will grow far better in Norway under predicted future conditions. A short growing season, low temperatures, and early frost currently limit the cultivation of maize at high latitudes (Mussadiq *et al*., 2012). Warmer temperatures along with the potential of developing short-season hybrids may also make possible the cultivation of maize in northern agricultural areas.

In most areas in Northern Europe, climate change is expected to increase forage production for sown as well as for permanent grasslands, including mountainous areas. This is due to increasing air temperature and CO_2 concentration (Ruget *et al.,* 2012). Based on the conceptual model presented above, we can therefore expect a decrease in herbage digestibility due to an increase in temperature. However, this is not taking into account the indirect effects of an increase in temperature that will favour species having an acquisitive growth strategy. This change in plant community functional composition could offset the direct effect of temperature. Long term ecological observatory grassland networks are needed to study these complex interactions between climate, plant community composition, species tissue composition, and potential management.

Perspectives for improving herbage nutritive value in less-favoured areas of cool regions

Rising demand for agricultural products in response to ever-growing world population, rising incomes, and increased non-food uses of agricultural products will put pressure on existing cultivated lands and will probably require a greater and more productive use of marginal lands and less-favoured areas. This is particularly true in the context of climate change because temperature limitations to agriculture production will be reduced in areas at the northern limit of production. This will require crops and cropping practices that are adapted to significant soil limiting factors (shallowness, acidity, low pH, and low fertility). Increased productivity will also have to occur in the context of a warmer, often drier, and more variable climate.

Sown herbage species in northern areas

Although the choice of species is limited in several northern areas of eastern Canada and the Nordic countries, species well adapted to local conditions are already available. As mentioned above, climate change will increase the 'tool box' available in those areas and this, in turn, might result in the production of more digestible herbage. Perennial herbage species like perennial ryegrass, tall fescue, and lucerne might see an expansion of their zone of adaptation to more northern areas. The possibility of using maize in northern areas also offers new opportunities to livestock producers in terms of crop rotations and ration formulation. The expansion to northern areas of crop species might come with new diseases, pests or increased disease or pest pressure, along with a greater demand on the land. The agricultural landscape of less-favoured areas of the north is, therefore, likely to change. Warmer temperatures in those areas, however, might result in a reduction of the herbage digestibility, although this could be partly compensated by increasing the harvesting frequency or grazing pressure.

Genetic selection

We discussed the negative relationship between herbage digestibility and yield. This suggests that cultivars with greater forage DM yield may have a lower herbage digestibility. The improvement of both herbage DM yield and herbage digestibility requires the identification of genotypes exhibiting a weaker negative relationship between DM yield and digestibility. Our studies confirm that improvements in the digestibility of the structural component, either taken as the true stems or herbage NDF, has the potential of improving forage digestibility with no negative impact on DM yield (Bélanger *et al.*, 2001). Herbage species of less-favoured areas of cool regions (e.g. timothy) have received less attention than other species (e.g. lucerne, ryegrass) from a breeding perspective. Considerable potential for improvements in nutritive value probably exists but it will require a greater genetic understanding and improved breeding strategies. Along with the more traditional production-oriented DM yield, digestibility, and N concentration, other attributes of nutritive value (e.g. fatty acids) should also be considered in the context of improving human, animal, and environmental health.

Management of species-rich grasslands

Harsh environments in mountainous areas favour grasslands composed of species having a conservative growth strategy leading to low herbage production (Duru *et al.,* 2010a). Promoting grasslands composed of species having an acquisitive growth strategy could be envisaged as a long term objective but it requires costly fertilisation and early hay harvest dates that are difficult to implement. In the short term, the choice of harvest or grazing dates of species-rich grasslands is the main farmer-controlled factor for manipulating the nutritive value (Duru *et al.,* 2008b). There is usually significant room to adjust the dates of grazing or harvesting although rainfall might be a barrier for advancing hay harvest dates. From a practical point of view, plant communities composed of species having an acquisitive growth strategy have higher herbage digestibility at early stages (grazing) while the opposite is true for later stages (hay or silage).

Simulating herbage nutritive value

Simulation models can assist in developing site-specific recommendations to optimize harvest yield and nutritive value attributes (e.g. digestibility and N concentration). Process-based models allow information integration and knowledge development of complex systems involving plant growth, nutritive value, and environmental conditions. For sown swards of timothy, the process-based CATIMO model (Bonesmo *et al.*, 2002) has been shown to simulate satisfactorily the nutritive value of the primary growth in eastern Canada and Finland (Jing *et al.*, 2013a). Simulations of the regrowth nutritive value, however, were not as successful and require more research. This simulation work of nutritive value of timothy is based on the conceptual model described above and involves a number of processes related to N uptake, the partitioning of growth into leaves, stems, cell walls, and cell contents. Improved knowledge of factors controlling the nutritive value of timothy summer regrowth along with

field data, including leaf-to-weight ratio and attributes of nutritive value of leaves and stems, are required to improve the accuracy of simulating its digestibility and N concentration.
A similar simulation model has been built for permanent grasslands (Duru *et al.,* 2008b). Based on the conceptual model described above, it takes into account the plant community functional composition and the herbage DM accumulation rate. For simplification, it is time- and temperature- (growing degree days) driven with specific equations for the reproductive spring growth and the following regrowths. Practically, it is coupled with an herbage growth model that allows both outputs to be considered simultaneously (Duru et al., 2010b).

Conclusion

Growing conditions in less-favoured areas of cool regions, including mountainous areas, are characterized by low temperatures, short growing seasons, harsh winters, and challenging soil conditions. The limited choice of herbage species in those areas or the environmental constraints imposed to (semi)natural grasslands might result in lower nutritive value than in more favoured areas. However, lower temperatures and longer days during the growing season, at least in more Northerly latitudes, partly mitigate the loss in nutritive value caused by the exclusion of high quality grasses such as perennial ryegrass. Climate change is likely to change the herbage landscape of less-favoured areas in cool regions while improvements in nutritive value through management and breeding will continue to offer new opportunities.

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Oral Presentations

Aggregated plant functional traits as affected by management practices impact on C stocks in temperate grasslands

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Abstract

Temperate grasslands contribute *ca.*10% of the global organic carbon (C) content in soils. Plants are the main way of C input in ecosystems and can constitute a lever to drive C sequestration. The aim of this study was to determine how aggregated plant functional traits as affected by management practices may modify C stocks in temperate grasslands. In a field study, a set of seven stations with contrasting management practices located in Basse-Normandie (France) was characterized in terms of floristic and functional composition (aggregated plant traits). Dry biomasses and soil organic matter (SOM) contents were measured from spring to autumn 2012 just before each grazing or mowing period. Grassland production, measured as the annual production above the cutting level, was lower in permanent grasslands than in temporary ones. Permanent grasslands were also characterized by high SOM content. Among measured aggregated traits, leaf dry matter content (LDMC) appeared to be the best predictor of SOM stock with a positive correlation. The high SOM stock observed in permanent grasslands may result in a slow degradation of plant tissues indicated by high values of aggregated LDMC.

Keywords: grasslands, SOM, carbon sequestration, plant communities, management practices, aggregated traits

Introduction

Carbon (C) sequestration is one of the ecological services provided by ecosystems and is of growing interest in the context of global changes. In temperate grassland ecosystems, C stocks are mainly located belowground, in roots and soil (IPCC, 2000; Soussana *et al.*, 2004). In Basse-Normandie (France), grasslands occupy almost half of the agricultural land area, and C sequestration and C pools are thus of great interest. C sequestration in soil is basically the result of a balance between C inputs by photosynthesis and C outputs due to respiration linked to organic matter decomposition. These two main C fluxes are closely related to the composition and functioning of plant communities that drive the quantity and quality of SOM (Soil Organic Matter) (De Deyn *et al.*, 2009). Plant community composition and functioning depend on grassland management practices. The community functioning can be apprehended by aggregated plant functional traits. These traits are calculated on the basis of the mass ratio hypothesis (Grime, 1998), which suggests that a species trait effect in a community is related to its contribution in the total biomass. Aggregated traits have been shown to impact on net primary productivity (Garnier *et al.*, 2004) and C-cycling (Bardgett *et al.*, 2005). The aim of this study was to determine how aggregated plant functional traits as affected by management practices may modify C stocks in temperate grasslands.

Materials and methods

Seven grassland stations were selected in the INRA Experimental Domain, located at Le Pin au Haras, Basse-Normandie, France (48°43'N, 0°11'E). For all stations, grassland grows on silty clay soils with pH ranging from 6.1 to 7.9, and calcium carbonate equivalent ranging from 0.7 to 1.5%. Management practices and floristic composition are given in Figure 1. *Lolium perenne* was the dominant species in most of the stations. High proportions of *Holcus lanatus* were observed in stations 1 and 2 corresponding to permanent grassland. In each station, a sampling design was established in a 400 m² square.

Stations	1	2	3	4	5	6	7
Permanent (P) / Temporary (T)	P	P	P	T	T	T	T
Grazed (G) / Mixed (M)	G	M	M	M	G	M	M

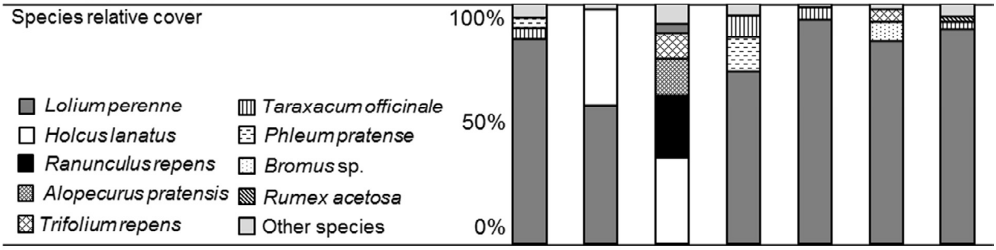

Figure 1. Management practices and floristic composition of the seven stations.

Leaves were collected according to Cornelissen *et al.* (2003) from the main species at each station (constituting more than 95% of the relative cover) in order to measure leaf traits (specific leaf area, SLA; leaf dry matter content, LDMC; leaf nitrogen content, LNC; leaf carbon content, LCC). Carbon stock measurements were carried out from spring to autumn 2012, just before each grazing or mowing period, in ten replicates per station. For each replicate, aboveground biomass was harvested from a 100 cm² ring and soil was collected as a core by using a 24 cm² × 10 cm depth cylinder. Aboveground biomass was collected in two compartments: above (>5 cm) and below (<5 cm) the cutting level. Grassland production was calculated as the sum of dry biomass sampled above the cutting level during the growing period. C and N contents in leaves were obtained by Isotope Ratio-Mass Spectrometry. SOM content was estimated by loss-on-ignition after combustion at 375°C for 16h.

Results and discussion

Grassland production and SOM content, which are negatively correlated (r = -0.780***), are both highly affected by the grassland type and, to a lesser extent, by the management and the interaction of both factors (Table 1). Production was lower and SOM content was higher in permanent grasslands than in temporary ones (Table 1). Among measured aggregated traits, LDMC appeared to be the best predictor of SOM content.

Our study suggests that permanent grasslands have a stronger potential than temporary grasslands for C sequestration in temperate areas. The relationship between plant-aggregated traits and C sequestration is the result of the balance between two conflicting processes (De Deyn *et al*., 2009). Because plant photosynthesis is the driving force of C input, the C stocks may increase with productive plants characterized by a low LDMC (Lal *et al.*, 1998). However, because SOM degradation is the major source of C output, C stocks may increase in non-productive systems, characterized by high LDMC, due to slow SOM degradation (Fornara *et al.*, 2011). In our study, the higher C stocks, as estimated by SOM content, observed in the lower productive grasslands may result in a greater impact of LDMC on SOM degradation than on photosynthesis.

Table 1. Production and SOM content for the seven stations (means and standard errors, n = 10). ANOVAs were performed to analyse the effect of stations, and then grassland types, management and their interactions on production and SOM content. Pearson correlation coefficients between LDMC and production and SOM content are given.

Station	Permanent (P)/temporary (T)	Mixed (M)/grazed (G)	Production (kg ha^{-1})	SOM (g kg^{-1})
1	P	G	3.4 (0.3)	146.0 (3.4)
2	P	M	7.5 (0.8)	103.6 (2.8)
3	P	M	8.0 (1.0)	114.5 (5.6)
4	T	M	12.2 (0.8)	77.5 (1.8)
5	T	G	15.3 (0.8)	86.3 (2.4)
6	T	M	15.5 (0.8)	71.7 (1.8)
7	T	M	20.4 (1.2)	66.5 (1.8)
Station effect			F = 44.31***	F = 127.80***
Grassland type (permanent / temporary) effect			F = 110.34***	F = 430.39***
Management (mixed / grazed) effect			F = 6.56***	F = 121.06***
Grassland type × Management effect			F = 3.78 ns	F = 24.60***
Pearson correlation with LDMC			r = - 0.628***	r = 0.540***

*P<0.05; **P<0.01; ***P<0.01; ns, not significant

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Grass-legume mixtures enhance yield of total nitrogen and uptake from symbiotic N_2 fixation: Evidence from a three-year multisite experiment

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Abstract

In a multisite field experiment conducted over three years, the amount of total nitrogen yield (N_{tot}) and yield from symbiotic N_2 fixation (N_{sym}) was quantified from grass-legume stands with greatly varying legume proportions. The five experimental sites spanned a climatic gradient from Atlantic to continental, and from temperate to arctic. N_{tot} of mixtures was, on average, greater than that of monocultures at all sites, the effect being most pronounced when legume proportions in mixtures were around 60%. N_{tot} was 256 kg ha^{-1} yr^{-1} when calculated across all years and sites from mixtures with 50% legume proportion, while the corresponding N_{sym} was 77 kg ha^{-1} yr^{-1}. Thus, 30% of N_{tot} originated from symbiotic N_2 fixation. Average N_{sym} in mixtures was positively related to mean site productivity (P=0.096), while percent N_{sym} (the ratio of N_{sym} to N_{tot}) was not (P=0.909). This indicates that the relative contribution of N_2 fixation to N_{tot} remained, on average, unchanged across a large productivity gradient. We conclude that the use of grass-legume mixtures can contribute to resource efficient, yet productive, agricultural grassland systems under a wide range of environmental conditions.

Keywords: N uptake, symbiotic N_2 fixation, climatic gradient, sustainable intensification

Introduction

Grass-legume mixtures offer the benefit of symbiotic nitrogen fixation (N_{sym}) of legumes to grassland forage systems, thereby increasing total harvest yield (Nyfeler et al., 2009), total N yield (N_{tot}) and forage quality (Carlsson and Huss-Danell, 2003). Because legumes are able to utilize atmospheric N_2 for their N requirements, the relative availability of soil N increases for grasses in mixtures due to 'N-sparing'. Therefore, the use of grass-legume mixtures could allow substantial reductions in the amount of industrial-N fertilisers used in agricultural grassland systems without compromising yield.

Although the amount of N_2 fixation has been quantified in temperate grassland (Boller and Nösberger, 1987; Nyfeler *et al.*, 2011), there are few data from arctic or continental ecosystems are much rarer. In such systems, low winter temperatures and/or scarce precipitation may hamper the legumes' growth and capability to fix nitrogen (Nesheim and Boller, 1991), and accordingly, levels of N_{sym} may decrease. In a coordinated field experiment conducted over three years across a large environmental gradient of Europe, N_{tot} and apparent

N_{sym} were quantified from grass-legume swards to investigate the benefit of N_2 fixation to grassland production at different levels of productivity. The five experimental sites spanned a climatic gradient from Atlantic (Ireland) to continental (Lithuania, Poland), and from temperate (Switzerland) to arctic (Iceland).

Materials and methods

At each site, monocultures and grass-legume mixtures were established with four important agronomic species. These were two grasses: *Lolium perenne* L. (*Phleum pratense* L. in Iceland) and *Dactylis glomerata* L. (*Poa pratensis* L. in Iceland), and two legumes: *Trifolium pratense* L. and *Trifolium repens* L. The experiment aimed at investigating grass-legume interactions, which was achieved by sowing swards with a large variation of legume percentages (0%, 20%, 50%, 80%, 100%). Eleven different mixtures, each containing all four species in varying proportions, and the four monocultures were established at two different seed densities for a total of 30 stands per site (see Kirwan *et al*. (2007) for a full description of the design). N concentration was determined by near-infrared reflectance spectroscopy (calibrated and back-validated by determinations of a CN-analyser) from a subsample of total harvested biomass per plot, from which N_{tot} was calculated. The influence of the swards' legume proportion on N_{tot} was analysed by linear mixed regression with a random grouping variable for sites. Seed density was never significant and was omitted from further analyses. The amount of apparent N_{sym} and percent N_{sym} (the fraction of N_{sym} to N_{tot}) was determined from the regression model estimates following the N-difference method (Ledgard and Steele, 1992). Detailed analyses of N_{tot} and N_{sym} based on the ^{15}N dilution method at one site (Switzerland, Nyfeler *et al*., 2011) suggested that the N-difference method was sufficiently reliable to draw conclusions on patterns of N dynamics.

Results and discussion

N_{tot} of mixtures was, on average, greater than that of monocultures ($P<0.01$ at three sites), and was significantly greater in mixtures over all sites ($P<0.001$; no figure shown). Higher N_{tot} in mixtures than monocultures can be explained by enhanced stimulation of N acquisition from symbiosis in legumes and - to a lesser extent - by higher N uptake of swards from non-symbiotic sources (Nyfeler *et al*., 2011).

N_{tot} was significantly affected by the relative abundance of legumes in mixtures, the effect being most pronounced when legume proportions were approximately 60% (Figure 1). Predicted N_{tot}, calculated across all years and sites from mixtures with 50% legume proportion, was 256 kg ha^{-1} yr^{-1}, while the corresponding N_{sym} was 77 kg ha^{-1} yr^{-1}. Thus, averaged across all years and sites, 30% of N_{tot} originated from symbiotic N_2 fixation. However, maximal N_{sym} at individual sites was as high as 220 kg ha^{-1} yr^{-1}, or 49% of N_{tot} (Figure 1). Nyfeler *et al*. (2011) reported N_{sym} in a temperate grassland with comparable legume proportion to be around 250 kg ha^{-1} y^{-1} or 53% of N_{tot}. Annual productivity (estimated from average grass monoculture biomass yield) at such grassland was 9.4 t ha^{-1} yr^{-1}, with biomass yields in mixtures as high as 18 t ha^{-1} yr^{-1}. In the present study, less-productive sites were also included: Iceland had average grass-monoculture yields of 3.5 t ha^{-1} yr^{-1}, and Lithuania had 6.9 t ha^{-1} yr^{-1}. The estimated N_{sym} at these sites was much lower (30 and 28 kg ha^{-1} yr^{-1}, respectively). This is in line with the concept that N_2 fixation of legumes is regulated by N-demand of the concomitant species, their growth being itself adapted to the local environmental conditions (Hartwig, 1998). Following this concept, legumes adapt the extent of N_2 fixation to the general level of productivity and account thereby for the gap between their N-demand (sink) and the N-availability (source) from non-symbiotic N-sources.

Further analyses showed that N_{sym} (predicted at each site from mixtures with 50% legume) was positively related to mean site productivity (P=0.096). Interestingly, Jacot *et al.* (2000) revealed that the reduction of N_{sym} across an altitudinal gradient from 900 to 2300 m a.s.l. corresponded to a general decrease of plant growth, but percent N_{sym} in legume plants was not diminished. In line with Jacot *et al.* (2000), percent N_{sym} was not correlated to average site productivity (P=0.909) in our study, indicating that on average, the relative contribution of symbiotic N_2 fixation to N_{tot} did not change over the large environmental gradient.

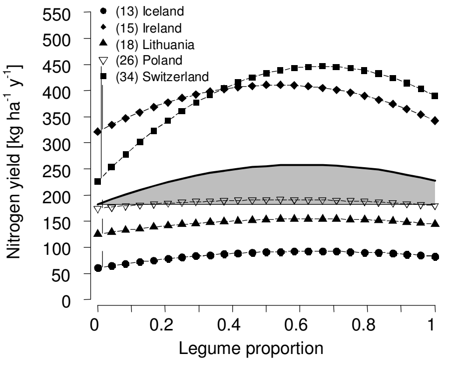

Figure 1. Total nitrogen yield (N_{tot}) as affected by legume proportion in swards of five sites across a climatic gradient. The bold, curved line is the weighted sum of individual site lines; the continuous horizontal line denotes estimated N_{tot} of grass monocultures, for which N is assumed to originate from soil. The shaded grey area is thus the apparent amount of N derived from symbiotic N_2 fixation of legumes (N-difference method, Ledgard and Steele, (1992)).

Conclusions

The use of mixed grass-legume swards can replace significant amounts of industrial N fertilisers with symbiotically-fixed N_2, while still achieving high levels of biomass yield across a wide range of environmental conditions. This is a significant contribution to resource efficient and sustainable agricultural grassland systems.

Acknowledgements

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Nitrous oxide emissions from white clover based grassland used for dairy production

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Abstract

The objective was to measure annual nitrous oxide (N_2O) emissions from grassland used for dairy production on soils seasonally saturated due to impeded drainage in Ireland (52°51'N, 08°21'W). N_2O emissions were measured between October 2008 and November 2011 from (i) white clover- (*Trifolium repens*) based dairy production system and (ii) white clover-ryegrass control plots receiving no input of N (background soil N_2O emissions). The dairy system was rotationally grazed by dairy cows. Herbage mass and N content was measured before each grazing to determine annual N uptake in herbage. Annual surplus rainfall (mm) was 701 in 2008/09, 687 in 2009/10 and 394 in 2010/11. Annual N uptake in herbage (kg ha^{-1}) was 254, 296 and 446 for each of the three years respectively. Mean (± SEM; 1.84) annual N_2O emissions from the dairy system (kg ha^{-1} of N) were: 2.7, 6.4 and 28.0 for the three years respectively (P<0.001). Relatively low surplus rainfall led to aerobic soil conditions and higher N mineralization that caused substantially higher N_2O emissions in 2010/11.

Keywords: mineralization, rainfall, soil moisture, annual variation

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential of up to 310 times that of carbon dioxide and it contributes to the destruction of the stratospheric ozone (IPCC, 2007; Ravishankara *et al.*, 2009). Nitrous oxide is produced by multiple processes in soil such as nitrification, denitrification, chemo-denitrification and dissimilatory reduction of nitrate to ammonium (Stevens and Laughlin, 1998). These processes are affected by soil factors such as inorganic N content, moisture, temperature, carbon, oxygen, pH and C:N ratio (Klemedtsson *et al.*, 2005; Saggar *et al.*, 2012). Changes in these controlling factors over time create considerable variation in emissions of N_2O, making it difficult to acquire accurate estimates of annual N_2O emissions. Therefore several years' data may be required to obtain a robust estimate of annual N_2O emissions. The objective of this study was to measure annual N_2O emissions from white clover-based grassland used for dairy production over three years and to assess annual variation in N_2O emissions.

Materials and methods

The study was conducted at Solohead Research Farm (52°51'N, 08°21'W). The predominant soils have a clay loam texture and are seasonally wet due to impeded drainage. The swards at

Solohead Research Farm range in age from 20 to 30 years and have been under permanent pasture for at least 60 years. White clover was established and maintained in the swards since 2000 through over-sowing white clover seed onto silage stubble. The experiment was a randomized block design with two treatments. Nitrous oxide was measured from (i) white clover-based dairy production system (WC) and (ii) white clover-ryegrass control plots (background soil emissions; WC_B) between October 2008 and November 2011. The WC consisted of three silage and three grazing paddocks ranging in size from 1.42 to 2.07 ha. The swards were dominated by perennial ryegrass *(Lolium perenne* L.*)* with an annual average white clover (*Trifolium repens* L.) content of 27% throughout the study. Grazing paddocks were rotationally grazed by Holstein-Friesian cows and surplus herbage was removed as silage. Silage paddocks were closed for first-cut silage in early April, harvested in late May and managed the same as grazing paddocks for the remainder of the grazing season. The WC had an annual average stocking density of 2.35 cows ha^{-1} and annual fertilizer N input of 100 kg ha^{-1}. Three WC_B were set up (11×3 m area) to measure background soil N_2O emissions and received no external input of N or grazing. The plots contained an annual average white clover content of 32% throughout the study. Herbage on WC_B was harvested at monthly intervals and discarded. Before each grazing or silage harvesting event herbage mass was measured on WC and the N content of herbage was subsequently determined and used to calculate annual N uptake in herbage dry matter (DM). N_2O emissions were measured using five static chambers per paddock in WC and one per WC_B. The N_2O sampling strategy consisted of weekly sampling with increased frequency following N fertilization. Rainfall and soil temperature at 0-10cm depth were recorded on a daily basis at the site.

Results

Annual surplus rainfall (rainfall - evapotranspiration) was 701 mm in 2008/09, 687 mm in 2009/10 and 394 mm in 2010/11 (Figure 1a). Mean annual soil temperature (°C) was 9.6, 9.2 and 8.6 for each of the three years, respectively. Annual N uptake (kg ha^{-1}) in herbage from WC was significantly higher (P<0.001) in 2010/11 than the previous two years (Figure 1b). With regard to N_2O emissions there was an interaction (P<0.05) between year and WC treatment. There was no difference in N_2O emissions between WC and WC_B in 2008/09 or in 2009/10, whereas there was a difference (P<0.001) between them in 2010/11. While there was no difference in emissions from WC_B between years, emissions from WC in 2010/11 was higher (P<0.001) than in each of the previous two years (Figure 1c).

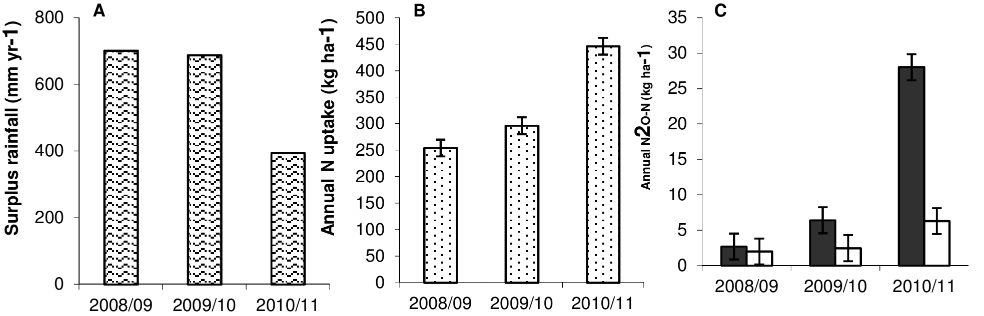

Figure 1. Annual surplus rainfall (A), annual N uptake in herbage DM (P<0.001) (B) and annual N_2O emissions, from white clover based dairy production system (WC ■) and background white clover plots (WC_B □), between 2008/09 and 2010/11 (P<0.05 interaction between year and WC treatment). Error bars represent the ± standard error of the mean.

Discussion

Relatively high annual surplus rainfall led to saturated anaerobic soil conditions in 2008/09 and 2009/10, whereas lower surplus rainfall resulted in soil drying and a shift to more aerobic soil conditions in 2010/11. This shift from anaerobic to aerobic soil conditions was conducive to soil organic N mineralization which is reflected in the increase in annual N uptake in herbage DM in 2010/11 (Figure 1b). There was a large degree of variation in annual N_2O emissions in this study, with two- to nine-fold differences between annual emissions, which is far greater than previously reported estimates from Irish grassland (Hyde *et al.*, 2006; Abdalla *et al.*, 2009; Rafique *et al.*, 2011). The saturated anaerobic soil conditions in 2008/09 and 2009/10 are likely to have caused the predominance of complete denitrification and hence relatively low N_2O emissions. Other studies have reported a decrease in the N_2O:N_2 ratio with increasing soil moisture content, which may have also been the case in this study (Rudaz *et al.*, 1999; Saggar *et al.*, 2012). In contrast the drier soil conditions in 2010/11 was more favourable to incomplete denitrification and the production of N_2O, which combined with high rates of N mineralization in the soil led to the very large emission of N_2O in 2010/11, particularly on WC where there was considerable quantities of N circulating within the system.

Conclusion

Differences between years in surplus rainfall impacted directly on soil moisture content and the mineralization of N on this heavy soil with impeded drainage. The extent of these differences was reflected in uptake of N in herbage DM and in N_2O emissions from the soil. The sharp contrast in annual emissions found in this study also suggests the need for long term studies when quantifying annual N_2O emissions from intensively managed grassland.

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Assessing quantity and quality of grazed forage on multi-species swards

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Abstract

Quantification of herbage intake by grazing livestock and estimation of forage quality of grazed herbage can be expected to be more difficult in multi-species swards than in simple species mixtures. The effect of sward composition (grass-dominated and diverse) on the indirect estimation of forage uptake and quality was investigated in a grazing experiment with sheep and cattle as grazer species. Standing biomass was quantified using a double-sampling technique analysed for crude protein and acid detergent fibre. Contrary to expectations, there was no evidence for a systematically higher difficulty of estimating quality and quantity of standing biomass in diverse swards. Estimates of herbage intake strongly varied depending on the degree of specificity of the chosen calibration model for standing biomass against sward height, but without a consistent sward-type effect. Higher sample numbers were necessary for precise calibration models of standing biomass than for precise estimates of herbage quality.

Keywords: pasture, multi-species swards, rising plate meter, herbage intake, CP, ADF

Introduction

Double-sampling methods of pre- and post-grazing standing biomass, using rising-plate-meter measurements in combination with calibration cuts, can be employed as an indirect method to quantify forage intake on pastures (Reeves *et al*., 1996). However, these methods have shown a lower performance on multi-species swards than on simple species mixtures (Martin *et al*., 2005). A more variable plant chemical composition and higher grazing selectivity in multi-species swards may also make it more difficult to assess the quality of the grazed forage. Against this background, we investigated the precision of biomass quantity and quality estimates on two pasture swards of different botanical complexity.
We expected (i) that rising-plate-meter calibration equations for standing biomass will differ between diverse and grass-dominated swards and that a better calibration model fit will be achieved for the latter. We further assumed (ii) that, particularly in diverse swards, at given sample sizes, estimates of quality parameters will show a higher variability than estimates of standing biomass using a double-sampling technique. These hypotheses were tested in a grazing experiment where diverse and grass-dominated swards were grazed by cattle or sheep.

Materials and methods

The grazing experiment was established in 2007 in the Solling uplands in Relliehausen, Germany, (51°46’N, 9°42’E; 200 m above sea level) on long-term permanent grassland (moderately species-rich *Lolio-Cynosuretum*). Diverse (untreated) and grass-dominated swards (herbicide application against dicots in 2006 and 2009) were grazed with ewes or suckler cows at stocking densities of 12 livestock units per ha in a rotational stocking system with three rotations. In each rotation, three blocks (A, B, C) were grazed consecutively.
During the second rotation in 2012 (block A: 12-17 June, B: 18-24 June, C: 25-27 June), sward height measurements were performed and calibration cuts were taken 5-7 days before and immediately after grazing in each block. Compressed sward height (CSH) was measured with a rising-plate meter at 50 randomly chosen points per plot. Calibration cuts were taken at 15 (A) or 8 (B, C) points per plot. There, CSH was measured and biomass was cut to ground

level, using scissors, on a circle of 30 cm diameter. Dry matter (DM) was weighed after drying the biomass samples. Their crude protein (CP) and acid detergent fibre (ADF) concentrations were determined by near infrared spectroscopy using an existing calibration.
For CP and ADF concentration, mixed effects models were fitted with grazer species (Gr), sward type (Sw), sampling time (T, pre- and post-grazing) and their pairwise and multiple interactions, as well as block (Bl), as fixed effects in the full model, and samples within plot as repeated measurements. Calibration equations of CSH on standing biomass (DM) were fitted using generalized least squares models, in which variance structure was modelled as a power function of CSH. Dry matter was modelled in response to CSH, with Gr, Sw, T and Bl and their two- and threefold interactions as possible further fixed parameters. Models were compared using Akaike's Information Criterion (AIC). The basic model included only CSH as a parameter and the three models with lowest AIC were used to calculate pre- and post-grazing biomass of each plot on the basis of the 50 CSH measurements. The difference of calculated pre- and post-grazing DM was used as an estimate of herbage intake.
The effect of sample size on quality and DM estimates was tested using data from block A, separately for sward type and sampling time, but pooling samples from cattle- and sheep-grazed plots. Mean CP and ADF concentrations of these variants were calculated from 5000 random draws of 14-16 samples each. Standing DM was estimated using simple linear regression based on the randomly drawn samples and the mean of 100 CSH measurements per variant. The coefficient of variation of the 5000 estimates for the mean of each parameter was calculated as a measure for the repeatability of the results.

Results and discussion

Over all blocks and treatments, pre- and post-grazing CSH were 8.4 and 5.3 cm, with mean within-plot standard deviations of 3.3 and 2.6 cm, respectively. ADF concentration was significantly influenced by sward type (P<0.001), grazer species (P<0.001) and sampling time (P<0.001), with higher values in post-grazing biomass, biomass from grass-dominated and from sheep-grazed swards (Table 1). CP was higher in diverse than in grass-dominated swards (P<0.001), but did not differ significantly between pre- and post-grazing samples (P=0.06). There were no interactions between the main effects. Diverse swards therefore had higher forage quality, but this did not lead to higher selectivity by grazing livestock.
There were considerable differences in the estimation of herbage intake, depending on the chosen calibration model (Table 2). The best model according to AIC took into account sward type, sampling time and block. In this model, differences of the calibration slope between grass-dominated and diverse swards were block-specific (significant CSH×Bl×Sw interaction, P=0.011). Fitting variance structure separately for grass-dominated and diverse swards did not significantly improve the model. Hypothesis (i) of specific regression slopes for the two sward types, and better model fit for grass-dominated swards, therefore had to be rejected.
Even at a simulated sample size of only n=4, the coefficients of variation of predicted mean CP and ADF concentrations were low at less than 10% (Figure 1), without differences between the two sward types. The predicted mean of standing DM, by contrast, varied more strongly, being as high as 6-11% even at a sample size of n=16, so disproving hypothesis (ii).

Table 1. Factor level means of crude protein (CP) and acid detergent fibre (ADF) concentrations in standing biomass. In brackets: mean standard deviation per plot.

Concentration (% of dry matter)	Sward type		Grazer species		Sampling time	
	Diverse	Grass-dominated	Cattle	Sheep	Pre-grazing	Post-grazing
CP	14.8 (2.79)	12.1 (2.73)	13.9 (2.94)	13.1 (2.58)	13.8 (2.95)	13.1 (2.57)
ADF	29.2 (3.46)	34.7 (2.80)	31.0 (3.49)	32.9 (2.77)	30.3 (3.31)	33.6 (2.95)

Table 2. Estimates of dry matter intake calculated as the difference between pre- and postgrazing biomass obtained by different calibration models, and model evaluation criteria (CSH: compressed sward height, Sw: sward type, Gr: grazer species, Bl: Block).

Sward type	Grazer species	Estimated dry matter intake (g m^{-2})			
		CSH*Sw*T*Bl	CSH*Sw*Gr*Bl	CSH*T*Bl	CSH
Grass-dominated	Cattle	44.8	53.0	46.9	52.6
Diverse	Cattle	51.1	46.3	31.8	38.2
Grass-dominated	Sheep	47.1	43.0	54.2	55.9
Diverse	Sheep	62.4	62.5	57.5	55.9
Akaike's Information Criterion		2107.9	2142.2	2179.2	2271.2
Root mean square error		31.21	33.19	32.69	37.44

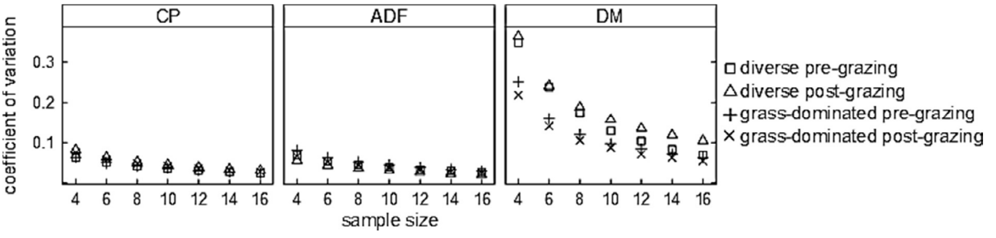

Figure 1.Coefficient of variation of predicted mean CP and ADF concentrations and standing dry matter (DM, double sampling technique) as a function of sample size, sward-type (grass-dominated/diverse) and sampling time (pre-/post-grazing) in a simulation with 5000 runs.

Conclusions

While forage quality differed between grass-dominated and diverse swards, there was no evidence for a consistently higher difficulty of estimating quality and quantity of standing DM in diverse swards. Contrary to expectations, fewer herbage samples were needed to reach the same level of precision for estimates of quality parameters than for estimates of standing DM, even if a double-sampling technique was used. Depending on the level of aggregation, calibration models for DM as a function of CSH delivered widely differing estimates of herbage intake. An independent quantification would be necessary to establish which level of aggregation delivers results of the highest adequacy.

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Forage value of leaf fodder from main European broad-leaved woody species

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Abstract

Leaf-hay was the principal feed for livestock during wintertime from the Neolithic until the first archaeological records of scythes dated to the Iron Age (700 – 0 BC). Despite the use of meadow hay, leaf fodder remained an important winter supplement until the present. Archaeological evidence has listed *Quercus*, *Tilia*, *Ulmus*, *Acer*, *Fraxinus* and *Corylus* as exploited tree species for leaf fodder, and *Fagus*, *Populus* or *Carpinus* as rarely used. The aim of our study was to test whether use of these listed woody species follows the pattern of their nutritive value. We collected leaf biomass and determined concentrations of N, P, K, Ca, Mg, and neutral- and acid- detergent fibre. There was no difference in the N concentrations among species, but they differed in all other parameters. *Fagus*, *Carpinus* and *Quercus* had the lowest values of P and K/(Mg+Ca) ratio together with the highest fibre content. Highly favourable concentrations of nutrients for livestock were recorded in *Fraxinus*, *Ulmus* and *Corylus*. Our results indicate that our ancestors' practice of woody species exploitation as leaf-hay for winter foddering followed the nutritive value of the species.

Keywords: agricultural history, leaf fodder, livestock feeding, Prehistory

Introduction

The practice of collecting leaves and twigs for domestic animals is probably the oldest form of fodder harvesting. Leaf-hay fodder played a significant role in feeding livestock especially during the wintertime since at least the late Neolithic (3000 BC) and has been widely used in the course of time. The first written records come from Roman authors, for instance Marcus Porcius Cato (234 – 149 BC, *De Agricultura*) or Lucius Junius Moderatus Columella (4 AD (?) – 70 AD, *De Re Rustica*) (Ash, 1941). Collecting of leaf-hay can be very efficient without use of special tools such as scythes or sickles. There are three main techniques for collecting leaf fodder: 1) pollarding, which consists of cutting off the top of the tree and harvesting the shoots; 2) shredding, which leaves the trunk and crown intact and the side branches are removed; and 3) coppicing based on cutting suckers or young shoots directly from the tree base.

Collecting of leaf hay has been widely practised over the whole of Europe, from Greece (Halstead, 1998) to Great Britain (Smith, 2010) and from Italy and France (Haas *et al.*, 1998) to Scandinavia (Slotte, 2001). In each region, different tree species were used in compliance with regional vegetation and tree species availability. Many archaeological records confirmed year-round leaf-hay foddering of cattle, sheep and goats in stables in the Alps by ash (*Fraxinus excelsior*), lime (*Tilia* spp.), hazel (*Corylus avellana*) or fir (*Abies alba*) (Rasmussen, 1989). In the northern part of Europe, exploited species were ash, alder (*Alnus glutinosa*), birch (*Betula* spp.), lime, poplar (*Populus* spp.) and several coniferous species, primarily Scots pine (*Pinus sylvestris*) or juniper (*Juniperus communis*) (Austad, 1988). However, the most important tree species for leaf-hay over Europe, considered of a high nutritive value, was the elm (*Ulmus* spp.). Consequently, the elm was suggested having been selectively harvested by Neolithic humans and this could have led to the marked general decline in elm recorded in pollen analyses. This theory was formulated by Iversen (1941) as the elm decline theory. On the other hand, hornbeam (*Carpinus betulus*) was not exploited to such an extent due to it being considered of low nutritive value (Halstead, 1998).

The aim of our investigation was to test whether selected main European broad-leaved woody species differ in the content of macro-elements, in terms of their nutritional value for livestock feeding, and consequently whether their use could follow the pattern of their nutritive value.

Materials and methods

Acer platanoides, Carpinus betulus, *Corylus avellana*, *Fagus sylavtica, Fraxinus excelsior*, *Quercus robur, Populus tremula*, *Tilia cordata* and *Ulmus glabra* were the selected species. We collected leaf biomass of each species from three individuals on three localities in the Czech Republic in late May 2012. A total of 36 samples were used to analyse the concentration of macro-elements and neutral- and acid- detergent fibre fractions (NDF and ADF). The total N concentration was determined by combustion using an automated analyser TruSpec (LECO Corporation, USA). P, K, Ca and Mg were determined after a microwave oven mineralization of the biomass sample mixed with HNO_3 and HCl in ratio 6:1 using ICP–OES (Varian VistaPro, Mulgrave, Vic., Australia). NDF and ADF contents were analysed by standard methods of AOAC. One-way ANOVAs followed by post-hoc comparison using the Tukey's multiple range tests were used to evaluate the obtained data.

Results and discussion

All woody species showed similar and relatively high concentrations of N, and were comparable to *Lolium perenne* or *Trifolium repens* and much higher than that of meadow hay and the requirements of dairy cattle (Table 1). This was due to the date of leaf-sample collection in the spring at the time of flushing. Nutritive value of woody species differed, however, in other macro-elements and K/(Ca+Mg) ratio (all P<0.001). P concentrations ranged from 1.97 to 3.22 g kg^{-1} and were thus lower than values recorded in *L. perenne*, *T. repens* or meadow hay and at the lower limit of, or below values of, diet requirements for cattle. K/(Ca+Mg) ratio, which should be 1:2.2 and narrower, showed balanced concentrations of K, Ca and Mg and leaf-hay does not present any risk of 'grass tetany' (Wilson *et al.,* 1969). Concentrations of NDF and ADF were relatively high in comparison with meadow hay and were higher than values recommended for lactating and dry cows. Such high concentrations of NDF and ADF may reduce forage intake and consequently animal growth or milk production.

The lowest values of P and the highest content of NDF and ADF were found in *Fagus*, *Carpinus* and *Quercus*, while the most favourable values of macro-elements were found in *Fraxinus*, *Ulmus* and *Corylus*. Three latter species were the most preferred species in the past (Rasmussen, 1989). In Norway, leaves of *Ulmus* and *Fraxinus* together with *Salix* and *Sorbus* have been used preferentially for milking cows and calves till the present (Austad, 1988) and their nutritional value was comparable to that of meadow hay. *Fagus*, *Carpinus* and *Quercus*, with their low nutritional values, were not reported in archaeological records in Central Europe as potential leaf-hay fodder for domestic animals; they were, however, used in other regions within different vegetation context, particularly in mountainous areas of Mediterranean regions (Haas *et al.,* 1998; Halstead, 1998). There was no difference in nutrient concentrations in leaves among localities.

Table 1. Concentration (means ± standard error of mean, n = 4) of nitrogen (N), phosphorus (P), neutral detergent fibre (NDF), acid detergent fibre (ADF) and K/(Mg+Ca) ratio in leaves of woody species. Differences (P<0.01) between tree species are denoted with different letters. Values for *Lolium perenne*, *Trifolium repens*, meadow hay and optimal value for cattle are from Kudrna (1998) and Whitehead (1995).

Plant species	N (g/kg)	P (g/kg)	NDF (g/kg)	ADF (g/kg)	/(Mg+Ca)
Acer platanoides	28.2 ± 1.2 [a]	2.7 ± 0.3[ab]	399.5 ± 24.3 [a]	313.2 ± 6.3[ab]	18 ± 0.07 [ab]
Carpinus betulus	30.7 ± 0.6[a]	1.9 ± 0.1[a]	437.0 ± 16.4 [a]	272.7 ± 10.1[ad]	69 ± 0.04 [a]
Corylus avellana	31.5 ± 1.4 [a]	2.3 ± 0.1[ab]	476.2 ± 23.5 [a]	342.2 ± 13.4[bc]	79 ± 0.06 [a]
Fagus sylvatica	31.4 ± 2.9 [a]	2.1 ± 0.2[a]	662.5 ± 11.6 [b]	480.0 ± 7.0[e]	71 ± 0.05 [a]
Fraxinus excelsior	33.2 ± 2.1 [a]	3.2 ± 0.2[b]	511.7 ± 77.8 [ab]	400.5 ± 13.9[c]	99 ± 0.05 [ab]
Populus tremula	28.9 ± 1.0 [a]	2.6 ± 0.2[ab]	452.0 ± 27.5 [a]	373.7 ± 24.9[c]	01 ± 0.09 [ab]
Quercus robur	32.9 ± 0.9 [a]	2.5 ± 0.1[ab]	449.5 ± 10.9 [a]	306.2 ± 5.3[ab]	97 ± 0.03 [ab]
Tilia cordata	36.0 ± 1.2[a]	3.0 ± 0.5[ab]	409.2 ± 21.4[a]	304.7 ± 7.0[ab]	46 ± 0.25 [b]
Ulmus glabra	34.3 ± 3.3 [a]	2.7 ± 0.1 [ab]	396.5 ± 41.2 [a]	243.0 ± 12.0[d]	18 ± 0.08 [ab]
Lolium perenne	20 - 35	3 - 6	-	-	
Trifolium repens	35 - 52	3 - 6	-	-	
Meadow hay	20.0 - 28.7	3.2 - 3.7	-	427 - 503	
Optimal values for cattle	19.2 - 25.6	2.3 - 3.7	330 - 450	190 - 300	

Conclusion

Our results suggest that our ancestors' practice of tree species exploitation for leaf-hay fodder in the past was not based only on their availability, but it followed the nutritional value of the harvested woody species within the vegetation context. The nutritional value of the investigated woody species, however, does not reach the level of dietary requirements of contemporary dairy cattle for their satisfactory performance.

Acknowledgement

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Environmental impacts of beef production systems (bull fattening and suckler cows) in different countries

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Abstract

Beef fattening systems can play an important role in maintaining and promoting grasslands. However, the effects of beef production on the environment have become a topic of great concern. Using the LCA-method SALCA, we analysed six different beef production systems: conventional bull fattening based on feeds from arable land (Germany, Switzerland), organic bull fattening based on grassland (Switzerland), grassland-based organic and conventional suckler cow systems (Switzerland) and extensive pasture based fattening (Brazil). The potential impacts on biodiversity were also assessed for the Swiss systems. The suckler cow systems had higher environmental impacts per kg liveweight in most categories. This was because no milk was produced and all environmental impacts were allocated to meat production, whereas in the other systems there was also an allocation to the co-produced milk. Still, they used less arable land and had lower impacts regarding deforestation and ecotoxicity. A trade-off between productivity and biodiversity was observed. Improvements regarding the overall system efficiency and a clear definition of the objectives of the particular production system are required.

Keywords: beef, life cycle assessment, biodiversity, arable land, grassland

Introduction

Beef production systems play an important role in maintaining and promoting grasslands, which occupy 70% of the Swiss agricultural area. However, the effects of meat production on the environment have become a topic of great concern. Beef production is under pressure, as life cycle assessment (LCA) studies show that impacts of beef are much higher than pork, poultry meat or milk (de Vries and de Boer, 2010). The present study was initiated to identify hot-spots in the beef supply chain and to determine reduction potentials for environmental impacts. A special focus was given to the use of arable land and grassland, the different levels of management intensity and their implications for biodiversity.

Materials and methods

By using the method SALCA (Swiss Agricultural Life Cycle Aseessment; Nemecek *et al.*, 2010), we analysed six different beef production systems: conventional bull fattening (Germany, Switzerland), organic bull fattening (Switzerland) and conventional and organic suckler cow systems (both Switzerland) and extensive pasture-based fattening (Brazil). The data for Switzerland came from model farms (Hersener *et al.*, 2010), those for Germany and Brazil from literature (details see Alig *et al.*, 2012). In addition, the potential impacts on biodiversity were assessed for the Swiss systems applying the SALCA-biodiversity method (Jeanneret *et al.*, 2008). The full results are reported by Alig *et al.* (2012).

Results and discussion

Two fundamentally different beef production strategies exist: bull fattening from dairy calves and suckler cow systems. The former is characterized by a relatively high production intensity (with the exception of the organic system), and it relies largely on concentrate feed and use of silage maize, and has therefore a high demand for arable land. Since the calves stem from milk production, most of the environmental burdens of the milk cows are allocated to milk production (economic allocation). In contrast, suckler cow systems produce only beef, to which all environmental burdens of the mother cows are allocated. Much more feed is thus used to produce 1 kg of beef and more emissions from animal husbandry occur (Figure 1).

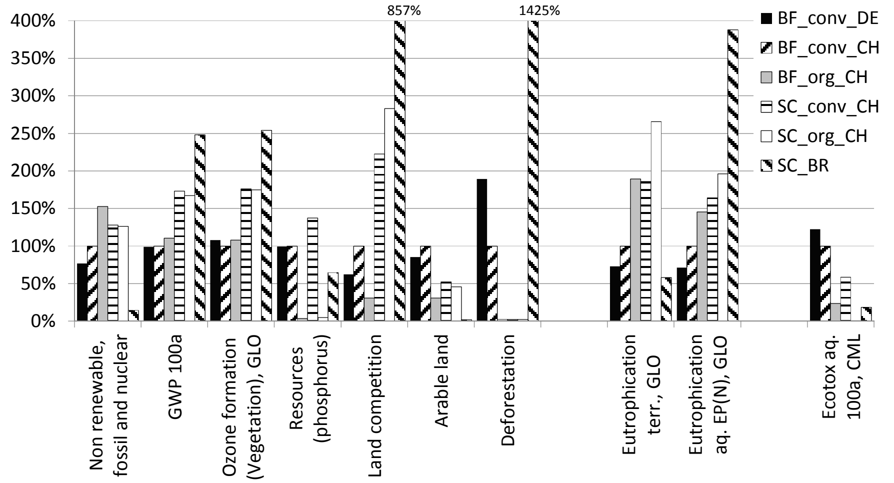

Figure 1. Selected environmental impacts per kg liveweight at the farm gate of six beef fattening systems: BF_conv_DE/BF_conv_CH: conventional bull fattening Germany/Switzerland; BF_org_CH: organic bull fattening system Switzerland; SC_conv_CH/SC_org_CH: conventional/organic suckler cow system Switzerland; SC_BR: suckler cow system Brazil. 100% = BF_conv_CH.

On the other hand, the Swiss suckler cow systems used less concentrate than conventional bull fattening, and therefore fewer mineral resources (P and K), and they had lower ecotoxicity and deforestation impacts, and lower use of arable land.

The environmental impacts of the bull fattening system in Germany were similar to those of the Swiss system. Higher impacts in the German system were observed for ecotoxicity and deforestation, due to the higher share of concentrate feed and silage maize in the diet. The energy demand, eutrophication and acidification were slightly lower in Germany, since feeding there is based mainly on silage maize and concentrates, whereas in Switzerland an important share of the feed is supplied by grass.

The Brazilian beef production is a low-input system leading to a very low energy demand, low use of mineral resources, and very low ecotoxicity. On the other hand, the fattening periods are long and the feed conversion is poor, which, combined with low pasture yields, led to 10-times higher land occupation than in the Swiss bull-fattening system. Therefore, impacts related to emissions from animal husbandry and to the use of land were very high. Furthermore, the system has also led to substantial deforestation.

The conflict between intensive production and the preservation of species diversity was apparent in the biodiversity analysis for the Swiss beef-production systems (Figure 2). The conventional bull fattening system used two to three times less area to produce 1 kg of beef compared to the suckler cow systems.

The overall species diversity was estimated to be lowest for the conventional beef fattening and higher for the suckler cow systems (Figure 2), due to generally higher species diversity in grassland than in crops. In particular less intensively and extensively managed grassland had higher scores for overall species diversity. However, the difference between suckler cows and bull fattening is reduced due to the fact that the calves and the milk powder in the latter stem from dairy farms, which are mainly grassland-based.

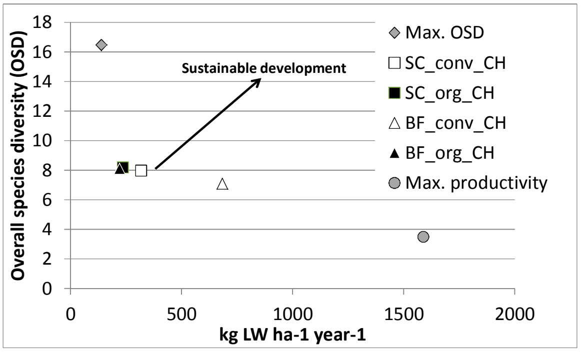

Figure 2. Comparison of overall species diversity (OSD) of four Swiss beef production systems (see Figure 1 for the abbreviations).

Two scenarios have been calculated to estimate the theoretical potentials for biodiversity and productivity of beef fattening: one with maximal biodiversity potential (Max. OSD), thanks to very extensive management, the other with maximal productivity (Max. productivity, see Figure 2). The large differences observerd indicate that the current beef production systems have a high potential for development both in terms of higher productivity or higher biodiversity. Research is needed to develop beef fattening systems which better reconcile a good productivity with a high level of biodiversity.

Conclusions

Three main factors determine the environmental impacts of beef production systems: 1) the design of the system (bull fattening vs. suckler cows), 2) its efficiency, and 3) the composition of the feed and feedstuff production. On the one hand, suckler cow systems rely on grassland and have advantages that are related to the low use of concentrate feedstuffs (less ecotoxicity, deforestation, and use of mineral resources). Furthermore, the biodiversity potential on the managed areas is higher. On the other hand, the mother cow has to be fully attributed to beef production, since no milk is sold, leading to high impacts in several categories. A combination of milk production with bull fattening, based on grassland could be a valuable alternative to make best used of the available grassland.

Acknowledgement

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Poster Presentations

Farming systems in mountain regions of NE Portugal: conversion from conventional production to organic production

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Abstract

The energy efficiency of a mountain mixed-farming system in NE Portugal was analysed for the period 2002-2003. The energy calculation included the energy of all inputs (fertilizers, fuels, concentrates for cattle) on farm production and the energy of outputs (bovine meat). The energy values were calculated by multiplying the quantities of inputs, indoor system production and outputs by their energy content. The efficiency of the farm (output/input) was 0.12. The results showed a low efficiency of the farm. However, these results were not due to the high inputs required by the farm (as in intensive systems) but it was due to the low use of available feed.

Keywords: agro ecosystems, sustainability, beef cattle

Introduction

The livestock sector has expanded rapidly in recent decades and demand for livestock products is expected to continue to grow strongly through the middle of this century, driven by population growth, rising affluence and urbanization (FAO, 2009). Decisive action is required to satisfy this growth in ways that support society's goals for poverty reduction and food security, environmental sustainability and improved human health (FAO, 2009). The agricultural landscape in the NE of Portugal is characterized by a pattern of small, fragmented farms that traditionally produced food mainly for family consumption. At the beginning of the 20th century, these farming systems integrated agriculture (mainly cultivation of cereals and potato) and livestock grazing into common long-term fallows, stubbles and rangelands, together with the private farm pasture areas. The aim of this study was to evaluate the energy efficiency of a farm in the highlands of NE of Portugal, as representative of the mixed-farming systems in these regions, and also to evaluate its suitability for organic animal production.

Material and methods

The farm was located in Salto, Montalegre region (NE Portugal), and was monitored and studied during 2002-03, recording all activities as well as the inputs, farm production and outputs. The altitude is around 950 m a.s.l., with annual precipitation and annual average temperature of 1455 mm and 9.9°C, respectively. The farm had an area of 35.8 ha divided into 33 fields: *meadows* (15 fields, 22.6 ha) used for grazing and hay cut (spring), *forage* that included maize, rye; and some vegetable gardens (7 fields, 4.15 ha), *shrubs and forest* (9 fields, 7.8 ha) used only for grazing and *chestnut* (2 fields, 1.3 ha). The farm produced beef cattle of the 'Barrosã' local breed with a stocking rate of 0.52 LU ha^{-1}. Livestock total live weight was 8942 kg in the base year 2002. Summer grazing on pasture was for approximately

231 days, about 8 h day^{-1}. Winter grazing was on meadows, and on rye forage. Meadow hay was part of the daily diet of adult cattle from October to April, and of calves throughout the year. Concentrates were also part of the diet of calves from 2-3 months until 6-8 months old, when they were sold. Maize complemented the diet from September to October (38 days). At the end of summer, land was ploughed and sown with rye for forage and part for grain. Farmyard manure was spread on meadows in autumn (28 Mg ha^{-1}), and before planting potatoes (vegetable garden) and sowing maize (forage) (152 Mg ha^{-1}) in spring, together with mineral N, P and K fertilizers. In order to determine the on-farm production (meadows for grazing and hay, rye and maize for forage, shrubs and forests) the following homogeneous areas were identified: two types of hay meadows, two types of grazed shrubs and forests areas, and three types of annual crop areas (for rye, maize and potatoes). In each area three enclosure cages were randomly distributed in all grazed crops. The samples were harvested inside the enclosure cages (0.25 m^2), at the beginning of spring, at the hay cut (June/July), at the end of summer and at the end of autumn. All samples were dried to constant weight at 60ºC (48 h) for yield determination. Maize yields were obtained by sampling (0.25 m^2), at the time of forage cutting during the growing season. All the remaining data were obtained by the farmer. The energy efficiency that describes the relationship between the energy outputs of a system and energy inputs needed to operate the system (Mikkola and Ahokas, 2009) was estimated (Figure 1). The energy values were calculated multiplying the quantities of inputs, productions inside the system and outputs, by the energy content values referred by Gliessman (2007) for fertilizers, farmyard manure and concentrates, by Demarquilly *et al.* (1980) for meadows and forage, by Leme *et al.* (2000) for meat and by Bayliss-Smith (1982) for fuels and machinery (including maintenance). There was no human labour from outside the system.

Results and discussion

From the flow diagram of this system (Figure 1), the following results were found: i) despite being a traditional mixed-farming system (Moreira, 1981), the output of vegetal component in this farm was nil (Figure 1) and therefore the animal component (bovine meat: 9986 MJ) was the only output from the farm; ii) there were reduced inputs, compared with previous studies (Kainz, 2005 and Funes-Monzote *et al.*, 2009), and outputs (bovine meat); iii) high importance of farmyard manure inside the system, to which the shrub litter used is indispensable (70-90 t ha^{-1} yr^{-1}); iv) low system efficiency (0.12), as a result of low outputs (only meat), since the inputs cannot be responsible for this value; v) this efficiency is explained by the low stocking rate (0.52 LU ha^{-1}), since it could be raised 2 LU ha^{-1}, the maximum allowed in organic farming; and vi) considering the amount of feed produced inside the system converted into meat using the efficiency of 3% (Spedding, 1979), the system efficiency could potentially be raised up to 1.83, a value near to that obtained by Intxaurrandieta and Arandia (2008) for livestock production in organic farming (2.19). Nevertheless, Spedding (1979) refers an efficiency of 0.18 for bovine meat production systems, when considering all the inputs into the system that applied in this case study.

Conclusions

The low efficiency of the farm is a result of the low stocking rate, which was not adjusted to the farm production (pasture and forage), and to there being no output of crops. The risk of unsustainability of this farm is only due to a low efficiency in the use of the forage and pasture resources. This farm is perfectly suited to organic farming, paying attention to the low inputs and local breed used.

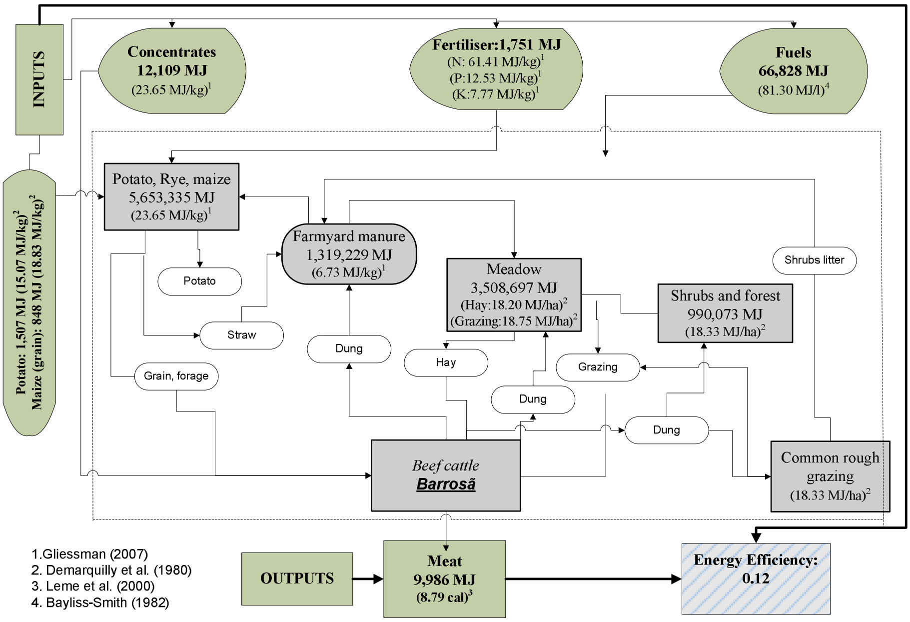

Figure 1. Flow diagram of the farm.

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The potential for improving the economic and environmental sustainability of dairy farming via farm diversification

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Abstract

The DAIRYMAN INTERREG project aims to enhance the environmental and economic sustainability of dairying in north-west (NW) Europe by improving the competitiveness and ecological performance of dairy farming. Accordingly, between 2009 and 2011, economic and environmental information (e.g. nitrogen and phosphorus balances) was collected from a network of 116 pilot dairy farms in 10 regions of NW Europe in order to evaluate and compare farm performances. For comparative purposes, five farm types were identified, including specialized dairy farms and mixed dairy farms with suckler-beef production, or commercial cropping, or both. In general, the production of commercial crops in mixed dairy farming systems significantly decreased farm N balances compared to specialized grassland-based dairy farms, whereas specialized dairy farms had the best economic performance. However, as a number of individual farms within both specialized and mixed farming typologies were able to operate with low N surpluses and high incomes, opportunities may exist for simultaneously improving the economic and environmental sustainability of dairy farming in both diversified and non-diversified farming situations.

Keywords: DAIRYMAN, dairy system, N and P balances, mixed farming systems, economy, environmental efficiency

Introduction

Dairy farming is an important economic activity in north-west (NW) Europe. Unfortunately, inefficiencies in the use of fertilizers, feeds and energy for dairy production can hamper the delivery of key environmental services such as clean water, clean air and biodiversity. Dairy farmers are also coping with the financial crisis in the Euro-zone, milk price volatility, high investment costs and narrow profit margins. The sustainability of dairying is therefore under threat environmentally and economically. Efforts are needed to improve resource management on dairy farms in order to lower external inputs, thereby minimizing costs and environmental impacts. One way of accomplishing this might be to diversify dairy farming systems to include crops and other livestock enterprises, since this may facilitate more internal recycling of resources, e.g. nutrients, and reduce the environmental impact of the farm (de Haan *et al.,* 1997; Oomen *et al*., 1998). Mixed farming systems, however, may require greater inputs of labour, equipment, buildings and land etc, than specialized farms, making them more expensive. The aim of this study, therefore, was to compare the economic and environmental performances of mixed dairy farming systems and specialized dairy farms, using data from a large network of dairy farms, to assess whether farm diversification has potential to improve the sustainability of dairy production.

Materials and methods

DAIRYMAN is a project funded under the INTERREG IV B NW Europe programme. It involves 14 partner organizations from the Netherlands, France, Germany, Luxembourg, Ireland, the United Kingdom and Belgium, and has a network of some 128 pilot dairy farms. Over a three-year period, 2009-2011, data were collected from the pilot farms and compiled to produce two standardized databases. The farm characteristics database contains information and data on farm structure (size of herds, land use, etc.), and the economics database contains information on sources of revenue (milk, animals, crops and subsidies), operating cost (related to herds, grassland, crops, building, and management), depreciation, and interest and taxes. Proportional Costs (sum of all input costs), Incomes (revenues minus input costs minus depreciation) and Inputs Efficiency *[In Eff]* (Revenues/Proportional) were evaluated for 116 of the 128 farms (for which economic data were available), as measures of economic performance. Mineral balances (kg of N and P balance ha^{-1}) and N *[N Eff]* and P *[P Eff]* efficiencies (ratio between output and input of nutrients at farm scale) were also calculated for the 116 pilot farms, as measures of environmental performance.

Five farm types were identified: (DS) Dairy-Suckler system, where >15% of animals were suckling cows; (DC) Dairy-Crops system, where >20% of utilizable area was used for commercial crops; (DSC) Dairy-Suckler-Crops system, where >15% of animals were suckling cows and >20% of utilizable area was used for commercial crops; (DG) Dairy Specialized system – Grassland, with <10% of forage maize in the fodder surface; and (DM), Dairy Specialized system – Maize, with >10% forage maize in the fodder surface. The economic and environmental performances of these different types were compared using non-parametric tests: the Kruskal-Wallis (comparison of mean ranks) and Mann-Whitney (structuration of means) (Jost, 2012) with the logiciel R (© 2011, The R Foundation for Statistical Computing).

Results and discussion

Table 1 shows the means and standard deviations obtained for the different environmental and economical indicators according to farm typology and the results of statistical comparisons. The level of specialization significantly influenced all indicators except In Eff and P Eff.

Table 1. Economic and environmental results according to typology (2010) - highest values in bold and lowest in bold italics.

Level of specialization	Dairy specialized		Dairy/Suckler	Dairy/Crops	Dairy/Suckler/Crops	*P* value
	Grassland	Maize				
Occurrence	49	19	7	33	8	
Economic results (1000€ ha^{-1})						
Revenue	4.4 ± 1.9	**7.1 ± 3.1**	3.2 ± 1.3	3.8 ± 1.6	***3.1 ± 0.4***	< 0.001 (***)
Income	1.7 ± 0.7	**2.0 ± 1.3**	1.1 ± 0.6	1.2 ±0.8	***0.9 ± 0.4***	< 0.001 (***)
Proportional Costs	1.4 ± 0.8	**2.4 ± 1.4**	1.2 ± 0.6	1.3 ± 0.8	***0.9 ± 0.2***	0.003 (**)
Inputs Efficiency (%)	3.6 ± 1.2	3.2 ± 0.8	2.9 ± 0.6	3.4 ± 1.1	3.4 ± 0.6	0.280 (ns)
Environmental results (kg of nutrient ha^{-1})						
N Balance	**182.3 ± 73.1**	**190.4 ± 61.1**	178.2 ± 89.1	***147.2 ± 73.2***	***130.6 ± 37.1***	0.015 (*)
N Efficiency (%)	28.3 ± 10.1	34.6 ± 9.1	***22.8 ± 7.0***	**37.0 ± 11.0**	**38.4 ± 11.3**	< 0.001 (***)
P Balance	5.7 ± 9.8	7.6 ± 9.1	***13.3 ± 9.2***	7.8 ± 13.8	***-0.6 ± 8.0***	0.034 (*)
P Efficiency (%)	100.2 ± 87.2	***77.1 ± 43.5***	80.6 ± 107.0	78.4 ± 36.3	**122.3 ± 72.6**	0.053 (ns)

Dairy specialized systems, and in particular dairy specialized systems with maize, provided higher incomes per hectare than mixed farming systems. On the other hand, mixed farming systems with cash crops performed best from an environmental perspective, with lowest N balances and highest N efficiencies. At first glance, therefore, it would seem that farm types giving good economic returns automatically have poor environmental performances, and vice versa. However, as shown in Figure 1, the relationship between N balance and farm income is highly variable, implying that there is scope within individual farm types to manage resources in such a way as to optimize both economic and environmental performances. However, performances observed can also be affected or enhanced by geo-political contexts and pedo-climatic conditions that differ between regions and lead to different potentials of production.

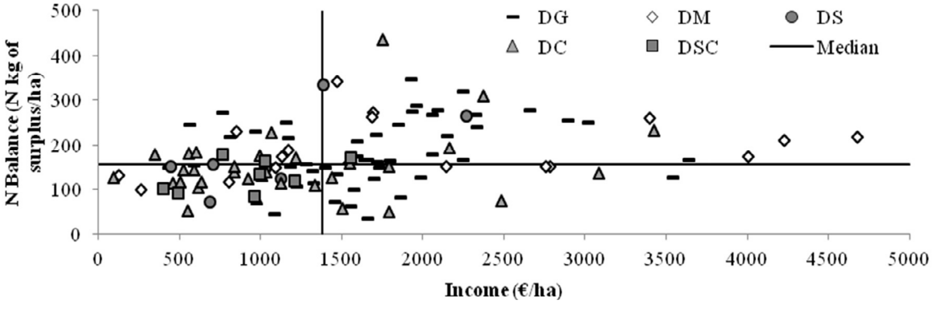

Figure 2. Plot of farm N balances versus farm income for 116 DAIRYMAN pilot farms (2010 data), with different symbols representing the 5 different farm types, and median values of N balance and farm income shown with black lines.

Conclusions

In general, dairy specialized systems provided higher levels of farm income per hectare than mixed farming systems, whereas mixed farming systems with cash crops had potentially the lowest environmental impact. However, since a number of individual farms within both specialized and mixed farming typologies were able to operate with low N surpluses plus high farm incomes, it would appear that opportunities for improving the sustainability of dairy farming exist for both diversified and non-diversified farming systems. The challenge now is to identify those farm structural characteristics and management practices within specific farm typologies that were responsible for facilitating the concurrent optimization of economic and environmental performances.

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A farmer's view on climate change in the North German Plain

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Abstract

Predicted climate change will affect grassland farming in Europe in various ways. Likely effects depend on current climate, soil conditions and farm management. A range of adaptation measures to the effects of climate change has been identified. However, an effective response to these challenges would include a requirement that the farmers were both knowledgeable about, and also accepted the idea of, climate change. Therefore, we need to know how grassland farmers perceive and anticipate postulated future climate change and whether their attitude is influenced by factors like, among others, region, farm size and socio-cultural background. To answer these questions, we conducted a survey with extensive on-farm interviews (n = 82) in four distinctive regions of the North German Plain on a gradient from more maritime to less-favoured areas with more continental weather. We found that with more continental climate and less rainfall and with increasing farm size, grassland farmers were more aware of the implications of climate change. However, some aspects of the farmers' socio-cultural background seemed to determine their views on climate change more strongly. More traditional and conservative farmers tended to be more critical of the idea of climate change compared to their future-oriented colleagues.

Keywords: climate change, farmers' survey, farming styles

Introduction

Climate change will likely have adverse impacts on grassland farming in numerous ways. Increased mean temperatures and CO_2-concentrations will probably lead to extended growth periods, water shortages and rainfall extremes during the growing season (Hopkins and Del Prado, 2007). A range of adaptation measures to the effects of climate change has been identified but, in practice, it is the local farmer who decides to opt for possible climate adaptations or not. Hence, understanding farmers' knowledge and attitudes on this subject is of importance, since passivity and ignorance mean rejecting these challenges. Apart from pure meteorological factors, other aspects like farmers' age, education and the way of farming (cf. 'farming styles', McRae-Williams, 2009) might have an influence on farmers' acceptance of the idea of climate change. We hypothesized that farmers' assessments of climate-change risks may differ according to their (agricultural) education, age, farm size, socio-cultural background, and, particularly, region in the North German Plain.

Material and methods

An on-farm survey (n = 82) was conducted in 2011 in four distinctive regions (from west to east: Diepholz n = 20, Uelzen n = 20, Fläming n = 21, Oder-Spree n = 21) in order to cover the sub-maritime and sub-continental climates of the North German Plain.
Face-to-face investigative interviews were conducted using a standardized questionnaire. The interviewer directly transcribed the answers given orally by the farmer. The questionnaire was divided into two major sections: In the first part, farmers' data, the farm business structure and

grassland management were collected. In the second part, their degree of agreement with a set of statements (climate change and nature- or environmental protection issues) were obtained with a five-point Likert scale (1 = totally agree, 5 = totally disagree). Precipitation and temperature data of the four regions are shown below (Table 1). All statistical tests were performed with the software R! Version 2.15.1

Table 1. Meteorological data of the four regions for the summer period (April-September); temperature (T), average rainfall as the long-term average (1961-1990) and as expected for 2041-2050 (Orlowsky *et al*., 2008).

		Diepholz	Uelzen	Fläming	Oder-Spree
1961-1990	Rainfall (mm)	355	373	302	315
2041-2050	Rainfall (mm)	319	311	272	264
1961-1990	T (°C)	14.0	13.6	14.8	14.7
2041-2050	T (°C)	16.2	15.8	17.0	17.0

Results

As shown in Figure 1, climate change has been recognized by two-thirds of the farmers as an evident key factor for their current and future land management. Twelve farmers (15%) totally denied the statement below, and 15 (18%) indicated this was an unlikely scenario.

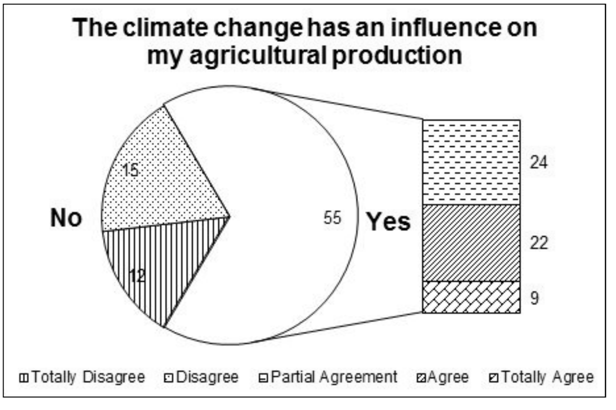

Figure 1. Total responses (n = 82) to the question: Does climate change have an influence on my actual agricultural production?

Table 2. Mean, standard deviation (s.d.) and farmers (n) per cluster of the factors region, farm size, optimist and traditionalist. High means (5) express high climate-change awareness, low (1) for a rejection of the idea. The items for optimist and traditionalist had been aggregated in accordance with the concept of 'faming styles' (Van der Ploeg, 1994). According to their answers, farmers were allocated to 5 classes describing their personal attitudes (absolute optimist/traditionalist to no optimist/traditionalist at all).

Region (*P*=0.074)	Mean	s.d.	n	Farm Size (*P*=0.081)	Mean	s.d.	n
Diepholz	2.65	1.18	20	Small (<50 ha)	2.71	1.07	15
Uelzen	2.80	0.83	20	Medium (51-250 ha)	2.31	1.20	42
Fläming	2.71	1.35	21	Large (251-1250 ha)	3.22	1.16	11
Oder-Spree	3.52	1.33	21	Huge (>1250 ha)	3.10	1.33	14
Optimist (*P*<0.001)	**Mean**	**s.d.**	**n**	**Traditionalist (*P*<0.001)**	**Mean**	**s.d.**	**n**
Not at all	3.50	1.50	2	Not at all	3.14	1.29	22
Hardly	4.00	0.00	2	Hardly	2.88	1.02	17
Restricted	2.92	0.95	12	Restricted	3.05	1.10	19
Definitely	3.05	1.18	37	Definitely	3.00	1.27	15
Absolutely	2.61	1.29	28	Absolutely	1.71	1.03	7

Farmers in the Oder-Spree district, characterized as sub-continental, showed a more distinct awareness of climate change (3.52) than their colleagues in the sub-maritime west (2.65, Diepholz) (Table 2).
The factors *education* (P=0.539) and *farm type* (P=0.375) had no significant influence on farmers' attitude towards climate change risks (not shown). The key determinant (P<0.001) was the socio-cultural background, here represented by the 'traditional farming style' and the personality, for example a positive outlook on life.

Discussion

The geographical influence, represented by the east-west gradient (increase in precipitation) was not as important as we assumed, but a trend (P=0.074) definitely exists. The last two years before the interview, with more rainfall than average especially in the Fläming and Oder-Spree regions, might have influenced the farmers' attitude. It was surprising to find that the factors *age* and *education* did not have an effect, despite the idea that young and qualified farmers are used to new information technologies and hence might be better informed about the on-going scientific discussion on climate change (Burger-Scheidlin *et al.*, 2009). In accordance with McRae-Williams (2009) traditionalists and more conservative farmers perceive climate change and its negative effects significantly more critically, and refer more to the natural climate variability than their future-orientated colleagues. On the other hand, we found that a positive attitude to life ('optimist') also increased the tendency to ignore the negative climate-induced changes such as drought damages and yield losses.

Conclusion

We found significant differences among farmers in their perception of climate change impacts and their willingness to consider and implement adaptation measures on their own farms. Moreover, the awareness of a farmer towards climate change seems to depend less on external factors such as farm size and type, meteorological factors or educational background, but is mainly determined by socio-cultural influences such as family background and personality.

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Evaluation of greenhouse gas budget of Icelandic *Grassland*

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Abstract

Emissions and removal of greenhouse gases (GHG) is an important factor in determining sustainability of management and land-use practice. Land-use related GHG emission is also one of the factors reported to the UN-Framework Convention on Climate Change. Here we describe the present status of the estimates for GHG emissions and removals for the land-use category *Grassland* in Iceland. This category is further divided into five subcategories based on land cover, land-use and management activities. Within these categories the evaluation of GHG budget is aggregated according to soil types, carbon pools and time series of management activities. The present estimate is that removal of CO_2 exceeded emission of CO_2 by 134 Gg CO_2 based on extent of land use in the year 2010. The estimated N_2O emission of 155 Gg CO_2 equivalents (eq) exceeds this removal, resulting in 21 Gg CO_2 eq as net emission from Icelandic *Grassland*.

Keywords: grassland, land-use, greenhouse gas, carbon pools

Introduction

Land-use and management can have variable impacts on carbon stocks and greenhouse gas (GHG) emissions (Bolin *et al*., 2000). To plan sustainable land-use and management, it is important to identify clearly all associated costs and benefits. All parties of the UN Framework Convention on Climate Change (UN-FCCC) are obliged to report anthropogenic emissions and removal of GHG regularly, including those associated with variable land-use. These reporting obligations led, in 2007, to the initiation of a project at the Agricultural University of Iceland (AUI) with the aim of evaluating land-use related emissions of all land not evaluated by other institutions. The evaluation of land-use related GHG emissions and removals is accordingly highly influenced by the methodology and categorization recommended by the convention. Two main approaches are recognized in the guidelines of the convention (IPCC, 2006). One approach is to estimate stock changes of defined carbon pools associated with certain land-use, land-use changes and management. The other is to evaluate the GHG fluxes related to the land-use and management. To calculate GHG emissions and removals associated with land-use, information on both the area under relevant land-use and the emission or removal per unit land is needed. In the approach of the AUI emphasis is placed on estimating the area of each land-use category defined and the size of associated carbon stocks. The Icelandic land-use category *Grassland* is defined in the UN-FCCC reporting as all land with >20% coverage of vascular plants, and not included under categories *Forest land, Cropland, Wetland* or *Settlement*. The area of *Grassland* so defined in Iceland is estimated 53,000 km^2 or 51% of the country. In this paper we report the observed carbon stocks on *Grassland* and summarize present estimates of GHG emission and removal.

Materials and methods

The information on area of *Grassland* subcategories (Table 1) is obtained from the National inventory report to the convention (Environmental Agency of Iceland, 2012). Carbon stock changes of subcategories *Revegetated land* and *Natural birch shrubland* were estimated by the Soil Conservation Service of Iceland (SCSI) and the Icelandic Forest Research (IFR) respectively, and will not be described further in this paper (Environmental Agency of Iceland, 2012). Carbon stocks of the subcategory *Other Grassland* are assumed stable as no changes in management are recorded. The CO_2 emissions from organic soils are estimated by applying emission factors (EF) 25 t C km^{-2} and 262.5 t C km^{-2} for *Drained Grassland* (IPCC, 2006) and *Abandoned Cropland* respectively. The EF for *Abandoned Cropland* is the average of EF for cropland and grassland organic soils (IPCC, 2006). The N_2O emission from organic soils is estimated from available EFs: 0.044 t N_2O-N km^{-2} and 0.099 t N_2O-N km^{-2} for grassland and cropland, respectively (Gudmundsson, 2009). For organic soils of *Abandoned Cropland* in conversion the average 0.072 t N_2O-N/km^2 is applied. The changes in mineral soil and above ground carbon pool of *Abandoned Cropland* and above-ground carbon pool of *Drained Grassland* are estimated from the samples obtained in the AUI project. Two carbon pools were sampled, i.e. SOC 0-30 cm depth and total above-ground carbon in an area of 100 cm^2, including both above-ground living biomass and dead organic matter. Sampling points were selected from grid points as described in Gudmundsson *et al.* (2010). The C and N content of particles < 2mm in soil samples were analysed in a Vario Max CN Element Analyser (Elementar Analysen systeme GmbH). The SOC on 1 m^2 was calculated from C content using average bulk density values for each soil type (Arnalds, 2004) and coarse fragment ratio of soil samples. The samples of above-ground organic matter were dried and separated to particles ≥2mm and <2mm. The organic matter (OM) of particle ≥2mm was determined as dry weight but the organic matter of particles <2mm was determined by loss on ignition. The C content of OM was assumed as 50%.

Results and discussion

The area and emissions of the five land-use categories and their subdivision according to soil type and conversion status are summarized in Table 1. Taking into account subdivisions with no area estimated, this adds up to twenty carbon pools to be estimated. Of these, four are estimated as being in equilibrium with no emission or removal.

Table 1. Area and estimated emission/removals of GHG from grassland in Iceland. NO = no area; NA = not applicable; NE = not estimated. Negative values stand for removal from the atmosphere.

	Area			Emission (+) removal (−) [Gg yr^{-1}]			
Land use category	Total area [km^2]	Organic soils [km^2]	Under conversion (there of organic soil) [km^2]	Total (CO_2)	Soil (CO_2)	Above ground (CO_2)	N_2O (CO_2 eq)
Natural Birch Shrubland	299	NO	NO	−18	NE	−18*	NA
Other Grassland	46,314	NO	NO	NA			NA
Drained Wetlands	3,436	3,436	(280)	315	315	0	74
Revegetated Land	2,486	NO	2,469	−516	−464	−52	NA
Abandoned Cropland	424	139	235 (98)	85	136	−51	81
Total	52,957	3,575	2,985 (378)	−134	−13	−121	155

*Only wood biomass

According to the IFR forest inventory, birch shrubs increased their biomass by 16.5 t C km^{-2} in the year 2010. Growth and losses in the birch shrubs are the only carbon pool changes presently estimated for *Natural Birch Shrubland*. Other components of the above ground carbon pool are not included yet.

The category *Other Grassland* is assumed to be in equilibrium as regards carbon stocks. Considering that this category encompasses almost half of the country it is important to improve the estimate of its emission/removal. The category *Drained Wetland* represents wetlands converted to *Grassland*, of which 280 km^2 were drained in the 20-year period 1991-2010. The emissions from organic soils are estimated from EF for both CO_2 and N_2O. No difference was detected between samples of above-ground carbon pool for *Drained Organic Grassland* and *Wetland* and hence are estimated as not changed.

The area and changes in carbon pools of *Revegetated Land* are estimated by the SCSI through a national inventory on *Revegetated Land* (Environmental Agency of Iceland, 2012). No changes are assumed in carbon pools of revegetation older than sixty years. No organic soils are reported under this category. The biomass and dead organic matter are estimated by SCSI to increase by 5.7 t C km^{-2} annually, and organic matter in mineral soil by 51.3 t C km^{-2} annually (Environmental Agency of Iceland, 2012). Accordingly, 516 Gg CO_2 are removed from the atmosphere annually through revegetation activity.

All abandoned cropland is assumed to be converted to *Grassland*. The estimate for this category is the aggregate of the two carbon pools separated on the basis of conversion period, (20 years) and soil type. Changes in all above-ground carbon pools and SOC in mineral soil are assumed to be limited to the conversion period, while emission from organic soils is assumed to continue. The estimated emission from organic soils is 3.8 Gg CO_2 and 94 Gg CO_2 for cropland abandoned for more than 20 years and cropland still in conversion, respectively. Emission due to changes in SOC of mineral soils is calculated from difference in SOC of *Grassland* and *Cropland* mineral soil samples. The SOC is according to soil samples 11 070 ± 740 t C km^{-2}, and 9 530 ± 460 t C km^{-2} for *Cropland* (n = 30) and *Grassland* (n = 191) mineral soil respectively. The annual decrease of SOC in the conversion period is thus 77 t C km^{-2}, and the total emission 38.7 Gg CO_2. Similarly, annual change in above ground carbon pool of abandoned cropland is estimated as 59 t C km^{-2}, from the size of the carbon pools, 210 t C km^{-2} (IPCC, 2006) and 1,398 ± 122 t C km^{-2} respectively for *Cropland* and *Grassland* (n = 145). This increase correspond to 51 Gg CO_2 removed from the atmosphere. *Abandoned Cropland* is thus estimated to emit 85 Gg CO_2 annually. Emission of N_2O from *Drained Wetlands* is estimated as 74.4 Gg CO_2 eq and for *Abandoned Cropland* as 80.6 Gg CO_2 eq. The *Grassland* category in Iceland as a whole emits 21 Gg CO_2 eq, based on these estimates. This result is still subject to large uncertainty for many categories. The estimate is expected to improve as more data will be available, both on carbon pools and area.

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Preparation of a LULUCF land-use map for Iceland: Development of the *Grassland* layer and subcategories

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Abstract

Reliable maps of land-use and land-use changes are key variables in estimating the impact of land-use and land-use changes on carbon stocks and greenhouse gas emissions at national level. In this paper we describe the preparation of a land-use map for Iceland, the determination of hierarchy of the map layers used, and the compilation process applied for compiling the 2010 land-use map. The resulting maps for the category *Grassland* and its subcategories are compared with other estimates of area. The comparison revealed considerable differences between area of grassland subcategories and other available area estimates. Only a proportion of these differences can be explained and more precise estimates and maps are needed to acquire consistency. Independent estimation for one subcategory is not available, but its areal estimate is mostly based on verified data.

Keywords: land-use map, Icelandic Geographic Land Use Database, GIS, Map compilation

Introduction

Land-use and land-use changes impact on carbon stocks and greenhouse gas (GHG) emissions (Bolin *et al.*, 2000). The role of land-use and land-use changes in relation to climate change has gained increasing attention within the arena of the United Nations Framework Convention on Climate Change (UNFCCC) as reflected by the guidelines for the reporting (Houghton *et al.*, 1997; Penman *et al.*, 2003; IPCC, 2006). The Agricultural University of Iceland has a legal obligation to assemble and report information on land-use and land-use changes relevant to the UNFCCC reporting (Alþingi, 2007; 2012). As geographical maps of many categories of land-use in Iceland were unavailable, in 2007 the university started to assemble data for an Icelandic Geographic Land Use Database (IGLUD). To respond to increased demand for information the decision was made to first compile available geographical data into a new land use map, and then gradually gather new data where needed. The objective was to be able to geographically identify at each time all main land-use categories requested for the inventory of Land Use and Land Use Changes and Forestry (LULUCF) to the UNFCCC. In this paper we describe the compilation of available maps into IGLUD towards a land-use map for the year 2010, and compare grassland subcategory maps to other available estimates of the total area of those categories.

Materials and methods

The data in IGLUD come from various sources. A total of 42 map layers representing individual land cover classes or specific land uses were identified as important data sources for the IGLUD 2010 land-use map. The Icelandic Farmland database was the most extensive of these sources (Arnalds and Barkarson, 2003) with 18 map layers. That database was prepared in two phases, with one phase covering around 60% of the country with 12 land-use classes and the other where the 12 classes were aggregated to 6 classes. In the first phase, extensive ground-verification was made to ensure >85% accuracy of the land cover classification (Arnalds and Barkarson, 2003). Other data used in IGLUD come from the

National land survey of Iceland, Icelandic forestry service, the Soil conservation service and the Agricultural University of Iceland (Environmental Agency of Iceland, 2012). The total area of all map layers used is much greater than the total area of the country, or 181×10^3 km^2 and 103×10^3 km^2 respectively, with the same area appearing in many map layers. To solve this, all the layers were ranked in hierarchical order to determine the fate of shared pixels. The selection of pixel values for the final map was done with overlay analysis operation of ERDAS IMAGIN 2011, which matches corresponding pixels from two or more map layers and selects the one from the layer highest in the hierarchy. The hierarchy rules of the compilation process have to be well grounded and in a logical dominance compared to other layers in the compilation. Before ranking of map layers a matrix of total overlays between individual layers was prepared. The hierarchical order of a map layer involves consideration of several factors: overlaps, possibilities of multiple land use, reporting guidelines, logical reasoning and map uncertainty. Based on those factors hierarchical order of all the map layers used for the land-use map was determined as described in the following decision tree (Box 1).

1. Does the land use mapped exclude other land use at the same time?
   - → Yes → rank the layer on top of the hierarchical order or next below other layers meeting the same criteria
   - → No → go to next criteria
2. Does the map layer overlap with other layers included?
   - → Yes → go to next criteria
   - → No → rank the layer anywhere e.g. next below the layers meeting the 1. Criteria or with other layers belonging to same land use category.
3. Is the hierarchical order of the map layer predetermined by IPCC guidelines?
   - → Yes → rank accordingly with other layers of the same land use category.
   - → No → go to next criteria
4. Is there a logical reason for the internal order of the map layer and the other layers with which it shares cells with?
   - → Yes → rank layers accordingly
   - → No → go to next criteria
5. Is there a relevant difference in the uncertainty of the map layer and other layers with which it shares cells with (age, scale, classification)?
   - → Yes → rank the layer with less uncertainty higher in the hierarchical order.
   - → No → Leave the hierarchical order of the layer as it is. (Consider if the map layer should be divided or merged with other layers on basis of the overlapping area or even excluded as data source)

Box 1. Decision tree for determining hierarchical order of map layers.

Having determined the hierarchy of the map layers, all layers were compiled to form the land use map and layers then grouped to represent the mapping categories for the UNFCCC inventory. Independent estimates of individual subcategories are available from the UNFCCC inventory (Environmental Agency of Iceland, 2012) for the categories *Natural Birch Shrubland* and *Revegetated Land* and from Óskarsson (1998) on drained wetlands.

Results and discussion

The area of *Grassland* and its subcategories as generated by the IGLUD mapping process is summarized in Table 1. Also included in the table are the available independent estimates of total area of comparable units. For the subcategory *Other Grassland* the land-use map is the only estimate presently available on their areal extent. Comparing the area generated by the IGLUD land-use map to these independent estimates shows considerable differences between these approaches (Table 1). The area of *Natural Birch Shrubland* estimated from the land-use map is 883 km^2 whereas the inventory-based estimate of the Icelandic Forestry Research for

Natural Birch Shrubland is 299 km^2 (Environmental Agency of Iceland, 2012). The difference is partly explained by different definitions, as 307 km^2 of land with birch, potentially reaching 2 m in height at maturity, are included in the mapped area but not in the inventory estimate where it is categorized as forest. The remaining difference of 277 km^2 is presently unexplained. The area of *Revegetated Land* before 1990 as estimated by the Soil Conservation Service of Iceland is 1637 km^2 whereas the area emerging form the land-use map is only 183 km^2. The difference is considered to result mainly from inadequate mapping of these areas (Environmental Agency of Iceland, 2012). For land revegetated since 1990 the mapped area yields 731 km^2, while an independent estimate of the Soil Conservation Service of Iceland gives 832 km^2. The difference is to some extent explained by inadequate mapping of certain revegetation activities and uncertainties in both numbers (Thorsson J., personal communication). The estimate for drained wetland includes other land-use categories than *Grassland,* such as *Cropland* and *Forest*. Having corrected for estimated drainage within other land-use categories, the estimated area from Óskarsson (1998) is 3889 km^2 compared with 3408 km^2 emerging from the land-use map: the difference of 481 km^2 is considered to be the result of uncertainty of both estimates. Of the far largest subcategory, i.e. *Other Grassland*, 75% of the area originates from map layers with >85% accuracy (Arnalds and Barkarson, 2003). The map of *Grassland* emerging from the IGLUD mapping can, as a whole, be considered reasonably accurate, but mapping of individual subcategories needs to be improved. The mapping presently is the only available estimate for many grassland subcategories.

Table 1. The areal extent of *Grassland* categories of the IGLUD 2010 land-use map compared with independent estimates.

Land use category	IGLUD land use map [km^2]	Independent estimate [km^2]
Natural Birch Shrubland	883	299
Grassland on Organic Soil	3,408	4,500
Revegetated Land before 1990	183	1,637
Revegetated Land since 1990	731	832
Other Grassland	47,425	-
Grassland total	52,631	-

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Micronutrient levels in pastures established in the north-east of Portugal

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Abstract

In this study we evaluate the effects of six types of fertilization: no fertilizer, lime, manure, lime + phosphorus, lime + phosphorus + boron and manure + inorganic fertilizer; and two types of pasture: spontaneous vegetation and sown pasture, on soil pH and micronutrient concentrations in herbage of pastures established in the NE of Portugal. The results showed that there were higher levels of Cu in the fertilizer treatments that promoted legumes. The 'no-fertilizer' treatment and inorganic fertilization with lime increased the percentage of grasses in the swards and herbage Mn levels.

Keywords: animal nutrition, meadows, fertilization, grasses, legumes

Introduction

Micronutrients such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are essential requirements for plants and animals, and play an important role in livestock diets as they may cause malfunctions as a result of either deficiency or of toxicity (Whitehead, 2000). Accumulation of these elements in plants depends on soil properties, total and plant-available amounts of elements, cultivation and fertilization system, climate, as well as plant properties. The objective of the present study was to evaluate the effects of six types of fertilization: no fertilizer, lime, manure, lime + phosphorus, lime + phosphorus + boron, and manure + inorganic fertilizer, and two types of pasture: spontaneous vegetation (unsown pasture), and sown pasture (sown), on the micronutrients levels of herbage in cattle-grazed pasture.

Material and methods

The experiment was carried out in Vila Meã, (NE Portugal) at 860 m a.s.l., on a Leptosol with an initial pH (water) of 4.5. Two types of pasture were studied: spontaneous vegetation (unsown), and sown pasture (sown) consisting on a mixture of annual legumes, perennial legumes, grasses and chicory (50%, 5%, 41% and 4%, of total seeding rate, respectively). Six fertilizer treatments were applied during the experiment: no fertilizer (NF), lime (Ca), manure (M), lime + phosphorus (CaP), lime + phosphorus + boron (CaPB), and manure + inorganic fertilizer (MCaPB). The experimental design was a hierarchical completely randomized split-plot with three replicates, where pasture type was the main plot and fertilization the sub-plots. Three herbage samples were taken from each sub-plot, using 0.25 m^2 cages, in 2005 and 2007. No samples were taken in 2006 as this year was for natural regeneration. Three herbage samples were harvested from inside the exclosure cages, from areas of 0.25 m^2 within sub-plots, in spring of 2005, and 2007 (and exclude the spring of 2006, when there was no grazing in order to allow for a natural reseeding). In the laboratory, plant species were hand separated to determinate botanical composition (grasses (G) + other species (OT) and legumes (L)). The samples were dried for 48 h at 60ºC to determine dry matter (DM), and then milled to provide samples for chemical analyses. After a microKjeldahl digestion, total Cu, Mn, Zn and Fe were analysed with a VARIAN 220FS spectrophotometer using atomic absorption. Data were analysed by Principal component analyses (PCA) based on a correlation matrix for the dependent variables, followed by multivariate and univariate analyses of variance (SYSTAT 12) and mean separation (Tukey's HSD test).

Results and discussion

PCA was significant (P<0.001) in the explanation of dependent variables (Figure 1). The first three PCA-axes explained 72% of the variation. PCA1 (40% of total variability) was positively related to legume percentage (L), DM yields and concentrations of Cu in the pasture, and it was negatively related to grasses percentage (G), no fertilizer (NF) and lime + phosphorus (CaP) treatments. PCA2 (17% of total variability) showed a negative correlation between Mn concentrations in pasture and manure + inorganic fertilizer (MCaPB). PCA3 (15% of total variability) showed a positive correlation between concentrations of Fe in pasture and lime fertilization (Ca).

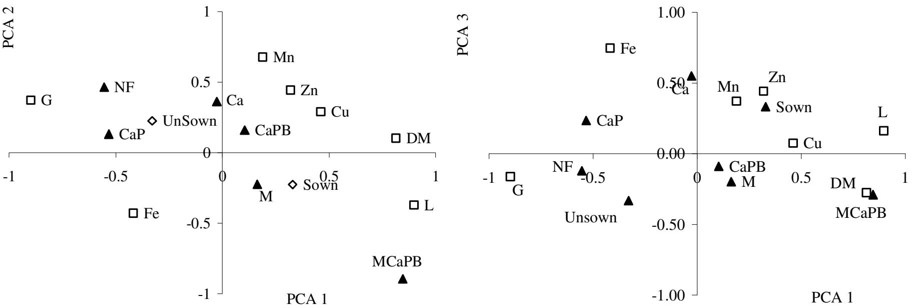

Figure 1. Loadings and scores of the first two PCAs and significant effect of pasture type and fertilizer treatment (P<0.05), where: ◊: pasture type (unsown and sown); ▲: fertilizer treatment (no fertilizer (NF), lime (Ca), manure (M), lime + phosphorus (CaP), lime + phosphorus + boron (CaPB), and manure + inorganic fertilizer (MCaPB)); □: dependent variables (G: % grasses and other species; L: % legumes; DM: DM yield, and mineral composition: Cu, Mn, Zn and Fe).

Results of ANOVAs showed that the interaction of pasture type × fertilizer treatment was significant (P<0.01) on Cu, Mn and pH. The lime+phosphorus+boron (CaPB) treatment significantly increased soil pH in unsown pasture, relative to the NF and the other fertilizer treatments that also applied Ca in 2007 (Table 1). In the case of sown pasture, pH was significantly increased by Ca treatment, when compared with the NF and M treatments. Despite the positive effect on soil pH of lime fertilization, the pH remained low (4.4-5.3) and at a level that usually indicates deficiencies in the availability of nutrients (Whitehead, 2000). In 2005, the MCaPB treatment significantly decreased the levels of Cu in unsown pasture, relative to the effects of the NF, Ca and CaPB treatments (Table 1). In the same year and in sown pasture, the level of Cu was significantly increased when MCaPB was applied, as compared with the M treatment. It is known that legumes and grasses have different abilities to accumulate mineral elements, even when grown under the same conditions. Thus, concentrations of Cu are reported to be higher in legumes than in grasses (Whitehead, 2000). In our case, the different effect of MCaPB treatment on Cu levels of herbage of sown and unsown pastures could be explained by the highest percentage of legumes found in the swards for this fertilizer treatment in sown pasture, as compared with the unsown pasture (75% *vs* 25% in sown and unsown pasture, respectively) (Pires *et al.,* 2004). In contrast to the other micronutrients, Mn concentrations in grasses are often higher than in legumes (Whitehead, 2000). Our results showed that in 2005, the M treatment significantly reduced the levels of Mn in unsown pasture relative to the effects of the NF, CaP and MCaPB treatments. This effect could be explained by the fact that the NF, CaP and MCaPB treatments had higher proportions of grasses (95-100%) than the M treatment (Pires *et al.,* 2004). In 2007, the levels of Mn were significantly increased in herbage of the Ca treatment relative to all fertilizer treatments except CaPB. It is known that in acidic soils (pH 5.0-5.5) the availability of Mn for plants is higher than in very strongly acidic soils (pH 4.5-5.0) (Jones and Jacobsen, 2005) because it increases the concentration of soluble Mn^{2+} (Watmough *et al.,* 2007), which is the most available form of Mn for plants. No significant differences were detected between treatments for Fe concentrations (average levels 510 and 208 mg kg^{-1}, in 2005 and 2007, respectively) and Zn (average levels 138 and 228 mg kg^{-1}, in 2005 and 2007, respectively). With regard to livestock requirements, the maximum Cu, Mn, Zn and Fe concentrations established by NRC (2000) for beef cattle were never exceeded. However, Cu and Mn concentrations were below or close to the minimum values needed for an adequate supply for beef cattle (NRC, 2000).

Table 1. Concentrations of Cu and Mn in pasture (mg kg^{-1}) in 2005 and 2007 and pH in 2007, on the two types of pasture (unsown and sown), and in the six fertilizer treatments: NF: no fertilizer, Ca: lime, M: manure, CaP: lime + phosphorus, CaPB: lime + phosphorus + boron, MCaPB: manure + inorganic fertilizer. Different letters indicate significant differences between fertilization treatments in the same pasture type, and in the same year (P<0.01). SEM: standard error of the means.

		Unsown							Sown						
		NF	Ca	M	CaP	CaPB	MCaPB	SEM	NF	Ca	M	CaP	CaPB	MCaPB	SEM
Year 2005	Cu (mg kg^{-1})	11a	8ab	4bc	5bc	6b	4c	0.88	2ab	12ab	1b	10ab	8ab	13a	0.96
	Mn (mg kg^{-1})	8a	7ab	5b	7a	6ab	7a	0.56	6	6	5	6	6	4	0.87
Year 2007	pH (H_2O)	4.4b	4.6b	4.6b	4.6b	5.2a	4.6b	0.09	4.4b	5.3a	4.4b	5.0ab	5.0ab	5.0ab	0.15
	Cu (mg kg^{-1})	10	10	12	10	11	8	1.05	13	10	10	12	12	12	1.11
	Mn (mg kg^{-1})	9	9	7	7	7	3	1.14	6b	13a	6b	8b	9ab	6b	0.91

Conclusions

The manure + mineral fertilization treatment increased the concentration of Cu in pasture only when this fertilizer treatment also promoted the presence of legumes in the sward. The 'no fertilizer' treatment and inorganic fertilization with lime increased the percentage of grasses in the pasture and the Mn concentrations in the herbage.

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Impact of different fertilization intensity on nutrient leaching in ley-based farming systems

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Abstract

Ley farming based on highly productive grass-clover mixtures can be an interesting amendment to permanent grassland, but during the course of grassland ploughing until the full establishment of the ley-farming vegetation there is an extended risk for nutrient leaching. By means of a perennial field experiment, equipped with gravitation lysimeters, nutrient leaching has been investigated in an intensive grassland region of Austria. High nitrate concentrations in the leachate (up to 360 ppm) and high nitrogen losses of up to 184 kg ha^{-1} occurred during the year of establishment. In the following period, nitrogen losses were reduced to a level comparable to permanent grassland. Therefore special attention has to be given to the fertilization level in the establishment year of ley-farming areas to avoid nutrient losses and negative environmental consequences.

Keywords: grassland ploughing, grass-clover mixtures, water quality, nitrate leaching

Introduction

Permanent grassland covers up to 90% of agriculturally used areas in mountainous regions of Austria and another 145,000 ha farmlands (≈ 10%) are temporarily cultivated with grass, clover or grass-clover mixtures. These ley farming areas provide around 19% of the net yield, 20% of the energy yield and 26% of the protein yield that is in total harvested from grassland-based areas (BMLFUW, 2012). The use of legumes in seed mixtures for ley farming and permanent grassland is an efficient strategy to reduce the external N-input on farms, which is especially important regarding the European-wide discussion about protein substitution in feeding. Although consisting of typical grassland plants, ley-farming stands are declared as arable land and have to be renewed within 5 years according to EC-legal regulations (Poetsch *et al*., 2007). Apart from high costs of establishment there are some ecological risks, which have to be considered seriously to avoid environmental problems, e.g. nitrate leaching.

Materials and methods

A field experiment was established at Winklhof in the province of Salzburg in 2007. This site is located at 452 m a.s.l and characterized by an average rainfall of 1500 mm yr^{-1} and a mean temperature of 9.1°C during the observation period of 4 years. After ploughing, a grass-clover mixture (80% grass and 20% clover) was sown at the end of April 2007. Two fertilization intensities (85 and 170 kg $N_{ex\ storage}$ ha^{-1} yr^{-1} using cattle slurry) were used and the plots were cut four times per year. To determine the amount and quality of leachate, gravitation lysimeter chambers (1.1 m diameter, 1.4 m depth) were permanently installed in the plots, including three replications of all treatments.

Results and discussion

In the year of establishment the yield productivity was at a disappointing low level, without significant differences between the fertilization intensities (Table 1). By reason of weed control two cleaning cuts were necessary in this year, followed by two late harvest cuts in September and October. In 2008 the yield level in both systems strongly increased but continuously dropped again in the following two years in both systems, due to unfavourable weather conditions with heavy rainfall during the growing season. Indeed, from 2008 to 2010 a significantly higher yield could be observed for the intensive fertilization level, but the potential of the ley-farming mixture, which usually provides up to 12 t ha^{-1} yr^{-1} in favourable regions, could not be obtained at the experimental site.

Table 1. Dry matter yield (t ha^{-1} yr^{-1}).

	Medium fertilization level					High fertilization level				
	$\bar{x}$	Median	σ	Min.	Max.	$\bar{x}$	Median	σ	Min.	Max.
2007	$3.88^{a, e}$	3.54	0.78	3.33	4.78	$4.25^{a, e}$	4.10	0.67	3.66	4.98
2008	$9.37^{a, f}$	9.29	0.23	9.19	9.63	$10.52^{b, f}$	10.34	0.44	10.20	11.02
2009	$7.17^{a, g}$	7.46	0.54	6.54	7.89	$8.66^{b, g}$	8.70	0.08	8.58	8.71
2010	$5.71^{a, g}$	5.99	0.50	5.13	7.50	$7.73^{b, g}$	7.88	0.30	7.38	7.93

a, b – indicate sign. differences between fertilization levels; e, f, g – indicate sign. differences between years

Compared with the seed mixture composition, legumes contributed a disproportionately large component of the yield in all years at both fertilization levels (Table 2). In grassland-based farming systems with a low input of external feedstuff the protein content of forage is of great importance. In our experiment the protein concentration in forage differed significantly between the two fertilization levels and showed a declining tendency in the course of the observation period. The removal of nitrogen, calculated on the basis of yield and its related protein content, clearly exceeded the nitrogen input via fertilization, which indicates that other N-sources such as biological N-fixation, N-deposition and N-mineralization also contributed. The high fertilization level (+ 85 kg N) representing the upper limit of the Council Directive 91/676 (EEC, 1991), indeed had a significant but disappointingly low effect on yield productivity and consequently on nitrogen removal (+ 14.5 kg N).

Table 2. Legume proportion (weight-%), protein concentration in forage (g kg DM^{-1}) and N-removal (kg N ha^{-1} yr^{-1}) - average data of four cuts yr^{-1}.

	Medium fertilization level			High fertilization level		
	Legumes	Crude protein	N-removal	Legumes	Crude protein	N-removal
2007	$38.0^{a, e}$	n.a.	n.a.	$37.7^{a, ef}$	n.a.	n.a.
2008	$47.3^{a, f}$	$155.1^{a, e}$	$232.5^{a, e}$	$44.9^{a, e}$	$151.4^{b, e}$	$254.8^{b, e}$
2009	$44.3^{a, ef}$	$148.8^{a, f}$	$170.8^{a, f}$	$42.7^{a, ef}$	$146.6^{b, f}$	$203.2^{b, f}$
2010	$36.0^{a, e}$	$138.8^{a, g}$	$126.8^{a, g}$	$35.0^{a, f}$	$141.3^{b, g}$	$174.8^{b, g}$

a, b – indicate sig. differences between fertilization levels; e, f, g – indicate sig. differences between years

In the establishment year an average nitrate concentration of nearly 80 mg L^{-1} leachate was detected with maximum values of more than 350 ppm (Table 3). In both fertilization systems more than 40% of all leachate analyses (15-20 yr^{-1}) were beyond the EU-wide existing nitrate threshold of 50 ppm whereas in the following years no values exceeded the threshold. The combination of low yield, high (but still legal) nitrogen load and an obviously strong mineralisation pulse in the soil system led to extremely high rates of N-losses via leachate in the year of establishment.

Table 3. Nitrate concentration in percolating water and nitrogen leaching data.

	Medium fertilization level				High fertilization level			
	mg NO_3^- L water^{-1}		N-leaching (kg ha^{-1} yr^{-1})		mg NO_3^- L water^{-1}		N-leaching (kg ha^{-1} yr^{-1})	
	x̄	Max.	x̄	σ	x̄	Max.	x̄	σ
2007	76.9$^{a, e}$	355.3	167.9$^{a, e}$	11.6	75.9$^{a, e}$	366.2	183.9$^{a, e}$	13.9
2008	9.9$^{a, f}$	47.1	17.2$^{a, f}$	10.7	7.1$^{a, f}$	26.8	14.1$^{a, f}$	7.9
2009	13.1$^{a, f}$	36.1	25.5$^{a, f}$	11.9	8.1$^{b, f}$	18.9	16.7$^{a, f}$	8.9
2010	10.3$^{a, f}$	23.6	15.2$^{a, f}$	6.1	8.8$^{a, f}$	24.7	15.1$^{a, f}$	4.7

a, b – indicate sig. differences between fertilization levels; e, f, g – indicate sig. differences between years

Nitrogen balances were established at the field level, including N-fertilization, N-deposition, biological N-fixation, N-removal, unavoidable gaseous N-losses and N-leachate. The total N-input was strongly influenced by biological N-fixation (up to 150 kg N ha^{-1}, estimated by N-difference method) whereas nitrogen leaching significantly contributed to the total N-output in the first year. Nitrogen balances should therefore not only be scaled to nitrogen fertilization and nitrogen removal by plants but should also consider other important partitions.

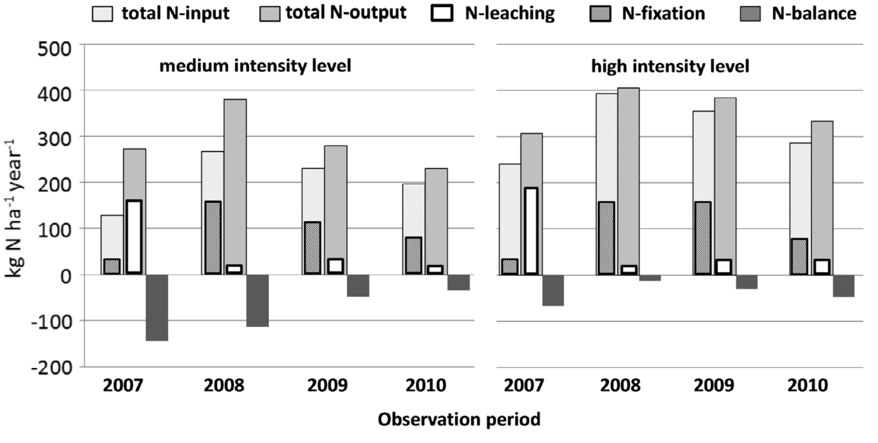

Figure 1. Nitrogen field balance in the lysimeter experiment at Winklhof, Austria.

Conclusions

Ley farming provides an attractive option to produce forage of high quality, but compared with permanent grassland there is a considerable risk for high nitrate concentration in the leachate and nitrogen losses, especially in the year of establishment. To avoid such environmental problems, the awareness of farmers has to be raised to adapt the fertilization level to the expected lower yields in this critical period.

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Grassland root biomass and carbon sequestration response to phosphorus and potassium supply

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Abstract

Carbon sequestration in grassland is largely controlled by the carbon input derived from roots. The impact of phosphorus and potassium supply on above- and belowground biomass and on soil organic carbon content (SOC) was studied in a long-term field trial on a light sandy soil in northern Germany. Nutrient deficiency significantly reduced aboveground yield and modified sward composition, but did not affect root biomass. SOC content in the 0-30 cm layer was influenced by phosphorus supply, whereas potassium had no effect.

Keywords: perennial ryegrass, root, soil organic carbon, phosphorus, potassium

Introduction

Grasslands represent an important component of the global C cycle. The net C exchange with the atmosphere is driven by many factors, such as the grassland management, which affects above- and belowground processes. While the nitrogen impact on root growth is well documented (Soussana *et al*., 2006), studies on the effects of other macro nutrients are still limited. The objective of the present study, therefore, was to investigate the effect of phosphorus and potassium supply on root biomass accumulation and on carbon sequestration of permanent grassland.

Materials and methods

The study was based on a field trial with five replicates, established in 1985 on a perennial ryegrass-dominated sward on a podzol (sandy sand, pH 4.8) in northern Germany. Treatments comprised the variation of either phosphorus or potassium in four levels (P_2O_5: 0, 45, 90, 135 kg ha^{-1} as superphosphate; K_2O: 0, 120, 240, 360 kg ha^{-1} as KCl), while the other nutrient was applied at the highest level to prevent any nutrient deficiency. Plots (5.6 m²) were cut 4 times a year and received a uniform application of 260 kg N ha^{-1} as calcium ammonium nitrate, split into four dressings (80-60-60-60). Sward composition was manipulated by resowing and herbicide treatments at irregular intervals in order to sustain productive swards. After 27 years of varied nutrient supply, samples for soil organic C (SOC) determination were taken in mid July 2012 from three soil layers (0-30, 30-60 and 60-90 cm). Samples were dried, sieved (< 2 mm), and ground to fine powder. Total C and N were measured using a CN-analyser (Vario Max CN, Elementar Analysensysteme, Hanau, Germany). The organic C content was obtained as the difference of total C and carbonate content (Scheibler, DIN ISO 10693). Soil bulk density was determined at 10-15, 40-45, and 75-80 cm depth, applying a core method. Additional samples were taken at the end of July in the two uppermost layers to quantify the root biomass (soil auger with 8 cm diameter, washing over a 0.63 mm sieve) and the corresponding C and N contents. The belowground data were supplemented by an analysis of yield data and of sward composition according to Klapp (1965). One-factorial analyses of variance were conducted to investigate the effect of phosphorus and potassium fertilization on SOC content and on root biomass, using SAS 9.2 Proc mixed. A two-way analysis of variance was performed to test the effect of nutrient level and year on aboveground DM yield, which had been recorded in irregular intervals in recent years. Multiple comparisons of means were conducted by the Tukey-Kramer method or by t-test and Bonferroni-Holm adjustment.

Results and discussion

The monitoring of the sward composition revealed a dominance of grass species in all treatments (Figure 1), probably due to manipulations in terms of resowing and herbicide application in recent years. A noticeable proportion of herbs (9.2%) was found only in the absence of phosphorus supply, whereas in the potassium treatments the herb proportion always remained below 1%. Legumes were absent in all plots.

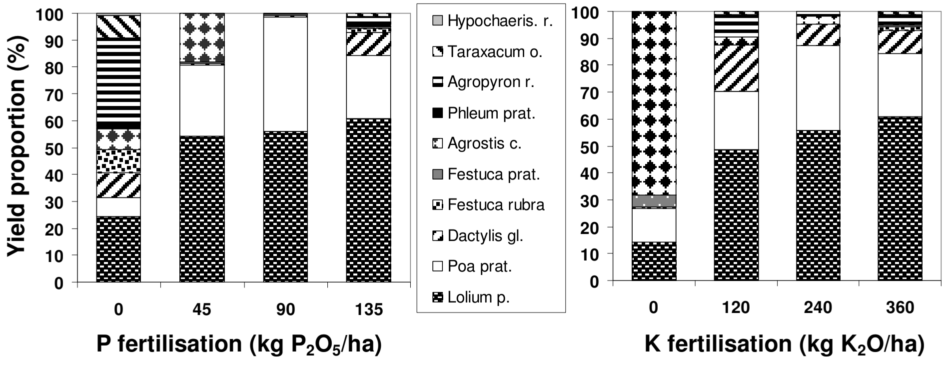

Figure 1. Sward composition provided as species yield proportions, monitored before the 1st cut in May 2012, as affected by phosphorus and potassium fertilization.

Aboveground annual DM yield was significantly affected by an interaction of fertilizer level and year, both for phosphorus and potassium. Except for two years (2000, 2011), phosphorus application gave higher yields than the control (P0), whereas levels P45, P90 and P135 produced similar yields (Figure 2). Compared to phosphorus, the absence of potassium supply caused a more severe yield decline. Apart from the significant difference between K0 and the remaining K levels, K120 achieved lower yield than K240 and K360 in 5 out of 9 years.

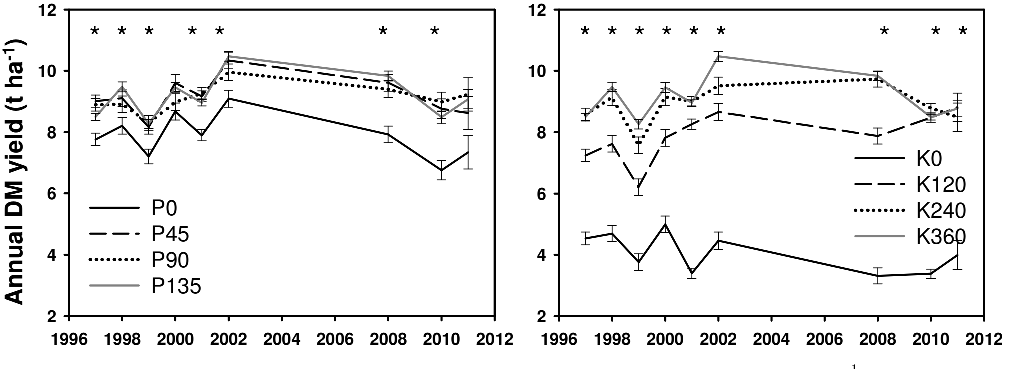

Figure 2. Impact of phosphorus and potassium fertilization on annual dry matter yield (t DM ha^{-1}), recorded at irregular intervals 1997-2011. Asterisks indicate significant differences among fertilizer levels within years.

According to the functional equilibrium theory and the significant impact of P and K on aboveground yield, we would have expected corresponding effects on root biomass and on SOC content. Previous research shows that nutrient deficiency reduces total plant biomass accumulation and modifies biomass allocation between plant organs. Low P availability was reported to accumulate carbohydrates in leaves and to shift biomass allocation in favour of roots. Potassium deficiency was found to accumulate carbohydrates but, due to impaired sugar export, apparently does not promote root biomass (Hermans *et al.*, 2006).

Root biomass varying between 10 and 14.7 t OM ha^{-1} in the 0-30 cm soil layer (Table 1), substantially exceeds values reported from a nearby sandy loam site (Chen *et al.*, unpublished). Presumably, drought stress occurring regularly during the vegetation period resulted in a generally higher root fraction. It should, however, be noted that a differentiation between dead and living roots is not possible by using the soil auger method. Rooting of the subsoil (30-60 cm) was very low. Nutrient supply tended to increase root biomass accumulation in the 0-30 cm layer for phosphorus and potassium, but differences were not significant (Table 1). The yield-reducing effect and differences in sward composition outweighed the opposite shift in biomass-allocation pattern. Soil organic C content was in the upper range reported by other studies (Springob *et al.*, 2001; Wiesmeier *et al.*, 2012). Similar to the root mass, SOC content tended to increase with nutrient supply in the uppermost soil layer. Yet, only P applications showed significantly higher SOC than the control. Belowground, K supply tended to decrease SOC, but no consistent pattern was detected for P.

Table 1. Soil organic C (t C ha^{-1}) and root biomass (t OM ha^{-1}) as affected by fertilization and soil depth. Different lower case letters indicate significant differences between fertilizer levels.

		Soil organic C content (t C ha^{-1})			Ash-free root biomass (t OM ha^{-1})	
		0-30 cm	30-60 cm	60-90 cm	0-30 cm	30-60 cm
Phosphorus fertilization (kg P_2O_5 ha^{-1})	0	97.24^{a}	49.49^{a}	19.68^{ab}	11.00^{a}	0.44^{a}
	45	109.86^{b}	51.08^{a}	27.40^{ab}	12.71^{a}	0.43^{a}
	90	113.44^{b}	64.83^{a}	32.72^{b}	14.69^{a}	0.49^{a}
	135	111.10^{b}	50.79^{a}	20.54^{a}	12.66^{a}	0.51^{a}
Potassium fertilization (kg K_2O ha^{-1})	0	104.42^{a}	60.05^{a}	31.83^{a}	10.03^{a}	0.34^{a}
	120	107.04^{a}	57.65^{a}	25.29^{a}	9.97^{a}	0.44^{a}
	240	109.23^{a}	49.37^{a}	21.62^{a}	12.38^{a}	0.57^{a}
	360	111.10^{a}	50.79^{a}	20.54^{a}	12.66^{a}	0.51^{a}

Conclusion

Inadequate P and K supply to grassland, as is often shown by soil analysis data on practical dairy farms, may not only have implications for forage yield and quality, but may also adversely affect the soil carbon sequestration potential. Further research is required to investigate the causal relationships.

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Effect of quick lime and superphosphate application on growth of *Rumex obtusifolius* in soils contaminated by cadmium and zinc

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Abstract

Rumex obtusifolius is widespread grassland weedy species and can be used for detecting soils contaminated by cadmium (Cd) and zinc (Zn). However, no information is available on how soil additives such as quicklime and superphosphate, which generally decrease mobility of metals in the soil, affect the biomass production of *R. obtusifolius* and Cd and Zn concentrations in its leaves and roots. In spring 2011, a pot experiment was laid out to test this. According to the control treatment, *R. obtusifolius* accumulated more Cd and Zn in roots than in leaves. Quicklime application substantially decreased Cd and Zn concentration in biomass. Superphosphate application caused only a small decrease of Cd and Zn, not significantly different to the control. Decrease of Cd and Zn concentration in leaves and roots increased biomass production in order control < superphosphate < quick lime. We concluded that high availability of Cd and Zn in soil substantially reduces growth of *R. obtusifolius*.

Keywords: broad-leaved dock, plant-available metal concentrations, pot experiment, soil additives, risk elements, yield

Introduction

Although areas contaminated by trace elements have been studied worldwide for a long time (Mulligan *et al*., 2001), not all contaminated sites have been identified so far. Contaminated soil represents a serious problem because of possible contamination of the food chain by high-risk elements (Alkorta *et al*., 2010). Broad-leaved dock (*Rumex obtusifolius* L.) is the one of the world's most widespread grassland weeds (Zaller, 2004) and it can thus be used as a possible indicator plant for contaminated sites. It is therefore necessary to obtain information about the growth of *R. obtusifolius* on contaminated soils and to evaluate its ability to accumulate high-risk elements. The mobility of many elements is affected by soil pH and soil P status. The aim of this study was to investigate the effect of lime and superphosphate application on growth, biomass and chemical properties of broad-leaved dock. In pot experiments we looked at how lime and superphosphate influenced the biomass production of *R. obtusifolius*, on the uptake of Cd and Zn and how the concentration of Cd and Zn is distributed between leaves and roots.

Materials and methods

Heavily contaminated Fluvisol located in the alluvium of the Litavka River in Trhové Dušníky village in the Czech Republic was used in the pot experiment, and was characterized as follows: pH_{CaCl2} 6.5, CEC 55 $mmol_{(+)}$ kg^{-1}, C_{org} 3.6%, 53.8 mg Cd kg^{-1}, 6172 mg Zn kg^{-1} (risk elements extracted by *aqua regia*). Legislation limits for total concentrations of Cd and Zn in light-textured soils are 0.4 mg kg^{-1} and 130 mg kg^{-1} respectively (Anonymous, 1994). Highly soluble quicklime and fast P-release superphosphate, both analytical grade, were used as the metal stabilization agents. At the start of the experiment, 5 kg of air-dried soil was mixed with 0.3g N, 016 g P, and 0.4 g K per kg of the soil. The quicklime (7.3 g CaO kg^{-1} soil) and superphosphate (1.3 g $Ca(H_2PO_4)_2$ kg^{-1} soil) corresponding to individual treatments, were thoroughly mixed with experimental soil and inserted to the pot. Five replications were used for each treatment (C – control, Ca – quicklime, P – superphosphate). Three seedlings of *R. obtusifolius* were planted in each pot and the pots were kept in an outdoor weather-controlled vegetation hall in Prague – Suchdol. Six months after planting, leaves and roots were harvested and fresh and dry biomass determined. Dried and ground samples were decomposed by microwave-assisted wet digestion system. Soil pH was determined in 0.01 mol L^{-1} $CaCl_2$ (1:5 (w/v)) extract. Soil samples were extracted with 0.01 mol L^{-1} $CaCl_2$ (1:10 (w/v)) for 6 hours (Novozamsky *et al.*, 1993). Plant-available Cd and Zn concentrations in soil extracts and total plant Cd and Zn concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian VistaPro, Varian, Australia).

Results and discussion

Application of quicklime significantly reduced plant-available Cd and Zn concentrations compared to the control and raised soil pH from 5.8 to 7.5 (Table 1). Immobilization of Cd and Zn after application of lime is well established (Mühlbachová and Tlustoš, 2006; Hejcman *et al.*, 2012; Vondráčková *et al.*, 2013). In contrast to quicklime, the effect of superphosphate on plant-available Cd and Zn was negligible.

Table 1. Mean plant-available Cd and Zn concentrations (± standard error of the mean; SE) and mean value of soil pH (± SE) in contaminated soil treated with different treatments. Calculated by one-way ANOVA followed by Tukey post-hoc test, treatments with same letter are not significantly different.

Treatment	Cd (mg kg^{-1})	Zn (mg kg^{-1})	pH_{CaCl2}
C	4.2 ± 0.2^a	179 ± 7^a	5.8 ± 0.01^a
Ca	0.1 ± 0.01^b	3.7 ± 0.7^b	7.5 ± 0.01^b
P	3.8 ± 0.2^a	174 ± 7^a	5.9 ± 0.02^a

Quicklime significantly decreased Cd and Zn concentrations in leaves and roots compared to the control (Table 2). Superphosphate application decreased Cd and Zn concentrations only slightly. Decrease in plant-available Cd and Zn concentrations in soil after application of additives was well reflected by decrease in Cd and Zn concentrations in leaves and in roots. There were generally higher Cd and Zn concentrations in roots than in leaves, but these differences were only significant in the control. Higher ability to accumulate Cd in roots than in leaves is typical for common plants without hyperaccumulation capability (McGrath *et al.*, 2002). *Rumex obtusifolius* is not a Cd and Zn hyperaccumulator because concentrations of Cd in its biomass did not exceed the threshold 100 mg kg^{-1} and concentration of Zn did not exceed 10000 mg kg^{-1} dry biomass, which is necessary for confirm hyperaccumulation ability (Alkorta *et al.*, 2004). By contrast, the minimum Zn requirement in normal plants is approximately 20 mg kg^{-1} dry biomass (Krämer, 2000).

High plant-available Cd and Zn concentrations in the control and superphosphate treatments suppressed biomass production (see Table 2). A decrease in dry biomass of common crops, because of high concentrations of high-risk elements in the biomass, was also observed by Anton and Mathe-Gaspar (2005). Quicklime increased biomass production. This increase was connected with high increase in soil pH and considerable immobilization of Cd and Zn in the soil.

Table 2. Effect of treatment on mean Cd and Zn contents (± SE) and on mean dry biomass (± SE) in leaves and roots of *R. obtusifolius* planted in contaminated soil. Calculated by one-way ANOVA followed by Tukey post-hoc test, treatments with the same letter are not significantly different within each organ of the plant.

Treatment	Cd (mg kg^{-1})		Zn (mg kg^{-1})		Dry biomass (g plant^{-1})	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
C	14 ± 2^{a}	29 ± 5^{a}	1260 ± 213^{a}	1479 ± 360^{a}	0.07 ± 0.03^{a}	0.40 ± 0.19^{a}
Ca	6 ± 1^{b}	7 ± 1^{b}	498 ± 105^{b}	329 ± 87^{b}	3.88 ± 0.55^{b}	11.5 ± 1.4^{b}
P	11 ± 1^{ab}	19 ± 4^{ab}	875 ± 66^{ab}	809 ± 180^{ab}	0.35 ± 0.16^{a}	0.99 ± 0.33^{a}

Conclusion

High availability Cd and Zn in soil substantially reduce the growth of *R. obtusifolius*. Soils with high availability of Cd and Zn can be identified during the field vegetation mapping according to decreased growth of *R. obtusifolius*. In contrast to superphosphate, quicklime application into slightly acidic soil considerably decreases mobility of Cd and Zn.

Acknowledgements

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Soil nutrient availability depending on arable land conversion to grassland

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Abstract

The aim of this research was to study changes in organic carbon (C_{org}), total nitrogen (N_{tot}), plant available phosphorus (P_{avail}) and potassium (K_{avail}) content in soil after arable land was converted into grassland. The study was carried out on a grass-clover sward (established in 2003; seed mixture composition *Trifolium repens, Phleum pratense* and *Lolium perenne*). The plant residues were either left on the growing plots or removed after cutting. Soil samples were collected at the 0-20 cm soil layer before the trial establishment in 2003, and in autumn 2007 at different depths of soil. The contents of C_{org}, N_{tot}, P_{avail} and K_{avail} were determined. The soil N_{tot} content increased after the four years under grassland, but the contents of C_{org}, P_{avail} and K_{avail} did not change significantly. However, P_{avail} and K_{avail} content in soil increased when the cut plant residues were left to decompose on the grassland surface, indicating that plant residues left on the growing plots act as organic fertilizers so that the rate of PK-fertilizers can be reduced.

Keywords: grass-clover, phosphorus, potassium, dry matter yield, land management, mowing

Introduction

Converting arable land into grassland is an option to enhance soil properties through increasing the content of soil organic matter (SOM). The SOM provides a vast reservoir of nutrients (i.e. nitrogen, phosphorus and potassium) for plants (Power, 1994). The SOM in grasslands originates primarily from root death and decomposition (Gill and Burke, 2002). In addition to the roots, soil organic matter input also is mediated through plant residues, especially in set-aside grasslands that are mown only 1-2 times per year to prevent scrub formation. Plant residues contain large amounts of nutrients, which will be available to the growing plants after their decomposition (Haynes and Goh, 1980). Leaving the plant residues onsite not only reduces the need for nutrients to support sward growth, but it can also save significant amount of fossil energy that would otherwise be used for the clippings as waste. The aim of this research was to study the changes in organic carbon (C_{org}), total nitrogen (N_{tot}), plant available phosphorus (P_{avail}) and potassium (K_{avail}) contents in soil after the arable land was converted into grassland. Nutrient availability dependence on cut plant residue management was also studied.

Material and methods

The field experiment was carried out at the Experimental Station of the Estonian University of Life Sciences in Tartu. The soil of the experimental field was a sandy loam *Stagnic Luvisol* according to WRB classification. The sward had been established in 2003 with a grass-clover mixture (*Phleum pratense, Lolium perenne* and *Trifolium repens*). The field was previously under barley for three years. In May 2003, before establishing the swards (sowing), soil samples (from depth of 0-20 cm) were collected. The content of C_{org} and N_{tot} at a depth of 0-20 cm was 14.7 g kg^{-1} and 1.49 g kg^{-1}, respectively; P_{avail} content was 39.6 and K_{avail} content 79.7 mg kg^{-1}. The experiment was arranged as a 2 × 2 factorial and set out in a randomized complete block design with four replicates. The plots were 10 m^2. The factors of the experiment were: (i) residue treatment: the cut plant residues were returned (RRT) to the plots or removed (RRM) from the plots after mowing; and (ii) fertilizer treatments: $N_0P_0K_0$ (N0) and $N_{80}P_{26}K_{50}$ (N80) kg ha^{-1}. The mowing frequency was 3-4 times per growing season, with a frontal bar mower. The sward dry matter (DM) yield was measured during 2004-2007. After each cutting, the harvested material was collected and weighed for the DM yield measurement. Cut plant materials were either returned (RRT) to the plots or removed (RRM) from the plots after weighing. Total nitrogen (N_{tot}), phosphorus (P_{tot}) and potassium (K_{tot}) content were determined from each cutting. The nutrient uptake by the plant was calculated based on the nutrient content in plants and DM yield. The soil samples from the 0-5, 5-10 and 10-20 cm depth were collected in September 2007. The C_{org}, N_{tot}, P_{avail} and K_{avail} content were determined. The soil C_{org} content was determined after Tjurin. The soil and plant N_{tot} content was determined by the dry combustion method on a varioMAX CNS elemental analyzer (ELEMENTAR, Germany). Acid digestion by sulphuric acid solution was used to determine plant P_{tot} and K_{tot} concentrations. The contents of plant available elements in the soil were determined by the AL-method.

Results and discussion

After the conversion of arable land to grassland, the C_{org} and N_{tot} content in top soil (0-5 cm) increased, the P_{avail} content did not change and K_{avail} content decreased (Table 1). The fertilization increased P_{avail} and K_{avail} contents in soil compared with the unfertilized treatments. The fertilization did not influence the C_{org} content, although in general it is found that there is more organic matter under grassland than under cropland (Cole *et al.,* 1993) due to higher organic matter input into soil and less soil disturbance. The higher N-availability in soil due to the fertilization may induce the soil organic matter decomposition (Mack *et al.,* 2004).

Table 1. Soil organic carbon (C_{org}), total nitrogen (N_{tot}), available phosphorus (P_{avail}) and potassium (K_{avail}) contents in autumn 2007.

Treatment	RRM	RRT	RRM	RRT	RRM	RRT	RRM	RRT
	C_{org}, g kg^{-1}		N_{tot}, g kg^{-1}		P_{avail}, mg kg^{-1}		K_{avail}, mg kg^{-1}	
				0-5 cm				
N0	15.9^{Ba}	18.2^{Bb}	1.65^{Ab}	1.62^{Ab}	39.0^{Aa}	71.0^{Ab}	51.5^{Aa}	199.0^{Bc}
N80	14.1^{Aa}	15.0^{Aa}	1.73^{Ab}	2.10^{Bc}	52.0^{Bb}	73.0^{Ac}	76.9^{Ba}	148.5^{Ab}
				5-10 cm				
N0	11.7^{Aa}	14.7^{Bb}	1.40^{Aa}	1.50^{Ab}	35.0^{Aa}	45.0^{Ab}	47.9^{Aa}	61.2^{Ab}
N80	10.3^{Aa}	12.7^{Ab}	1.41^{Aa}	1.39^{Aa}	36.0^{Aa}	54.0^{Bb}	48.7^{Aa}	59.5^{Ab}
				10-20 cm				
N0	12.5^{Aa}	11.9^{Aa}	1.21^{Ab}	0.74^{Aa}	35.9^{Aa}	45.9^{Ab}	48.2^{Aa}	53.1^{Aa}
N80	12.7^{Aa}	11.3^{Aa}	1.38^{Ab}	0.99^{Ba}	38.5^{Aa}	48.9^{Ab}	51.0^{Aa}	52.7^{Aa}

Different capital letters within column indicate significant difference of the mean values at $P<0.05$
Different small letters within each row indicate significant difference of the mean values at $P<0.05$

The average DM yield of the grass-clover sward was 5168 kg DM ha^{-1} during 2004-2007. The fertilization increased the sward DM yield but there was no influence of the fertilization on the plant nutrient contents (Table 2). The fertilization influenced plant P and K content only in the RRT treatment. The amount of the plant residues left on the sward surface after mowing varied from 7465 to 8162 kg DM ha^{-1} depending on fertilization.

Table 2. The average dry matter (DM) yield of grass-clover sward during 2004-2007, nutrients content in plants and uptake by plants in 2007.

Treatment	RRM	RRT	RRM	RRT	RRM	RRT
	DM yield, kg ha^{-1}		N_{tot}, g kg^{-1}		N uptake, kg ha^{-1}	
N0	5168^{Aa}	7465^{Ab}	24.0^{Aa}	24.4^{Aa}	124.0^{Aa}	182.1^{Ab}
N80	6336^{Ba}	8162^{Ab}	24.0^{Aa}	26.4^{Bb}	152.3^{Ba}	215.4^{Bb}
			P_{tot}, g kg^{-1}		P uptake	
N0			2.8^{Aa}	2.7^{Aa}	14.6^{Aa}	20.3^{Ab}
N80			2.6^{Aa}	2.7^{Aa}	16.5^{Ba}	21.8^{Bb}
			K_{tot}, g kg^{-1}		K uptake	
N0			15.3^{Aa}	19.9^{Ab}	79.1^{Aa}	148.8^{Ab}
N80			15.5^{Aa}	22.7^{Bb}	98.4^{Ba}	185.2^{Bb}

Different capital letters within column indicate significant difference of the mean values at $P<0.05$
Different small letters within each row indicate significant difference of the mean values at $P<0.05$

The plant residues did not influence the P content in plants. This result may have been caused by the fact that the amount of P returned to the sward with the residues was significantly lower (20.3-21.8 kg P ha^{-1}) when compared to the amount of N (182.1-215.4 kg N ha^{-1}) and K (148.8-185.2 kg K ha^{-1}). Returning the plant residues increased N_{tot}, P_{avail} and K_{avail} contents compared with the content in the arable land before the sward establishment. The changes were greater in the top soil layer.

Conclusions

The C_{org} and N_{tot} content in the soil of a grass-clover sward increased, but plant available P and K contents in the soil remained the same. Furthermore, there is tendency for decreasing (i.e. K_{avail} content) when the sward is unfertilized. Leaving the plant residues to decompose on the sward surface after the mowing had a positive effect on the plant available P and K content in soil compared with the arable soil before the sward establishment. The need for externally supplied nutrient inputs can be reduced because nutrients in plant residues become mineralized during decomposition and are potentially available to the growing plants.

Acknowledgements

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Lucerne (*Medicago sativa*) or grass-clover as cut-and-carry fertilizers in organic agriculture

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Abstract

On-farm nitrogen fixation is a driving force in organic agriculture. The efficiency with which this nitrogen is used can be increased by using lucerne (*Medicago sativa*) or grass-clover directly as sources of fertilizer on arable land: cut-and-carry fertilizers. In two arable crops, the use of lucerne and grass-clover as fertilizers was compared with the use of poultry manure and slurry. The nitrogen-use efficiency at crop level was comparable or better for the cut-and-carry fertilizers as compared to the animal manures. The relative P and K content of these fertilizers came closer to the crop demand than that of the poultry manure. Crop yields were comparable or better when using lucerne or grass-clover as fertilizer. It is concluded that cut-and-carry fertilizers are a serious alternative for manure as part of an overall farm soil fertility strategy.

Keywords: organic farming, cut-and-carry fertilizers, lucerne, grass-clover

Introduction

The objective of this study was to address the issue of developing intensive cropping systems that facilitate more effective use of on-farm N-fixation (Antichi *et al*., 2008). This was achieved by developing cropping systems based on grass-clover or lucerne used as fertilizer Burgt *et al*., 2013). Nutrients accumulated by these crops can be used as soil amendment rather than being sold off-farm as forage. This is desirable because the revenues from these crops are rather limited, whereas the on-farm nutrient-use efficiency can be improved.

Materials and methods

The experiments were located on an organic farm in the centre of the Netherlands on a well-drained clay soil with 2.6% of organic matter. During 2009 the use of fresh cut grass-clover, fresh cut lucerne and lucerne silage were compared with the application of chicken manure as a nutrient source for spinach. All materials were applied in four replicates of 3×15 m per plot five weeks before the sowing of spinach. After application, the fertilizers were mixed with the soil till 8 cm depth. The aim was for 200 kg N ha^{-1} to be applied. The realized N application rates are presented in Table 1.

Table 1. Nitrogen applied in 2009.

Treatment	Applied days until sowing	Fertilizer applied t ha^{-1} fresh	N-content kg t^{-1} fresh	N applied kg ha^{-1}
Control		0	0	0
Lucerne fresh late	10 days	27.0	6.1	165
Lucerne silage	36 days	18.2	11.0	200
Poultry manure	36 days	8.2	24.6	202
Grass-clover fresh	36 days	23.3	11.4	266
Lucerne fresh	36 days	24.4	11.1	271

In 2010 the experiment was repeated in a potato crop, using slightly adapted treatments. The treatments, again in four replicates with plots of 3×15 m, were: control, fresh-cut lucerne, lucerne silage, lucerne silage early application, poultry manure and a mixture of cattle slurry with vinasse. Lucerne early application was realized when the potatoes were planted; the other applications took place three weeks later when the ridges were formed. The aim was 125 kg N ha^{-1} to be applied. This was realized for all treatments, except for the mixture of cattle slurry and vinasse, which received 93 kg ha^{-1}.

Results

Fresh yield of spinach in 2009 was the highest with the use of fresh-cut grass-clover and fresh-cut lucerne, applied 5 weeks before sowing (Figure 1). Compared with chicken manure, use of lucerne and grass-clover applied 5 weeks before sowing increased N-production efficiency by 32-44% (Table 2). However, delaying application to 10 days before sowing did not result in an appreciable improvement of N-production efficiency. Mineral removal rates amounted to 67-126 kg N, 13-17 kg P and 122-233 kg K ha^{-1}. The P and K content of the forage crops closely matched actual crop demands of spinach resulting in only relatively slightly positive nutrient balances (Table 2). The P surplus of the poultry manure was very high.

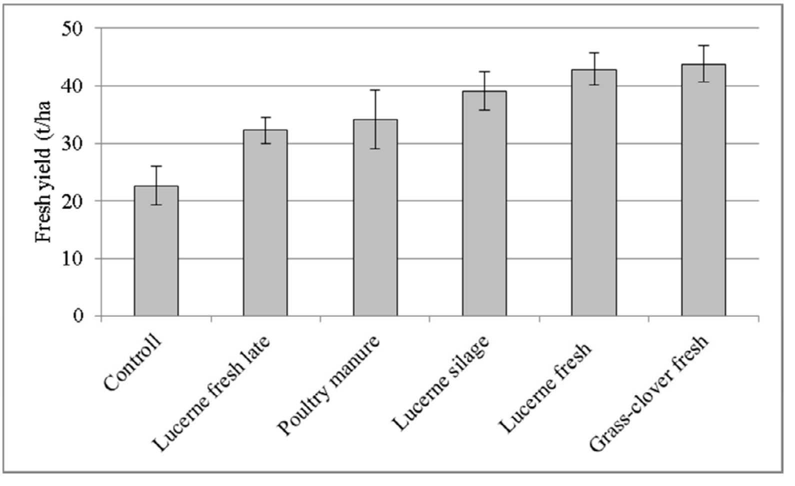

Figure 1. Fresh yield and standard deviation of spinach in 2009 in different fertilizer treatments.

Table 2. Mineral balance of phosphorous and potassium and ANR for spinach in 2009.

	P (kg ha^{-1})*		K (kg ha^{-1})		ANR(%)* §	
Control	-7	E	-31	C	-	
Lucerne fresh late	36	B	70	A	21	ab
Lucerne silage	21	C	36	Bc	23	a
Poultry manure	141	A	61	Ab	15	b
Grass-clover fresh	20	C	89	A	22	ab
Lucerne fresh	14	D	59	B	22	ab

* Significant for *P*<0.05 after ANOVA

§ Apparent Nitrogen Recovery: N-yield treatment^{i} minus N-yield control, divided by N applied in treatment^{i}

When the ridges were formed in the 2010 potato experiment, soil mineral N in the lucerne silage early-application plots was, on average, 38 kg ha^{-1} higher than on the other plots. The marketable yield of the potatoes was in all treatments higher than in the control. The lucerne silage early-application treatments had the highest yields, but differences were not statistically significant. Nitrogen removal rates varied from 64 to 92 kg ha^{-1}.
The P content of forage crops closely matched actual crop demands of potatoes resulting in only relatively slightly positive nutrient balances (Table 3). For K, all treatments except the slurry/vinasse showed negative balances. The apparent nitrogen recovery (ANR) was lower for the poultry manure.

Table 3. Mineral balance of phosphorous and potassium and ANR for potato in 2010.

	P (kg ha^{-1}) §	K (kg ha^{-1}) §	ANR(%) *	
Control	−16	−139		
Lucerne fresh	11	-83	20	b
Lucerne silage early	2	−68	22	b
Lucerne silage	3	−58	20	b
Poultry manure	132	−28	11	a
Slurry/vinasse	−10	26	18	b

§ P and K based on measured input and default P and K content
*Significant for $P<0.05$ after ANOVA

Discussion

The nitrogen provided by lucerne and grass-clover gave a comparable or better ANR then nitrogen provided by poultry manure or slurry/vinasse. At crop level the P balance was much better using lucerne or grass-clover in both crops. Use of chicken manure resulted in a hyper-accumulation of phosphorus of 132-141 kg P ha^{-1}. The K balances were more ambivalent. This shows that cut-and-carry fertilizers such as lucerne and grass-clover have a high potential as providers of nitrogen fertilizer, while at the same time substantially reducing the risk of unbalanced P applications.
The overall effect on the mineral balance of introducing cut-and-carry fertilizers and reducing manure purchase in a farming system will strongly depend on the starting situation and the choices made by the farmer. An economic evaluation study indicated that the cut-and-carry fertilizers are of interest with prices above 12 € per ton of cattle slurry (€3.50 kg N^{-1}).

Conclusions

It is concluded that a cut-and-carry fertilizer system facilitates an effective use of perennial leguminous forage crops for sustaining inherent soil fertility. Based on studies with crops of spinach and potato it appears that use of fresh cut or silage materials from such leguminous crops will result in comparable yields, while also reducing the dependence of arable farms on external animal manures by closing nutrient cycles more effectively.

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Short-term fertilizer values of spring applied cattle slurry on grass fields

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Abstract

Four experiments were established in 2010, and five in 2011, to estimate the manure fertilizer values (MFV) of 30 Mg ha^{-1} spring-applied cattle slurry on semi-permanent well managed grass fields on Icelandic dairy farms. The slurry application was compared with mineral nitrogen (N) and phosphorus (P) fertilizers at different rates. Experimental plots were cut twice in the growing season for harvested dry matter (DM), N and P determination. Apparent N efficiency (ANE) for DM yield ranged from 16-26 kg DM kg N ha^{-1} in the mineral fertilized plots, compared to 9-10 kg DM kg total N ha^{-1} in the slurry plots. The MFV of the slurry was 0.40-0.42 for DM and 0.33-0.34 for N yield. In this study, the N in 30 Mg slurry equalled 40 kg N in mineral fertilizers for DM yield, and for N yield, 32-34 kg N. The MFV for P ranged from 0.82-0.89 corresponding to 7 and 10 kg fertilizer P in in the 30 Mg slurry. It was concluded that MFV in conjunction with ANE and P-recovery coefficients are good tools to estimate fertilizer values in slurry but more research is needed.

Keywords: cattle slurry, manure fertilizer values, N efficiency, phosphorus recovery

Introduction

Most of the cattle manure in Iceland is stored as low DM slurry (40-80 kg DM Mg^{-1}) and is surface spread on grass fields in autumn or early spring. Normal application rates are 20-40 Mg slurry ha^{-1}. The fertilizer value is estimated using database values that give average nutrient contents in cattle slurry, based on analysis from Icelandic research projects, and with subjective correction coefficients made by the farmer or adviser. The objective of this study was to evaluate with experimentation fertilizer values (DM, N and P yield) for spring applied cattle slurry on semi-permanent dairy farm grass fields.

Materials and methods

Nine experiments were established in 7 selected forage grass fields on two adjacent dairy farms in the Hörgár valley, North Iceland. These fields are on soils ranging in pH from 5.3-6.6 and from 12-48% w/w organic matter (in 0-10 cm soil profile). Weather data for this area is shown in Table 1. Dominating forage species varied between grass fields with mixtures of *Phleum pratense*, *Poa pratensis, Alopecurus pratensis* and *Deschampsia cespitosa.*

Table 1. Meteorological data during the growing seasons from Möðruvellir Station (at 65°46'N, 18°15'W), which is within 5 km of all experimental sites.

Month	Daylight hours	Mean air temp., °C		10 cm soil temp., °C		Precipitation, mm	
		2010	2011	2010	2011	2010	2011
May	18.5	5.1	6.3	5.2	4.6	19	10
June	21.4	6.9	11.5	7.6	10.2	10	4
July	20.2	11.7	11.1	10.4	11.9	8	25
August	16.6	9.5	11.2	10.3	12.4	34	42
Mean/sum	19.1	8.3	10.0	8.4	9.8	70	81

Experimental plots were laid out at 4 sites in 2010 with 30 Mg slurry ha^{-1} or four rates of N-P mineral fertilizers (0-150 kg N and 0-36 kg P ha^{-1}), and at 5 sites in 2011 with 30 Mg slurry ha^{-1} or 6 rates of N-P-K fertilizer (0-200 kg N, 0-33 kg P and 0-58 kg K ha^{-1}). Samples from the slurry were taken at the time of application for standard composition analysis (Table 2). All treatment plots were randomly allocated in three blocked replicates on each site. Mineral fertilizers and slurries were surface applied manually. All plots were cut twice during the growing season and weighed and sampled for standard yield, DM, N and P determination.

Table 2. Mean composition of cattle slurry applied in 2010 and 2011.

Year	DM, %	Total N	NH_4-N	P	K
		kg 30 Mg^{-1} cattle slurry			
2010	5.1	96	33	12	47
2011	5.6	99	37	8	55

The following calculations were made on total DM, N and P yield: (i) Apparent N efficiency from mineral fertilizer or slurry $(ANE)_i = (Y_i - Y_0)/N_i$ kg ha^{-1} (Beckwith *et al.*, 2002) where Y_i is the DM or N yield in $treatment_i$ and Y_0 is DM or N yield in plots with no fertilization; (ii) P_i recovery (PR) = PY_i/P $applied_i$ (Johnston and Syers, 2009); (iii) Manure fertilizer value (MFV) for DM, N or P yield = ANE_s/ANE_f where $_s$ = slurry and $_f$ = fitted fertilizer ANE by regressions of the general formula $y = a + b_1x^2 + b_2x + error,$ and x = N or P applied, a, b_1, b_2 are constants. Standard statistical regression models and variance analysis were made with the software JMP Discovery^{TM} to compare ANE and PR in mineral fertilizers and cattle slurry.

Results

In the absence of significant interactions between sites and treatments on ANE for DM and N yield and P recovery, only mean treatment effects for 2010 and 2011 are presented (Figure 1). ANE for DM yield ranged from 16-21 kg kg^{-1} N in 2010 and 18-26 kg kg^{-1} N in 2011 in the mineral fertilizer treatments, and 9 and 10 kg kg^{-1} N in 2010 and 2011 respectively, in the slurry treatments (Figure 1a,b). The difference between slurry and mineral fertilizer ANE for DM yield was highly significant (P<0.0001) in both years but not significant between fertilizer N rates. The MFV for DM yield was on average 0.42 in 2010 and 0.40 in 2011. ANE for N yield was 0.57-0.68 N in 2010 and 0.44-0.70 N in 2011 in the mineral fertilizer treatments, but 0.21 and 0.23 N in slurry treatments in 2010 and 2011 respectively (Figure 1c,d). The difference between slurry and mineral fertilizer ANE for N yield was highly significant in both years and between mineral fertilizer rates in 2011 (P<0.0001). MFV for N yield was 0.33 and 0.34 for 2010 and 2011 respectively. MFV for DM and N yield is similar on average between years in spite of temperature differences (Table 1). 30 Mg slurry equalled 40 kg N in mineral fertilizers for DM yield and for N yield, 32-34 kg N. PR (P removed with harvest/P applied) declines with increased fertilizer rates (Figure 1e,f). Break-even mineral fertilizer rate (P applied = P removed with harvest) was 20 kg P ha^{-1} in 2010 and 12 kg P ha^{-1} in 2011. The MFV for P yield was 0.82 P in 2010 and 0.89 in 2011 and therefore the break-even slurry rate was 24 kg P ha^{-1} in 2010 and 13 kg P ha^{-1} in 2011. This difference between years is attributed to extremely low spring and early summer temperatures in 2010 (Table 1).

Conclusions

MFV in conjunction with ANE and PR are good tools to estimate fertilizer values in slurry but more research on application timing and fertilizer × slurry interactions are needed to improve the picture for Icelandic conditions.

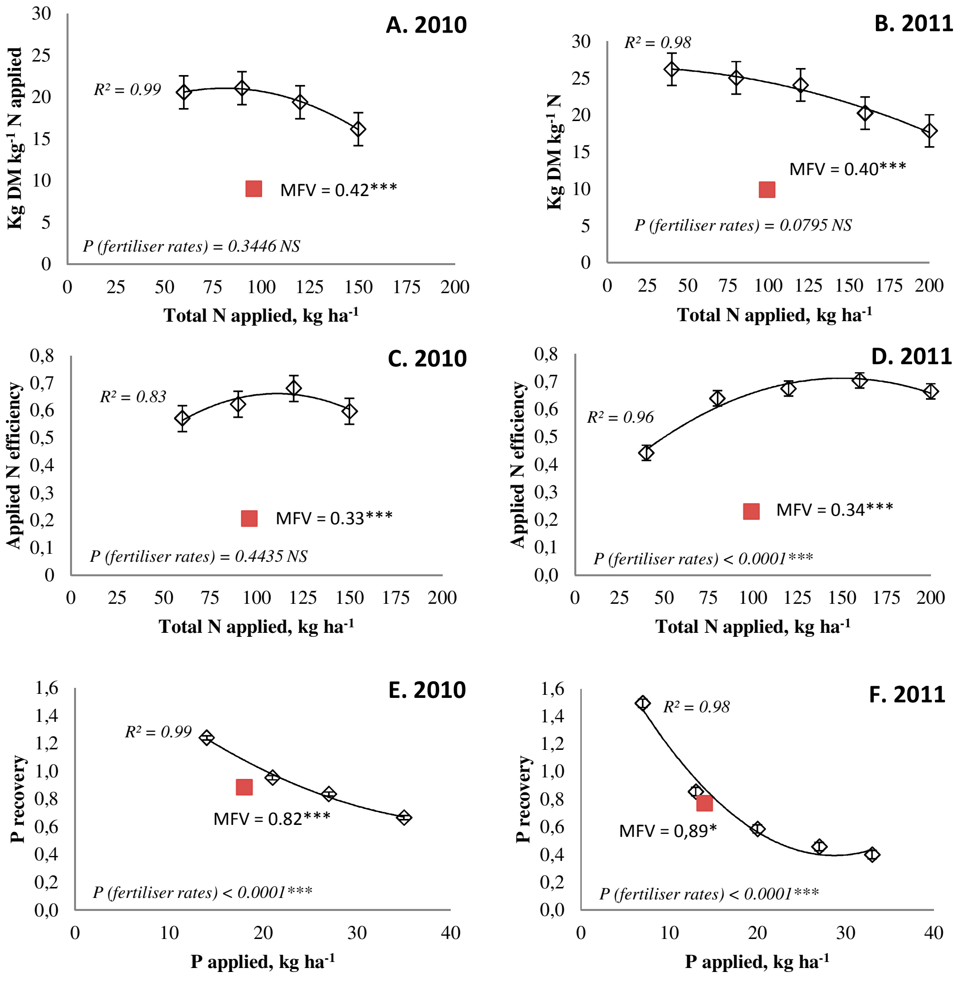

Figure 1. The effect of N application on apparent N efficiency (ANE) on dry matter (DM) (A, B) and N (C, D) yield and P application on P yield recovery (E, F) in grass fields on two dairy farms in North Iceland. Mean responses from 4 sites in 2010 and 5 sites in 2011. Open diamonds and rectangles show N efficiency and P recovery means from mineral fertilizer and slurry applications respectively. MFV = manure [slurry] fertilizer value. Vertical bars are 2* mean standard errors (error d.f.; 9 in 2010, 16 in 2011). *P* = probability levels, NS = not significant between rates, * = significant levels. R = regression line fit with the general formula; $y = a+b_1x^2+b_2x$, were x = N or P applied, a,b_1,b_2 = constants.

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Evaluation of temporal nutrient dynamics in pasture-based beef production systems

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Abstract

Achieving optimal mineral nutrition and animal performance in pasture-based livestock systems requires maintaining adequate mineral concentrations in plant tissue and supplements. Numerous factors cause variation in pasture-herbage mineral concentration. The objective of this study was to determine how herbage mineral concentrations vary between months throughout the growing season and to determine the relationships between mineral concentrations and environmental variables. Twelve sets of cool-season paddocks stocked with 7 or 8 beef cow-calf pairs were evaluated during a 4-year experiment in Virginia, USA. Pasture herbage was harvested once per month from April to October and analysed for mineral concentration. Herbage tissue mineral concentrations fluctuated throughout each growing season, with lowest average concentrations in July and August. A multivariate technique, redundancy analysis (RDA), was employed to determine the relationship between herbage nutrient concentrations and environmental variables. Soil moisture appears to be well related to variation in the concentration of many nutrients. A better understanding these relationships may lead to predictive modeling of pasture herbage nutrient concentrations for periods of abnormal weather and allow for the adjustment of mineral supplements to optimize animal performance.

Keywords: pasture herbage, mineral nutrient, seasonal variation, redundancy analysis

Introduction

Maintaining adequate mineral concentrations in pasture herbage for pasture-based beef production is essential in order to optimize animal performance. Several metabolic disorders occur as a result of improper mineral nutrition of livestock. Mineral nutrients have been shown to vary across the growing season. In addition to plant maturity, several abiotic environmental factors have been shown to influence mineral nutrient concentration in plants. Understanding the quality of the seasonal variation in nutrient concentration is important for the management of livestock supplements to prevent livestock mineral imbalances and unintended losses to the environment. Laboratory experiments have shown effects of soil moisture, soil temperature, humidity, and light intensity on plant nutrient concentrations (White, 2012). The effects and interactions of these environmental factors become complex *in situ*. Multivariate techniques allow for a more comprehensive analysis of a range of nutrients and other variables. The objective of this study was to explain the seasonal variability of 10 essential plant nutrients by several environmental variables. This work could lead to the ability to predict changes in expected seasonal herbage nutrient concentration variation and allow for adjustment of livestock mineral supplementation.

Materials and methods

Twelve groups of 7 or 8 beef cow-calf pairs were rotationally-stocked in eight 0.8 ha paddocks per group. The pasture consisted of *Schedonorus phoenix* (Schreb.) Dumort., *Poa pratensis* L. and *Trifolium repens* L., and was well established on Frederick and Christian silt loams. A stratified sample of 20 of the 96 total paddocks was selected for this study, and a randomly selected 0.75 m × 3.5 m swath within each paddock was harvested at 8 cm once per month from April-October of 2007-2011. Herbage samples were extracted with microwave digestion, and analysed for P, K, S, Ca, Mg, Fe, Mn, Al, Cu, Zn, B by Inductively Coupled Plasma Atomic Emissions Spectroscopy. Nitrogen was analysed by the combustion method. Average daily soil temperature and percent moisture at 10.2 cm, precipitation, air temperature, and relative humidity were measured at the site. Day length was calculated (Hijmans *et al.* 2012) as a proxy for light intensity. Soil temperature and moisture, precipitation, and air temperature were averaged for 15 days prior to each harvest date and 5 days prior for relative humidity and day length.

Each nutrient was analysed separately using ANOVA, including month as treatment with paddock sampled and year as blocking factors, and a month by year interaction term. Residuals were checked for normality. Fe and Al showed a large number of outliers, likely due to soil contamination. Fe and Al distributions could not be corrected through transformation and were not included in subsequent analyses. Remaining nutrients were transformed by $\log_{10}$. Mean separation was determined using Tukey's HSD ($P < 0.05$).

For RDA, the mean nutrient concentration for each harvest month and year was taken and assigned to the corresponding environmental variables. RDA was performed, using the 'vegan' package in R, to explain the variability of nutrient concentrations by the environmental variables (Oksanen *et al.*, 2012). Significant environmental variables were selected using permutation. The final model included soil temperature, soil moisture, humidity and day length.

Results and discussion

Nutrient concentrations by month differed significantly among each nutrient ($P<0.05$) (Table 1). Lowest concentrations of N, P, K, S, Ca, Mg, Cu, Zn occurred in July and August. For most nutrients, the highest concentrations occurred in April and May, diminished to lowest values in July and August, and increased again in September and October. Calcium and Mg tended to be at higher concentration in August and September. Concentrations of Mn and B fluctuated throughout the growing season. A month by year interaction was found but was caused by aberrations in the autumn 2009 concentrations (data not shown).

Table 1. Pasture herbage mineral nutrient concentration by month (four year averages). For a given row, letters indicate significant difference between months based on Tukey's HSD ($P<0.05$). Analysis was performed with log-transformed data; results are not transformed.

Nutrient	April	May	June	July	August	September	October
N (g kg^{-1})	31.7^{a}	25.3^{b}	19.8^{c}	15.8^{e}	17.8^{d}	24.1^{b}	23.9^{b}
P (g kg^{-1})	2.8^{a}	2.7^{a}	2.5^{b}	2.0^{c}	2.3^{b}	2.4^{b}	2.4^{b}
K (g kg^{-1})	21.9^{a}	22.2^{a}	21.2^{ab}	15.3^{d}	15.5^{d}	19.8^{bc}	19.7^{c}
S (g kg^{-1})	2.4^{a}	2.1^{bc}	2.0^{cd}	1.6^{e}	1.9^{d}	2.1^{b}	2.0^{bc}
Ca (g kg^{-1})	5.5^{ab}	4.7^{cd}	5.7^{a}	4.5^{d}	5.5^{ab}	5.6^{a}	5.0^{bc}
Mg (g kg^{-1})	2.3^{d}	2.2^{d}	2.5^{c}	2.3^{d}	2.8^{b}	3.1^{a}	2.7^{b}
Mn (mg kg^{-1})	105.4^{a}	99.9^{abc}	78.5^{c}	83.3^{bc}	93.0^{ab}	87.3^{bc}	86.9^{bc}
Cu (mg kg^{-1})	11.4^{a}	9.0^{b}	7.8^{c}	6.8^{d}	8.1^{c}	8.3^{bc}	8.7^{bc}
Zn (mg kg^{-1})	35.6^{a}	30.6^{b}	25.1^{b}	22.2^{b}	24.0^{b}	24.4^{b}	26.1^{b}
B (mg kg^{-1})	6.6^{a}	5.0^{b}	6.5^{a}	4.3^{bc}	4.9^{b}	4.7^{bc}	4.0^{c}

The RDA captured 60.9% of the unconstrained variability and 39.1% of the variability constrained by the environmental variables (Figure 1). These values are acceptable in reference to other ecological studies using RDA (Ter Braak and Verdonschot, 1995). Variation was well explained along the first dimension, which explains variability in soil moisture and soil temperature. Three groups of nutrients emerged in the RDA ordination plot.

Nitrogen, P, K, S, and Cu were correlated to each other, high soil moisture, and to a lesser extent, low humidity and soil temperature. Manganese, Zn, and B appear proximate to the origin, indicating that their correlations to the environmental variables were weak. Calcium and Mg were correlated to each other, high soil temperature, low soil moisture and short day-lengths. The separation of Ca and Mg from the remaining nutrients is supported by the difference in seasonal variation shown by the ANOVA.

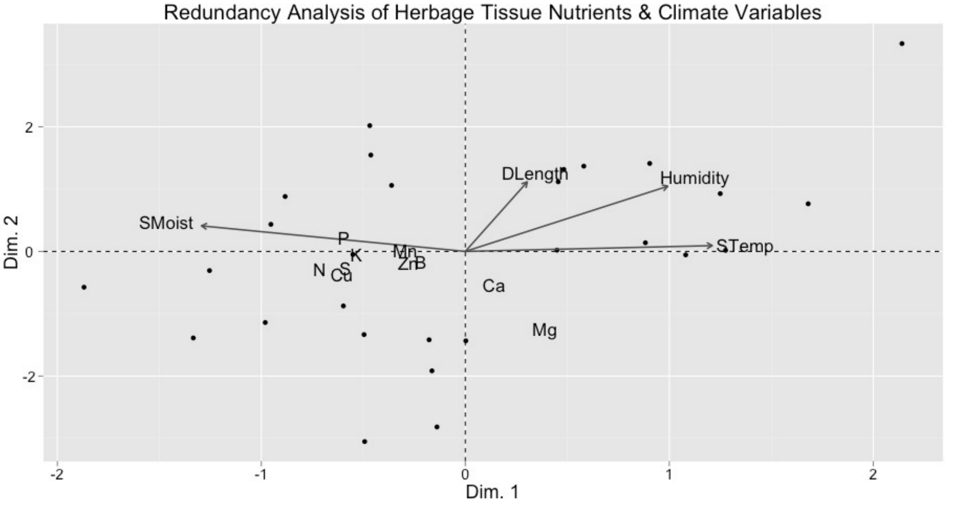

Figure 1. RDA ordination plot. Points are harvests. Environmental variables are indicated with arrows.

Conclusions

Many factors and interactions influence nutrient uptake and subsequent concentration in plants. This study showed that a portion of the variability in herbage nutrient concentration can be explained by several environmental variables. Soil moisture was most closely related to the concentrations of plant macronutrients. While monthly species composition was not measured, it probably explained little of the temporal variation in nutrient concentration. Grasses and legumes vary in terms of mineral composition, but the overall quality of pasture herbage is the important factor for livestock production. Further use of environmental variables and multivariate analysis could lead to the development of predictive models that better optimize livestock mineral supplementation, prevent livestock health disorders, reduce production costs and limit nutrient losses to the environment.

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Optimization of mineral nutrition for perennial ryegrass seed production

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is a popular forage grass species in Latvia. More information is needed concerning its fertilization requirements for the purpose of seed production because grass seed production is a very important factor for farmers' incomes in Latvia. This paper presents results on the effect of different application rates of NPK fertilizers on perennial ryegrass seed production, and it further determines relationships among perennial ryegrass seed trials.
Two years of field experiments were carried out on a sod podzolic sandy loam soil. The following mineral fertilizer rates were used: N and P each 0, 13, 26, 39, 52 and K of 0, 33, 66, 100, 133 kg ha^{-1}. This research examines the effects of varying the mineral fertilizer rates on seed yield by using the perennial ryegrass cultivar 'Spidola' and in the context of the local Latvian weather conditions. Lodging was higher on treatments with high fertilization rates. The simple correlation indicated that seed yield was highly significantly related to seed weight and generative tiller numbers. The seed yields were 584-1019 kg ha^{-1}, average of 2 years, during the first year of use.

Keywords: perennial ryegrass, fertility levels, seed production

Introduction

Perennial ryegrass *(Lolium perenne* L.) is one of the most important grass species of temperate regions (Wilkins, 1991). In Latvia, perennial ryegrass is also a species that has a great seed production potential. At present there is no research in Latvia on which it is possible to make good fertilizer-use recommendations and to provide advisory support for farmers involved in perennial ryegrass seed production. Perennial ryegrass is a major component in different seed mixtures that are used for grassland management and forage production (Slepetys, 2001). This grass species plays an important role in grassland productivity and forage quality. Seed yield response of cool-season grasses to spring-applied N is usually limited because of lodging (Young *et al*., 1999). Lodging of perennial ryegrass plant is a widespread problem. Crop lodging reduces seed yield and interferes with seed harvest (Young *et al*., 1996). Lodging is usually associated with conditions such a high rainfall, mild temperatures and high levels of available soil N that would otherwise favour rapid plant growth and high seed yields. Both the time and severity of lodging influence seed yield. Seed weight is often thought to be important in determining seed yield and, even if not always, it is also a factor in obtaining a good establishment (Negri and Falcinelli, 1990).
The objectives of this study were to examine the effect of fertility level on perennial ryegrass seed production and determine relationships among perennial ryegrass seed trials.

Materials and methods

Field experiments were carried on sod podzolic sandy loam soil with a pH_{KCl} of 6.5, plant available P_2O_5 of 110 and K_2O 204 mg kg^{-1}, and organic matter in soil 21 g kg^{-1}. The plots were established according to a randomized complete block design with four replicates. The plot size was 16 m^2. Five fertilizer rates were applied in both years. The following mineral fertilizer rates were used: N and P each 0, 13, 26, 39, 52 kg ha^{-1} and K at 0, 33, 66, 100, 133 kg ha^{-1}. Perennial ryegrass (12 kg ha^{-1}) was sown using a Nordsten seed drill in May 2010 and 2011 after field preparation. Weed control was carried out using MCPA herbicides. Lodging of the perennial ryegrass stand was evaluated during the growing season using a scale from 1–9 (1: the stand is completely lodged; 9: lodging is not observable). Seed yield was recorded from the first year sward use. Analysis of yield components and other parameters were also recorded. Microsoft Excel was used for mathematical processing analysis of the data subprogram (Berzins, 2002).

Results and discussion

Perennial ryegrass cv. 'Spidola' is a tetraploid cultivar. This cultivar shows a preference for certain growing conditions: it is better suited for mineral soils, prefers loamy or loamy sand soils, and does quite well on clay soils, but is somewhat less responsive for light soils; it is suitable for inclusion in seed mixtures planned for establishment of permanent pastures and is a late-maturing pasture grass, good for late grazing (Bumane, 2010).

All mineral fertilizer rates applied had a positive effect on seed yield of perennial ryegrass. The seed yield was increased on average by 14 to 74%, depending on mineral fertilizer rates applied and the ratio of fertilizer elements (Table 1). Mineral fertilizer-use efficiency was significantly affected by meteorological conditions over the trial years.

The number of generative tillers is a component of the potential grass seed yield established during vegetative plant development and before flowering (Hebblethewaite *et al.*, 1980). The seed yield increased with the growth of generative tiller numbers. The greatest seed yield was obtained with average generative tiller numbers 1200-1300 per m^2. The seed yield and generative tiller numbers is in positive correlation (R^2=0.53).

Table 1. Effect of fertility levels on seed yield of perennial ryegrass 'Spidola' (average of 2 yrs, first year of use).

Levels of fertilizer element, kg ha^{-1}			Seed yield, kg ha^{-1}	1000-seed weight, g	Lodging resistance, 1-9	No. of generative tillers m^{-2}
N	P	K				
0	0	0	584	2.9	8.8	1003
0	26	66	809	3.0	8.6	1059
30	13	33	663	2.9	7.0	1128
30	13	100	670	2.9	6.2	1163
30	39	33	772	2.9	6.9	1269
30	39	100	776	2.9	6.6	1222
60	0	66	808	3.0	5.7	1267
60	26	0	839	2.9	5.6	1330
60	26	66	838	3.0	5.0	1298
60	26	133	823	3.1	4.6	1221
60	52	66	793	3.0	4.9	1228
90	13	33	844	3.1	3.8	1212
90	13	100	883	3.1	3.4	1328
90	39	33	906	3.1	3.5	1364
90	39	100	923	3.0	3.6	1204
120	26	66	909	3.1	2.5	1307
120	52	133	1019	3.0	2.6	1318
	Average		805	3.0	5.2	1235
	$LSD_{0.05}$		92	0.1	0.8	133

In our experiments the highest seed yields were obtained using balanced NPK doses, which were as follows: N_{120} P_{52} K_{133} – 1019 kg ha^{-1} and N_{90} P_{39} K_{100} – 923 kg ha^{-1}.

The average 1000 seed weight was 2.9-3.1 g; seed yield correlates with seed weight (R^2=0.42). The density of productive stems was 869 per m^2 in the unfertilized treatment. In

plots treated with NPK fertilizer, the density of productive stems was in the range from 1121 to 1298 per m^2 or 25-43% more than from the unfertilized plots.
Lodging resistance is one of the parameters that are important for seed production (Young *et. al.*, 1996). The estimated lodging resistance in perennial ryegrass plots that by received no NPK fertilizer was 8.8.

Conclusion

Applications of balanced application rates of NPK fertilizers provide comparatively high seed yields (84 to 1019 kg ha^{-1}) of perennial ryegrass cv. 'Spidola' under Latvia's agroclimatic conditions.
The fields of grass seed are more productive with estimated lodging resistance scores of 6-7.
Optimization of mineral nutrition in perennial ryegrass seed production fields has a positive effect on yield structure and seed quality parameters.

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Nutrient uptake in soil niches affected by plant species and drought stress

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Abstract

Benefits of grassland mixtures over monocultures are hypothesized to be based on niche complementarity among plant species. The objective of this experiment was to use tracer methods to assess the below-ground niche differentiation between deep and shallow rooting species under benign and drought conditions. A field experiment was established with plots containing monocultures and mixtures of *Lolium perenne* (Lp), *Cichorium intybus* (Ci), *Trifolium repens* (Tr) and *Trifolium pratense* (Tp) according to a simplex design. Using rainout shelters, half of the plots were subjected to a drought treatment of 10 weeks summer rain exclusion. During the drought period, a rubidium chloride solution was applied in 50×50 cm sub-plots at 5 and 35 cm depth and Rb concentrations and uptake in the individual plant species was measured. Results show that Rb uptake was much higher from 5 cm depth compared to 35 cm depth for all species. There was a relative shift of Rb uptake to the deeper soil layer under drought conditions for all species. The deep rooting species (Ci and Tp) were shown to have relatively higher Rb uptake at 35cm, and the shift to deeper root layers under drought conditions was larger.

Keywords: grassland mixtures, Rubidium, rare elements, niche differentiation

Introduction

Growing grassland mixtures compared to monocultures can result in increased yields, greater stability in response to disturbance, reduced invasion by weeds and improved nutrient retention. The proposed mechanisms behind these responses include positive interspecific interactions and niche differentiation (Hooper *et al.*, 2005). For example, the interaction between species with and without the ability for symbiotic N fixation, has been shown to result in significant yield benefits (Nyfeler *et al.*, 2011). One potential mechanism for which there is little evidence in agronomic grassland systems is soil niche complementarity between deep-rooting and shallow-rooting species. This mechanism may be of particular importance under drought stress, when soil nutrients may be less available in dry top layers compared to relatively moist deep layers.
Tracers, and more specifically rare elements (Rb, Cs, Li, Sr) can help to identify patterns in nutrient uptake from different depths for species grown in mixtures (Fitter, 1986). Rb is an analogue for K+ (Marschner, 1995) and previous work has shown Rb to be a reliable tracer, in terms of stability in soil and uptake potential (Hoekstra *et al.*, 2012).
The objective of this experiment was to use Rb as a tracer to assess the belowground niche differentiation in grassland mixtures and monocultures of shallow-rooting and deep-rooting species under benign and drought conditions.

Materials and methods

In August 2010, monocultures and mixtures were sown in 66 plots of 3×5 m at Tänikon research station, Switzerland, in a completely randomized design with 3 replicates. Four model species were selected based on their ability for symbiotic N_2 fixation (N-fixing, N and not N-fixing, Z) and rooting depth (deep rooting, D and shallow rooting, S): *Lolium perenne* (Lp, Z-S), *Cichorium intybus* (Ci, Z-D), *Trifolium repens* (Tr, N-S) and *Trifolium pratense* (Tp, N-D). The plots were sown according to a simplex design (Kirwan *et al.*, 2007), consisting of four monocultures, six binary stands (50% of two species), and one equal stand (25% of each of four species).

Plots were cut 7 times in 2011, including a clearing cut in April. Using rainout shelters, half of the plots were subjected to a drought treatment of 10 weeks summer rain exclusion in 2011 (spanning two regrowth periods).

In order to assess the root activity of the different species under control and drought conditions, tracers were applied four to five weeks before the end of the drought period. A solution containing RbCl (0.17 mol L^{-1}) was injected at 5 and 35 cm depth in two separate sub plots (50×50 cm) per plot in 25 holes per sub-plot (5×5 grid). In each hole, 1.5 mL of tracer solution was injected, using a 50 mL multipipette with a four-sideport needle attached via a rubber tube.

Plant material was harvested at the end of the drought period and separated into the four sown species. The Rb concentrations in the plant samples were determined with an inductively coupled plasma mass spectrometer (ICP-MS, 820 Varian).

The Rb concentration was corrected for the background Rb concentration (measured in same species that did not receive Rb injections). Plant Rb uptake (RbU) was divided by the sown proportion of the individual species, to correct for the difference in species dry matter yield in monocultures and mixtures. The proportion of RbU from 35cm was calculated as RbU_{35} / ($RbU_5 + RbU_{35}$).

Results and discussion

During the drought period a total of 306 mm precipitation was excluded from the drought plots, which is equivalent to 33% of total annual precipitation. This resulted in a reduction of soil moisture content during the tracer injection experiment of 31% and 5% at 5 and 35 cm soil depth, respectively.

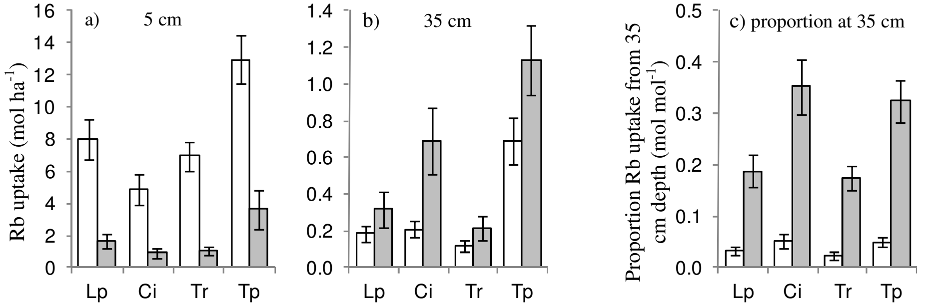

Figure 1. The Rb uptake (mol ha^{-1}) from a) 5 cm depth, b) 35 cm depth and c) the proportion of Rb taken up at 35 cm depth (mol mol^{-1}) for the four plant species under control (open bars) and drought (shaded bars) conditions. Error bars represent the SE.

At 5 cm injection depth, the corrected RbU was significantly higher (P<0.001) for control compared to drought plots for all species (Figure 1a). This confirms that the reduction of soil moisture was sufficiently strong to limit the root activity in the top soil layer. There was also a significant species (P<0.001) effect and RbU decreased in the order Tp > Lp > Tr > Ci.

RbU at the 35cm injection depth was on average a factor 11 lower than for 5cm. At 35cm, RbU was significantly (P<0.001) higher for the drought compared to the control plots (Figure 1b). This indicates that under drought conditions, the water and nutrient availability at 35cm

depth was much less restricted than in the top soil. Additionally, there was a significant (P<0.001) species effect on the RbU from 35 cm, which was higher for the deep-rooted species Ci and Tp compared to shallow-rooted species Lp and Tr.
As a result, the proportion of RbU from 35 cm was significantly (P<0.001) higher for drought compared to control plots (Figure 1c), indicating a shift of nutrient uptake to deeper soil layers under drought conditions. This would be in line with the concept that plant roots increase activity or even grow towards zones with higher water content and therefore nutrient availability (Skinner, 2008).
Additionally, the proportion of RbU from 35 cm was significantly (P<0.001) higher for the deep rooting species Ci and Tp. Also, the shift to deeper layers under drought conditions was stronger for these two species compared to Lp and Tr (significant species × drought interaction, P<0.01). There was no effect of whether the species were grown in monoculture or mixture on the RbU or the proportion of RbU from 35 cm, for the different species.

Conclusions

The tracer method showed a clear difference in niche occupation between shallow and deep rooting species, with deep rooted species having increased tracer uptake at deeper depths. The drought treatment also resulted in a shift towards uptake from the deep soil depth, particularly for deep rooting species.
The next step will be to determine whether these findings translate into i) yield advantages of deep rooting species under drought stress and ii) yield advantages of combining deep and shallow rooting species in mixtures.

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Herbage composition and nutrient content in dairy pasture as influenced by long-term potassium fertilization

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Abstract

The experiment has been carried out in a long-term pasture since 1961 at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The focus of this study was to estimate the productivity, botanical composition and herbage nutrient content of the pastures in relation to the long-term potassium fertilizing regimes. Potassium is considered to be an essential nutrient, although it has a weaker effect on increasing pasture productivity than nitrogen. In our experiment, the botanical composition in old pasture swards depended on climatic factors and potassium fertilizer rates. The greatest number of forb species was found during droughty vegetation periods, compared with wetter ones. Non-fertilized treatment produced a significantly lower yield than when fertilized. The structure of pasture was dynamic and depended on the weather conditions during the experimental period. During a droughty vegetation period, the proportion of legumes decreased while that of grasses and other herbs significantly increased. The data suggested that with a regular application of potassium in a long-term pasture it is possible to maintain a good sward with a sufficient amount of legumes and a stable herbage nutrient content.

Keywords: potassium, dry matter yield, legume, crude protein

Introduction

Sustained forage production is a key element for successful grazing systems and may be influenced by plant diversity in natural grassland ecosystems (Tilman, 2001). As a result, the pasture botanical and herbage chemical composition on dairy farms may be affected by a long-term grassland management system. Potassium has received less attention than nitrogen, and phosphorus is at the core of interest for agriculture and the environment. However, potassium remains one of the relevant elements, although it has a weaker effect on increasing pasture productivity than nitrogen (Kayser and Isselstein, 2005). Not only the number of forage species, but also their interaction with one another, play a significant role in achieving greater forage productivity (Sanderson *et al*., 2005).

Materials and methods

The long-term experiment was carried out on an old pasture established in 1961. The soil of the experimental pasture is loam-textured *Endocalcari-Epihypogleyic Cambisol*. Soil potassium (K) concentration ranged from 76-112 mg kg^{-1}, soil phosphorus (P) ranged from 77-98 mg kg^{-1}. The experiment was designed as a randomized complete block with four replicates. The total size of a treatment was 25 m^2. The seed mixture consisted of white clover (*Trifolium pratense* L.) 15%, red clover (*Trifolium pratense* L.) 5%, timothy (*Phleum pratense* L.) 15%, Kentucky bluegrass (*Poa pratensis* L.) 25%, and meadow fescue (*Festuca pratensis* L.) 40%. Four rates of potassium chloride (K): 0, 30, 60, 90 kg ha^{-1} in combination with superphosphate (P): 30 kg ha^{-1} were tested. The fertilizers were applied every spring before resumption of vegetation. The grazing season started in the middle of May and lasted until the beginning of October. During the whole experiment period, the pasture was grazed by 2-2.5 cows ha^{-1} yr^{-1}, on average 4 times per season, which lasted for 150 days. The pasture was grazed when the sward had reached an average height of 20-25 cm. Before each grazing, the dry matter (DM) was estimated. The cut herbage was immediately weighed, two 0.5 kg samples of fresh herbage were taken for DM and botanical composition determination. The botanical composition of the samples was established after separation.

Results and discussion

During the final experimental period 2007-2009, the percentage of legumes (white clover) increased, especially in a wet year (2008), compared to that at the beginning of the experiment (1969). The proportion of legumes over 49 years varied from 7.9 to 15.7 % depending on the fertilization level (Figure 1).

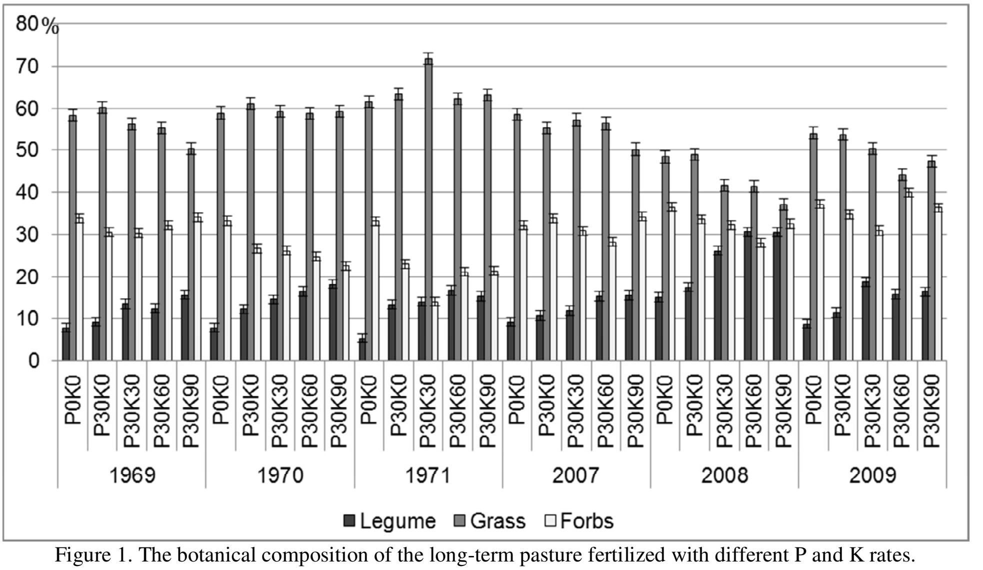

Figure 1. The botanical composition of the long-term pasture fertilized with different P and K rates.

The content of legumes ranged from 9.3 to 15.6% after 37 years, depending on the fertilization level. Grasses accounted for the largest share (37.0-63.4%) in the herbage during the experimental period. The proportion of sown grasses and legumes in each experimental year depended on fertilizer rates. All sown species persisted throughout the entire experimental period, except for red clover. The proportion of forbs increased in all K-fertilized treatments. Consequently, the percentage of grasses significantly decreased in those treatments. The long-term K fertilization indicated its importance to white clover. Low soil K availability has been reported to reduce legume growth (Høgh-Jensen, 2003), which also agrees with our findings suggesting that the share of legumes increased having fertilized with K. In the final experimental years, herbage yield varied from 3.29 to 3.42 t ha^{-1} in the treatments with K fertilization (Table 1). Significant differences were observed between the lowest and the highest K fertilizer rates. Non-fertilized pasture produced significantly lower DM yield compared with fertilized pasture. In the final years, DM yield was twice as high as that 40 years ago. Fertilization at $P_{30}K_{30}$ significantly increased DM yield compared with non-fertilized and P_{30} – fertilized plots. Higher K fertilizer rates did not exert any significant effect on DM yield compared with the lowest (K_{30}) rate. However, according to K fertilizer rates, the trend of DM yield increasing was observed.

Table 1. The concentration (g kg^{-1}) of crude protein (CP), phosphorus (P) and potassium (K) in herbage and dry matter (DM) yield (t ha^{-1}).

	Averaged over 1969–1971				Averaged over 2007–2009			
Treatments	CP	P	K	Yield, DM	CP	P	K	Yield, DM
P_0K_0	158	2.9	26.5	2.17	186	2.8	23.9	5.10
$P_{30}K_0$	170	3.6	26.3	3.29	186	4.3	20.7	6.25
$P_{30}K_{30}$	173	3.6	27.6	3.32	194	4.6	22.8	6.74
$P_{30}K_{60}$	177	3.6	29.7	3.48	197	4.6	25.1	7.41
$P_{30}K_{90}$	176	3.7	30.4	3.62	196	4.2	28.5	7.42
$LSD_{0.05}$	6.19	0.33	1.61	0.30	8.16	0.30	2.26	0.70

LSD – least significant differences at P<0.05

The long-term experiment showed that the CP concentration in herbage met the needs of cows in all the cases studied. Potassium fertilization significantly increased CP concentration. Cows given 2.4 g kg^{-1} P in diets produced significantly less milk and lost body weight. There were no significant differences in the variables measured between cows given dietary P concentrations of 3.2 and 4.2 g kg^{-1} (Call *et al.*, 1987). Potassium fertilization did not exert any significant effect on P. However, K concentration in herbage was significantly increased by the lowest K_{30} rate. In final years, the concentration of K in herbage was by 1.9-5.6 g kg^{-1} lower compared to that in the beginning of the experiment.

Conclusion

The effect of long-term PK fertilization regimes maintained sustainable pasture. After 49 years, the sown legumes and grasses accounted for 60-71.8% in the herbage yield. The nutrition value of herbage was high and the concentrations of CP, P and K met the needs of cows. In terms of pasture productivity parametres, K_{60} rate had a significant advantage over K_{30} rate.

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Farm-gate phosphorus balances and soil phosphorus concentrations on intensive dairy farms in the south-west of Ireland

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Keywords: farm-gate balance, Phosphorus balance, soil phosphorus, dairy farming

Abstract

This study examined farm-gate P balances and soil test P (STP) concentrations on 21 dairy farms in the south-west of Ireland over four years. Average stocking density (and standard deviation) on the farms was 2.4 (0.4) livestock units per ha. Annual mean import of P onto farms was 21.6 (1.9) kg ha^{-1}. Fertilizer P accounted for 0.47 (0.041), concentrates 0.35 (0.060) and organic manures 0.18 (0.034) of imported P. The mean annual P balance per farm was 9.4 (1.2) kg ha^{-1}, ranging from -3 to 22 kg ha^{-1} and mean P-use efficiency was 0.71 (0.05) ranging from 0.24 to 1.37. The mean soil test P (STP) per farm, following extraction using Morgans solution, was 8.13 (2.9) mg L^{-1} of soil and ranged from 4.2 to 17.5 mg L^{-1}. There was a correlation (R^2=0.34; P<0.01) between STP and P balance; farms with a deficit of P tended to have agronomically sub-optimal STP and vice versa. The high variation between and within farms in STP indicates that farmers were unaware or were not making efficient use of STP results, causing agronomically sub-optimal soil P status in some fields and potentially environmentally damaging excesses on others often within one farm.

Introduction

Phosphorus is an essential nutrient for plant and animal production and is an important input on dairy farms. Once P is applied at a rate that closely matches crop requirements, the potential for surface and subsurface P loss to water is minimized (Kurz *et al*., 2005). Agriculture is the single biggest contributor of P to Irish waters and eutrophication remains Ireland's most serious environmental pollution problem (McGarrigle *et al*., 2010). P inputs to grassland in Ireland have been regulated since 2006 by Statutory Instruments (SI); the most recent version being SI 610 2010 (Anon., 2010). In the SI, soil P concentrations in grassland soils are divided into four categories: Indices 1 to 4 (Table 2). Data collected over the four years (2003 to 2006) in this study were used to investigate the extent to which the farms in the present study would be able to comply with the limits on P use in the run up to the implementation of these regulations, first implemented in 2006.

Materials and methods

Data were collected from 21 dairy farms located in the south-west of Ireland. The selection of the farms involved and much of the on-farm recording was described by Treacy *et al.* (2008). Permanent grassland-based milk production from spring-calving cows was the main enterprise on the farms. Eight soil samples were taken on each of the farms during the study period and analysed for soil pH and soil P (extracted using Morgan's solution; Byrne, 1979). Farm-gate P balances were calculated for each calendar year taking account of P imports and exports. Imports included fertilizer, concentrates and organic manures. Exports of P include the recovery of P in agricultural products such as milk and livestock. The farm-gate balance was the difference between imports of P onto the farm and export of P in products. P-use efficiency was calculated as the proportion of imported P recovered in products.

Table 2. Index system 1 to 4 for soil P and actual Morgan soil test range for P in the Statutory Instruments (SI) 378, 2006, SI 101, 2009 and SI 610, 2010 (Anon. 2006; 2009; 2010).

Soil Index	1	2	3	4
	Soil P ranges (mg dm^{-3})			
SI 378, 2006; 101, 2009; 610 2010#	0.0 – 3.0	3.1 – 5.0	5.1 – 8.0	> 8.0

#Anon. (2006; 2009; 2010)

Table 3. Mean values for soil pH and soil P, imports of P in fertilizer, concentrates and organic manures, exports of P in milk and livestock, farm-gate P balances and P-use efficiency for 21 dairy farms over 4 years.

	Year				s.e.	*F*-test
	2003	2004	2005	2006		
Farm-gate P imports (kg ha^{-1})						
Fertilizer	12.0	10.7	9.3	8.9		
Concentrate	8.4	6.3	6.3	9.3	0.42	***
Organic Manures	4.1	3.6	4.5	3.1		
Total	24.4	20.6	20.2	21.3		
Farm-gate P exports (kg ha^{-1})						
Milk	7.4	7.2	7.2	7.7	0.14	*
Livestock	5.8	4.7	4.5	4.4	0.35	*
Total	13.2	11.8	11.7	12.1	0.38	*
Farm-gate P surplus (kg ha^{-1})	11.2	8.8	8.5	9.2		
P-use efficiency	0.65	0.77	0.74	0.68		

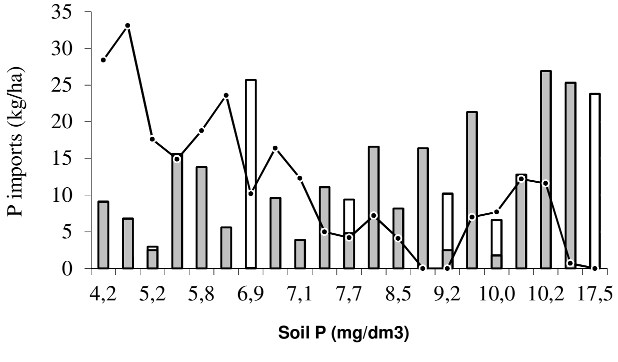

Figure 3. Imports of P in fertilizer (□) and in manure (□) relative to the amounts (line) allowed under Statutory Instruments 610, 2010 (Anon., 2010), after deducting P in concentrates imported onto farms, across the range of average soil P concentrations on 21 intensive dairy farms.

Results and discussion

Farm production during the 4 years has been presented by Treacy *et al.* (2008). Soil pH for the entire set of soil samples from the 21 farms ranged between 5.0 and 6.8, with both a mean and median value of 6.0. Soil test P concentrations (mg L^{-1}) ranged between 2.7 and 21.3; mean 8.1 and median 7.1. The overall mean annual quantities of fertilizer P imported onto the farms decreased from 12.0 (9.7) to 8.9 (9.3) kg ha^{-1} between 2003 and 2006 (Table 3). Highest (P<0.05) exports of P were recorded in 2003 and 2006, although the relative contribution of exports of P in milk and in livestock changed during the study. Milk accounted for 0.56 of total export in 2003 and 0.64 in 2006, reflecting the trend for higher dairy cow numbers and fewer beef cattle on farms during the study. There was no significant difference in mean annual P balance during the study, averaging at a surplus of 9.4 (9.8) kg ha^{-1}. Mean annual P-use efficiency on farms ranged from 0.24 to 1.37. With regard to the overall management of fertilizer P, and P in organic manures imported onto farms, it is evident that best practices were not being implemented. There was a positive correlation (R^2=0.34) between soil P concentrations and P balance, whereas, if the recommendations were being followed, there should have been an inverse relationship: Farms with high soil P status should import less P, and vice versa. This is also evident in Figure 3 which shows that eleven farms with high P inputs were in fact the farms that would have been allowed little or no P imports under the SI. Four of these farms were importing pig manure, presumably for purposes of disposal rather than maintaining agronomic STP levels. Eight farms importing small amounts of P would have been allowed to import more P than they actually did. These farms had the lowest levels of STP and needed to import more P to avoid agronomically sub-optimal STP levels. Only two of the farmers involved in the study were importing P at the rate recommended in the SI.

Conclusion

This study looked at farm-gate P balances and STP levels on 21 dairy farms in south-west Ireland in the years preceding the introduction of the Statutory Instruments. Although the farmers involved in this study had regular close contact with Teagasc farm advisors it is evident that best practices were not always followed. Over half of the farmers involved in the study were importing excessive amounts of P which increases the potential for pollution of nearby water bodies. Eight farmers were importing less than they were allowed, which could lead to a reduced amount of grass growth due to sub-optimal STP levels. This study has highlighted the need for educating farmers on the correct use of P, which will in turn have a positive effect in terms of finance and the environment.

Acknowledgements

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The effects of fertilization with anaerobic, composted and pelletized sewage sludge on the amount of soil organic matter in a silvopastoral system under *Fraxinus excelsior* L.

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Abstract

Agroforestry systems have been considered a good tool for carbon (C) sequestration under the Kyoto Protocol because of their ability to absorb CO_2 from the atmosphere and store C mainly in the soil. In terrestrial ecosystems, soil organic matter is considered the most important store of C. The objective of this study was to evaluate the effect of the fertilization with municipal sewage sludge that has been stabilized using anaerobic digestion, composting, or pelletization, on the amount of soil organic matter, as compared to control treatments (mineral and no fertilization) in a silvopastoral system under *Fraxinus excelsior* L. and a sown sward (*Dactylis glomerata* L., *Lolium perenne* L. and *Trifolium repens* L.) in Galicia (Spain). The results showed that silvopastoral systems have great potential to enhance C sequestration compared with pastoral systems, and therefore their implementation should be considered as a land-use option in Europe. Moreover, composted sewage sludge reduced soil C sequestration more than the control treatments, probably due to the improvement of soil pH observed with this type of sludge which increased the mineralization rate of soil organic matter.

Keywords: agroforestry, climate change, carbon sequestration, sowing, afforestation

Introduction

The reduction of the atmospheric concentration of greenhouse gases, particularly CO_2, has captured the world's attention during the recent past. The establishment of silvopastoral systems, a type of agroforestry system promoted by the EU as a sustainable land management technique (EU, 2005), has been recognized as a possible greenhouse gas mitigation strategy under the Kyoto Protocol because of the potential for C storage in multiple plant species and in the soil. Soil organic matter (SOM) represents the most important pool of C storage in terrestrial ecosystems, accounting for about 75% of total stored C (Mosquera-Losada *et al.*, 2011). In silvopastoral system, fertilization with sewage sludge may reduce or increase SOM content. In Europe sewage sludge should be stabilized before using it as a fertilizer, in order to reduce the health hazards resulting from its use. The stabilization process could cause differences in the mineralization rates of sewage sludge (EPA, 1994) and therefore in the concentration of SOM. The aim of the present study was to evaluate the effect of the fertilization with municipal sewage sludge that has been stabilized using anaerobic digestion, composting, or pelletization, on the amount of SOM compared with that of control treatments (mineral and no fertilization) in a silvopastoral system under *Fraxinus excelsior* L.

Materials and methods

The experiment was conducted in an agriculturally abandoned land area in A Pastoriza (Lugo, Galicia, NW Spain) at an altitude of 550 m above sea level. The pasture was sown with a mixture of *Dactylis glomerata* L. var. Artabro (12.5 kg ha^{-1}), *Lolium perenne* L. var. Brigantia (12.5 kg ha^{-1}) and *Trifolium repens* L. var. Huia (4 kg ha^{-1}) in autumn 2004. *Fraxinus* *excelsior* L. shoots with naked roots were planted at a density of 952 trees ha^{-1} after sowing the pasture. The experiment was arranged as a randomized complete block design with three replicates and five treatments. Each experimental unit had an area of 168 m^2 and 25 trees planted with an arrangement of 5×5 stems, forming a perfect square. Treatments consisted of (a) no fertilization (NF); (b) mineral fertilization (MIN) of 500 kg ha^{-1} of 8:24:16 compound fertilizer ($N:P_2O_5:K_2O$) at the beginning of the growing season, and 40 kg N ha^{-1} after first harvest; (c) fertilization with anaerobically digested sludge (ANA) with an input of 320 kg total N ha^{-1} before pasture sowing; (d) fertilization with composted sewage sludge (COM) with an input of 320 kg total N ha^{-1} before pasture sowing, and (e) application of pelletized sewage sludge (PEL), which involves a contribution of 320 kg total N ha^{-1} split as 134 kg total N ha^{-1} just before pasture sowing in 2004 and 93 kg N ha^{-1} at the end of 2005 and 2006. The calculation of the required amounts of sludge was conducted according to the percentage of total N (EPA, 1994) and taking into account the Spanish regulation RD 1310/1990 (BOE, 1990) regarding the heavy metal concentration for sewage sludge application. To estimate the concentration of SOM, a composite soil sample per plot was collected at a depth of 25 cm as described in the RD 1310/1990 (BOE, 1990), in February 2006, and in January 2007 and 2008. In the laboratory, soil samples were air dried, passed through a 2-mm sieve, and ground with an agate mortar. The SOM concentration was determined by using the Saverlandt method (Guitián and Carballás, 1976). Data were analysed with repeated measures ANOVA (proc glm procedure). The Tukey's HSD test was used for subsequent pair wise comparisons (P<0.05; a=0.05) if the ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

Results and discussion

In this experiment, the ANOVA analysis showed that the SOM was modified by the interaction treatment × year (P<0.01). In Figure 1it can be observed that the amount of SOM was between 73.8 and 190.5 g kg^{-1}. These values of SOM are similar to those observed by Rigueiro-Rodríguez *et al.* (2011a) in a silvopastoral system under *Pinus radiata* D. Don, which was also fertilized with mineral and anaerobic sewage sludge (122.5-173 g kg^{-1}), but they are high when compared with the range obtained by Traspar-Cepeda *et al.* (2008) in treeless cropped soils in the same zone of this experiment (25.3-109.5 g kg^{-1}). The higher SOM found in our study than in the experiment of Traspar-Cepeda *et al.* (2008) may contribute to the hypothesis that recently established silvopastoral systems have greater potential to enhance C sequestration compared with treeless cropped systems. However, SOM depends on weather conditions, which may explain the reduction of SOM from 2006 to 2007 or 2008. In general, the research conducted so far has shown that the tree component of silvopastoral systems implies greater inputs of C into the soil through litterfall and root decomposition compared with pastoral systems (Mosquera-Losada *et al.*, 2011).

Regarding the effect of the treatments, in 2006, the NF treatment and the application of MIN resulted in a greater increase in SOM (initial SOM: 80 g kg^{-1}) than fertilization with COM. However, in the following years (2007 and 2008) there were no significant differences between treatments. The reduction of SOM due to fertilization with COM could be explained by the increment of the SOM mineralization rate as a result of the higher soil pH observed in this treatment than in the other treatments (NF and MIN) (Rigueiro-Rodríguez *et al.*, 2011b), which could, therefore, reduce the soil capacity to sequester C when COM was applied. The same result was found by other authors, e.g. Rigueiro-Rodríguez *et al.* (2011a) in silvopastoral systems established also in Galicia (Spain), when lime and a higher rate of application of anaerobic sewage sludge (480 kg N ha^{-1}) than that used in our study were applied together. In general, under Galician conditions, when the soil pH is improved it is not easy to find an increment of SOM concentration because the SOM mineralization rate is high due to the high temperature and precipitation rate.

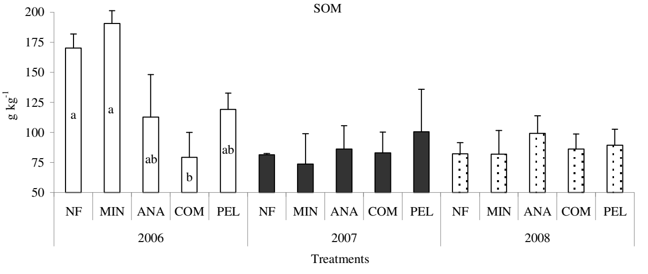

Figure 1. Soil organic matter under different fertilizer treatments in 2006, 2007 and 2008. NF: no fertilization, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletized sludge. Different letters indicate significant differences between treatments in 2006. Vertical lines indicate mean standard error.

Conclusions

Silvopastoral systems have great potential to enhance C sequestration compared with common treeless cropped systems, and therefore their implementation should be considered as a land-use option in Europe. However, SOM depends on weather conditions. Composted sludge reduced the soil C sequestration more than the control treatments (no fertilization and mineral) probably due to the increment of the SOM mineralization rate as a result of the improvement of soil pH observed in this treatment.

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The value of silvopastoral systems in the mitigation of greenhouses gas emissions: A case study from NW Spain

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Abstract

Carbon sequestration is promoted as a practice to offset the negative consequences of greenhouse gas emissions. The aim of this study was to estimate carbon sequestration in silvopastoral systems established with *Pinus radiata* D. Don and *Betula pubescens* Ehrh. The soil compartment proved the most significant carbon sink, and carbon sequestration tended to be higher under *P. radiata* due to the higher growth rate of this species compared to *B. pubescens*.

Keywords: livestock, Monterey pine, Downy birch, carbon sequestration, silvopasture

Introduction

The European Union has agreed to reduce its greenhouse gas emissions (GHG) by 20% by 2020, from base-year levels (1990) (EEA, 2011). Silvopastoral systems (SPS), when compared to systems that are exclusively agricultural, can contribute to the mitigation of climate change by acting, to a greater degree, as sinks for GHG. In this type of systems, tree species and an adequate management of the pasture carrying capacity (PCC) will contribute to increased carbon sequestration. Carbon storage in a SPS is counterbalanced by the emissions of GHG (CH_4 and N_2O) produced by the ruminants that feed on it. The quantity of GHG emitted by livestock depends on the stocking rate, which depends on the pasture production and which is affected by tree development after afforestation. The objective of this study was to estimate the C balance in two SPS established with *Pinus radiata* and *Betula pubescens* for the 11-year period that elapsed since the planting of the trees.

Materials and methods

The experiment was conducted in Lugo (NW Spain) at 439 m a.s.l. The experimental design was established using randomized blocks with three replicates. Two forest species were planted (25 trees per plot): *Pinus radiata* D. Don (Monterrey pine: pine) and *Betula pubescens* Ehrh. (Downy birch: birch) at 833 trees ha^{-1}. In 1995 the plots were sown with *Dactylis glomerata* L. and clovers (*Trifolium repens* L and *Trifolium pratense* L.). Plots were fertilized with dairy sludge in 1995 at 154 m^3 ha^{-1}. In 1996 and 1997, the plots were not fertilized, but fertilization ensued in 1998 and continued to 2005 at levels of 500 kg ha^{-1} of 8:24:16 (N: P_2O_5: K_2O) in March, and 40 kg of N ha^{-1} in May. To compare the C balance of the system (Figure 1), three main components were considered: Tree, Soil and Pasture (including livestock losses).

<u>Tree:</u> Diameter at breast height (DBH) measurements was taken in 2005 from the inner nine trees, to eliminate border effects. Based on DBH, tree biomass (trunk, fine and thick branches, leaves and roots) was calculated via allometric equations determined by Montero *et al.* (2005).

Soil: from soil samples taken at 25 cm depth in 1995 and 2006, the total soil C content was determined using the Saverlandt method (Guitián and Carballás, 1976). Soil C losses were estimated following IPCC (2001).

Pasture: *Aboveground biomass*: from 1995 to 2005 pasture was harvested (May, June, July and December) between six of the nine most central trees to eliminate border effect. Two samples (100 g each) were taken to determine, after hand separation, the relative proportions of the litterfall and pasture components. These samples were oven-dried (72 hours at 60ºC) to quantify the contribution (Mg DM ha^{-1}) of litterfall and pasture components to the C balance model. *Belowground biomass*: to determine the C content in roots (diameter <2 mm), three samples were taken during the autumn in 2005 at a depth of 15 cm. C roots (diameter >2 mm) was determined by Montero *et al*. (2005).

Livestock: With the goal of quantifying the potential GHG effect of the animals, we determined an average annual PCC that the system could support based on actual annual pasture production in each treatment (Steinfeld *et al*., 2006). Livestock were in the pasture for approximately seven months and stabled for the remaining five months, during which the animals were fed grass silage. Estimation of livestock GHG (CH_4 and N_2O emissions) was calculated using IPCC (2001) methodology. C in soil, DBH and annual pasture production were analysed by a factorial ANOVA. The significant differences between means were determined using the LSD test (SAS, 2001). For more detail on methodology, see Fernández-Núñez *et al*. (2010).

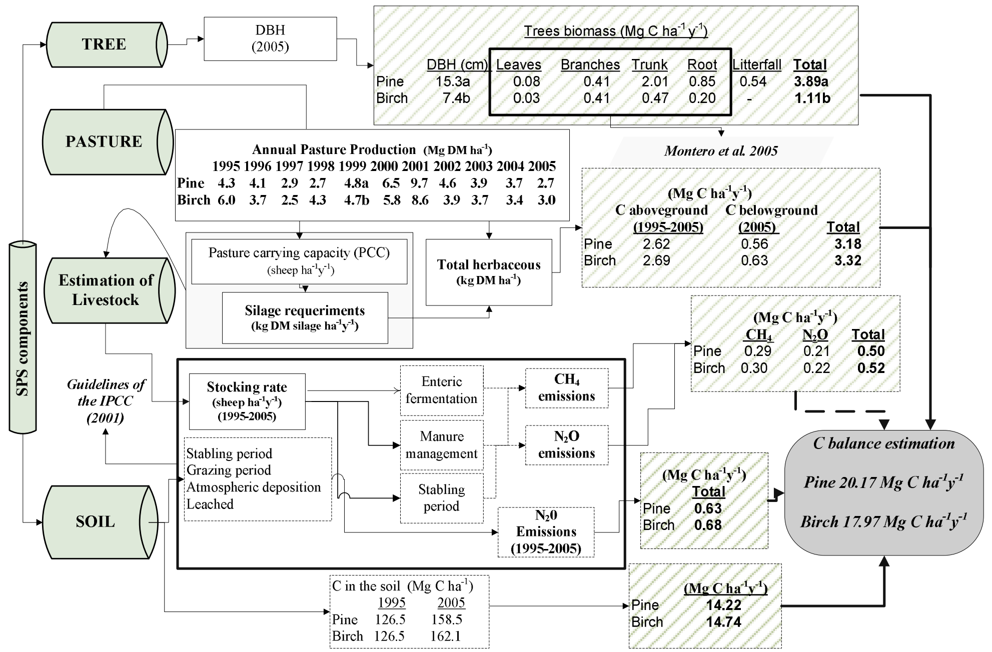

Figure 1. Components of the system considered in order to evaluate the C balance in the study and C balance estimation (Mg C ha-1 yr-1) for those systems. The sampling period or year used to estimate the balance is shown between brackets. DBH: diameter at breast height. SPS: silvopastoral systems. Different letters indicate significant differences between treatments (P<0.05).

Results and discussion

DBH reached by pine was significantly greater (P<0.001) than that of birch (Figure 1). Increased growth translated into an increased tree biomass accumulation and a significant increased in C content (3.89 and 1.11 Mg C $ha^{-1}year^{-1}$ under pine and birch, respectively). In both forest species, the highest biomass accumulation occurred in the aerial component, with the root component contributing little (3:1). Fertilizer treatments had a significant effect (P = 6%, P=11% and P<0.05 in 1995, 1998 and 1999, respectively) on annual pasture production in those years when weather conditions allowed tree growth. Annual pasture production was low in those years when fertilization was not applied (1996 and 1997) because there was not a residual effect of inorganic fertilization (Rigueiro-Rodriguez *et al.*, 2000). In both SPS, the highest level of pasture production was found in 2001 as a result of the low pruning in the systems and an unusually rainy summer (238 mm). From the year 2001 onwards, pasture production was drastically reduced in both established systems because of the negative effect tree canopy development was having on pasture production. Mean C content of the aerial herbaceous stratum (pasture + silage) (1995-2005) was very similar under both systems (2.62 and 2.69 Mg C ha^{-1} under pine and birch, respectively). On the other hand, the estimated amount of C in the roots (year 2005) was 0.56 Mg C ha^{-1} under pine and 0.63 Mg C ha^{-1} under birch. The relative contribution of pasture component (aboveground + belowground) to the global carbon sequestration was 15% (under pine) and 18% (under birch). These percentages were higher than those obtained by Fernández-Núñez *et al.* (2010) (11% under pine and 15% under birch) in SPS where competition between trees and pasture was reduced because fertilization was not applied. Large differences were not found in the annual global balance of carbon sequestration in the studied systems (2.2 Mg C ha^{-1} yr^{-1} more under pine). However, the capacity for carbon sequestration (Mg C ha^{-1} yr^{-1}) among the different components of the system was different under the two SPS. Under pine, the soil showed the highest level of C fixation, followed by tree cover and then pasture (soil > tree > pasture); whereas under birch, the C stored in the pasture component was higher than that of the tree (soil > pasture > tree) due to lower rate of tree growth.

Conclusion

The largest stock of C was found in soil, independent of canopy cover. Tree growth was greater in the pine plots than in the birch plots, which contributes to the higher capacity for carbon sequestration under pine. GHG emissions (from soil and livestock) were compensated for by the C that was sequestered in the pasture component.

Acknowledgments

This study was carried out thanks to CICYT, XUNTA (Consolidation) funds.

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Meadows fertilized with compost, anaerobic and pelleted sewage sludge: effects on annual pasture production, botanical composition and alpha biodiversity

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Abstract

Application of sewage sludge to agricultural soil is a common practice in the European Union (EU) because of low costs and recycling of nutrients achieved. The aim of this study was to evaluate the effects of different types (anaerobic, pelleted and composted sewage sludge) and application rates of sewage sludge on annual pasture production, botanical composition and species richness, compared with an unfertilized control treatment, in meadows established in Galicia (Spain). The results showed that annual pasture production was increased by anaerobic and pelleted sewage sludge in the first year of the study. However, no effects of treatments were found on botanical composition and species richness later in the study.

Keywords: biosolids, dose, sowing, waste, ryegrass, species richness

Introduction

The EU promotes the use of sewage sludge as a fertilizer due to its specific organic matter and macronutrient contents, particularly nitrogen (MMA, 2006). The use of sewage sludge in agriculture includes several operations that improve efficiency of crop production, compared with mineral fertilizers, which are related to the stabilizing process before spreading. Anaerobic digestion and composting are two sewage sludge stabilization processes that are promoted by the EU (EEA, 2000) before the sludge is used as a fertilizer in agriculture. Pelletized sewage sludge is derived from the thermic treatment of anaerobic digested sewage sludge in order to reduce water content to 2%, which consequently reduces storage, transport and spreading costs compared with anaerobic or composted sludge (Mosquera-Losada *et al*., 2010). The objective of the present study was to evaluate the effects of different types of sewage sludge (anaerobic, compost and pelleted) at different application rates (160 and 320 kg total N ha^{-1}) compared with a control treatment (no fertilization) on annual pasture production, botanical composition and species richness in meadows established in the Atlantic bioclimatic region.

Materials and methods

The experiment was established in Pol (Galicia, N.W. Spain; altitude 527 m a.s.l.) in spring 2003. The experimental design was a randomized complete block with three replicates and seven treatments. At the beginning of the experiment (March 2003) the soil was ploughed, and the pasture was sown with a mixture of *Lolium perenne* L. var. Brigantia (12.5 kg ha^{-1}) and *Trifolium repens* L. var. Huia (4 kg ha^{-1}). Fertilization treatments consisted of three types of sewage sludge: anaerobic (A), pelleted (P) and composted sewage sludge (C) applied at two different application rates (doses) in the first year of the study: 160 kg total N ha^{-1} and 320 kg total N ha^{-1}. No fertilization (0N) was used as a control. The calculation of the required amounts of sludge was conducted according to the percentage of total nitrogen (EPA, 1994) and taking into account the Spanish regulation (R.D.1310/1990) regarding the heavy metal concentration for sewage sludge application. Plots (4×1.45 m) were harvested in June and December 2003, and in May, June and December 2004. The fresh forage was weighed in situ and a representative subsample was taken to the laboratory. At the laboratory, one pasture sample (95-100 g) was dried for 72 h at 60ºC and weighed to estimate DM content and hence annual pasture DM production. The other sample (95-100 g) was separated by hand to determine botanical composition (percentage of grasses (%G), legumes (%L) and other species (%OT)). The different species were weighed separately to determine dry weight (72 h at 60ºC) to estimate species richness (SR). Data were analysed by principal component analysis (PCA) based on a correlation matrix for the dependent variables (annual pasture production, botanical composition and SR). General linear models procedure (SAS, 2001) was used for ANOVA, and the difference among means was detected by LSD (P<0.05).

Results and discussion

PCA was significant (P<0.000) in the explanation of dependent variables. The first three PCA-axes explained 89% of the variation. PCA1 (48% of total variability) was positively correlated with SR, %L, %OT and C_{160N} treatment, and negatively with %G and A_{320N} treatments (Figure 1). PCA2 (22% of total variability) was negatively related with %OT and A_{160N} and positively related with %L and C_{320N}. Finally, PCA3 (19% of total variability) was highly positively correlated with annual pasture production and A_{160N}.

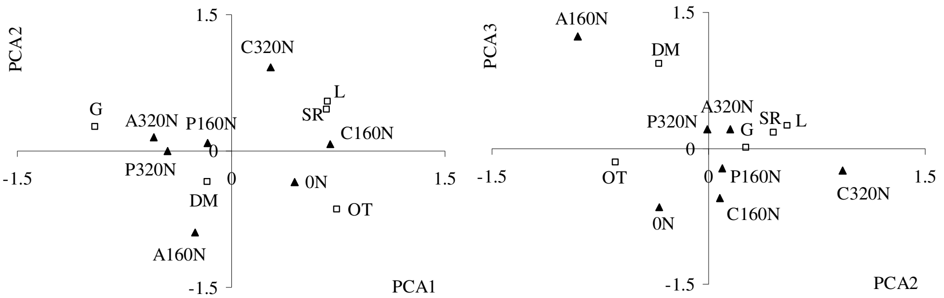

Figure 1. Loadings and scores of the first three PCAs and significant effect of fertilizer treatment (P<0.05), where: A, C, P: anaerobic, composted and pelleted sewage sludge, respectively; 0N: 0 kg total N ha^{-1}, 160N: 160 kg total N ha^{-1} and 320N: 320 kg total N ha^{-1}.; □: dependent variables (G, L, OT: percentage of grasses; legumes; and other species, respectively; DM: annual pasture production, and SR: species richness).

Pasture production levels were lower than that reported by Mosquera and González (1999) in Galicia (6-12 Mg DM ha^{-1}) (Table 1). These lower levels could be explained by the fact that the study was established in a soil with low pH (4.9-5.3) and low effective exchange capacity (< 6 cmol (+) kg^{-1}): conditions which usually indicate deficiencies in the availability of cations and, therefore, limit pasture production (Whitehead, 2000). In 2003, pasture production was significantly increased when anaerobic sludge (A_{160N} and A_{320N}) was applied with respect to no fertilization (0N), composted (C_{160N} and C_{320N}) and pelletized sludge at a high rate (P_{320N}). Furthermore, P_{320N} had a positive effect on pasture production with respect to no fertilization (0N) and composted sludge (C_{160N} and C_{320N}). The lower pasture production in composted sewage sludge was probably due to the lower mineralization rate and the N availability of this treatment compared with that of the other treatments (EPA, 1994). Annual pasture production was higher in 2004 than in 2003 due to higher and more favourably distributed rainfall in spring and summer of 2004 (data not shown). Significant differences were not detected between treatments in pasture production in 2004 because there was no further residual effect of sewage sludge application. No effects of fertilizer treatments were found on botanical composition and SR (Table 1). However, in 2004 the percentage of legumes and other species increased in all treatments and contributed to increased SR.

Table 1. Pasture production (DM), botanical composition and species richness in 2003 and 2004. %G, %L, %OT: percentage of grasses, legumes and other species, respectively; A, C, P: anaerobic, compost and pelleted sewage sludge, respectively; 0-160-320N: 0, 160 and 320 kg total N ha^{-1}, respectively. Different letters indicate significant differences between treatments ($P<0.001$).

	Year 2003					Year 2004				
Treatments	Mg DM ha^{-1}	%G	%L	%OT	SR	Mg DM ha^{-1}	%G	%L	%OT	SR
0N	1.09c	60.19	6.64	33.17	9	1.99	51.49	9.74	38.77	15
A160N	3.97a	84.30	0.13	15.57	7	3.73	51.33	9.87	38.80	13
C160N	0.87c	52.88	4.23	42.89	10	1.98	49.55	19.70	30.75	19
P160N	1.70bc	86.87	0.27	12.85	9	2.38	59.62	10.69	29.69	16
A320N	2.68ab	86.89	1.49	11.62	11	2.63	79.97	2.21	17.82	15
C320N	0.61c	86.91	0.47	12.62	10	2.39	45.65	33.92	20.44	17
P320N	2.45b	91.64	0.63	7.73	8	2.59	67.57	4.99	27.44	16

Conclusions

Anaerobic and pelleted sewage sludge enhanced pasture production compared with compost sludge in the first year of application in meadows established in the Atlantic bioclimatic region. No residual effects of sewage sludge were found.

Acknowledgments

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Application of mineral fertilizer at different times of the year

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Abstract

In the years 2006-2012, five experiments with application of fertilizer at different times of the year were performed at the Experimental Station Korpa in Reykjavík. Nine different application times were included and one treatment without fertilizer. The earliest application time was in early September but the last one in early May when onset of growth had started. All plots in the experiment were harvested in late July the following summer. Total yield of dry matter and nitrogen content was observed in all plots. Yield was always lowest in plots without fertilizer but the plots fertilized beyond conventional dates gave in average 80% of the yield observed in the spring plots. Similar pattern was found for nitrogen uptake.

Keywords: mineral fertilizer, application time, winter application

Introduction

The traditional application time for grass leys in Iceland is at the onset of growth in spring (often in early May). However, manure is sometimes applied in winter time in Iceland and that was the initial motivation to these experiments. Furthermore, the functionality of the roots during the dormancy period is of interest. Several experiments have been performed with application of mineral fertilizer in late summer and autumn in Iceland (Hólmgeir Björnsson and Hermannsson, 1987; Björnsson, 1998; Thorvaldsson, 2007). These experiments have shown that fertilizer applied in autumn gives a yield in the following year that is not much lower than that from fertilizer applied the following spring. When roots from the autumn-applied plots were dug up in early spring a large part of the fertilizer was found in the roots (Björnsson, 1998). In northern Sweden experiments have also shown similar utilization of nitrogen after application in late September as in spring application (Palmborg *et al.*, 2011). Because of these results it was decided to perform experiments with 9 different application times from early September to early May.

Materials and methods

The experiments were located at the experimental station at Korpa in Reykjavík (64°09'N; 21°45'W). Five experiments were performed in the years 2006-2012. New plots were used each year. Each experiment included 9 different application times and one treatment without fertilizer. Plot size was 14-24 m^2 and varied between experiments. The experiments were designed as randomized complete blocks with four replications. The amount of fertilizer was 60 kg N ha^{-1} supplied in a mixed fertilizer that included N, P, K, S and Ca. The fertilizer was not applied when snow or ice covered the plots but the soil could be frozen. This explains the variation in application dates between years. The plots were harvested in late July or in August. Table 1 show the application and harvest dates in each experiment. Nitrogen was measured in samples from each plot with NIR and Dumas method.
In 2006 the experiment was conducted on an old, fertile grass field with mixed vegetation. The main species was *Agrostis capillaris* but *Festuca rubra*, *Descampsia caespitosa*, *Phleum pratense*, *Alopecurus pratensis* and *Rumex acetosa* were also of importance. Eleven other species were also found. In 2007 the experiment was on an old grass field, with low fertility and with mixed vegetation. Main species were *Agrostis capillaris* and *Festuca rubra* but 11

other species were also found. In 2008 the experiment was on a young and very unfertile grass field with pure *Poa pratensis*. In 2010 the experiment was on a young and fertile grass field with almost pure *Phleum pratense*. In 2011 the experiment was on a young and unfertile grass field with almost pure *Phleum pratense* but *Vicia cracca* had started to invade the experiment.

Table 1. Application time and harvest dates (date.month) during the experimental years.

Application time	2006–2007	2007–2008	2008–2009	2010–2011	2011–2012	Mean
1	4.9.	2.9.	31.8.	5.9.	5.9.	3.9.
2	25.9.	24.9.	18.9.	15.10.	24.9.	23.9.
3	17.10.	18.10.	8.10.	7.11.	18.10.	20.10.
4	13.11.	8.11.	5.11.	25.11.	12.11.	13.11.
5	30.1.	2.1.	29.12.	2.1.	4.2.	7.1.
6	28.2.	18.2.	18.2.	17.2.	13.3.	20.2.
7	28.3.	14.3.	18.3.	4.3.	30.3.	19.3.
8	21.4.	17.4.	6.4.	4.4.	12.4.	12.4.
9	7.5.	5.5.	6.5.	7.5.	27.4.	4.5.
Harvest date	20.7.	29.7.	20.8.	26.7.	24.7.	30.7.

Results and discussion

Dry matter yield for each year and each application time is shown in Table 2 and the yield of N in Table 3. These two tables show a similar pattern but utilization of nitrogen in these experiments was generally low, in the spring-fertilized plots also. The yield was usually highest when fertilized at the conventional time in spring. On average, plots fertilized at unconventional time yielded about 80% of the yield of plots that were fertilized in early May.

Table 2. Dry matter yield of swards fertilized at different application times and in different years (hkg ha^{-1}).

	Year					
Application time	2007	2008	2009	2011	2012	Mean
1	52.7	28.6	13.3	30.6	40.8	33.2
2	50.5	21.9	15.6	39.7	48.5	35.2
3	47.3	21.1	15.8	31.5	49.8	33.1
4	51.3	18.9	19.1	20.2	49.5	31.8
5	50.4	21.4	18.8	30.6	54.8	35.2
6	49.5	20.9	21.0	49.1	54.6	39.0
7	51.5	18.7	20.3	52.3	61.4	40.8
8	53.8	24.3	18.9	53.3	59.4	41.9
9	57.3	25.5	26.9	58.8	65.4	46.8
Unfertilized	45.7	6.8	7.2	20.6	16.9	19.4
Average yield	51.0	20.8	17.7	38.7	50.1	
SD	3.3	7.7	3.6	4.9	6.5	

There were, however, differences between years in the utilization of fertilizer. On average, there was slightly better utilization of the fertilizer applied in the period February-April than for earlier application dates. However, autumn-fertilized plots could give results that were as good as the results for spring-fertilized plots. Few dates of application resulted in values lower than 60%. Application dates with low utilization of fertilizer could often be related to high precipitation around the fertilization dates, or frozen soil surface and cold weather, but not always.

Unfertilized plots yielded about 40% of the dry matter yield of plots fertilized in early May and about 55% of the N yield measured in plots fertilized in early May.

Green colour was often seen in the plots a few weeks after fertilization during autumn and winter. However, in middle of the winter it took longer time for the colour to be visible compared with the plots fertilized in early autumn, and the colour was weaker.

Table 3. Nitrogen yield (kg ha^{-1}) from swards fertilized at different application times and in different years.

Application time	2007	2008	2009	2011	2012	Mean
1	87	32	11	22	35	37
2	89	24	13	29	39	39
3	83	26	13	22	44	38
4	91	21	16	17	31	35
5	84	24	16	24	39	35
6	82	24	17	32	32	37
7	90	22	17	37	40	41
8	96	30	16	37	42	44
9	101	32	22	39	44	48
Unfertilized	74	9	7	16	2	26
Average yield	88	24	15	28	37	

Conclusions

Perennial grasses in Iceland seem to be able to utilize mineral fertilizer even if it is applied during winter when the plants are dormant. On average, production in the following year was 80% of the yield obtained from spring-fertilized plots.

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Leaching of dissolved organic nitrogen under white clover pure stand *vs*. grass-clover mixture

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Abstract

Leaching of dissolved organic nitrogen (DON) is a considerable loss pathway in grassland soils. We investigated the importance of white clover for presence of DON in soil water. White clover was leaf labelled with ^{15}N-urea and dissolved inorganic N (DIN) and DON ^{15}N-enrichment was determined under perennial ryegrass (*Lolium perenne*) - white clover (*Trifolium repens*) mixture and white clover pure stand. Soil water was collected during autumn and spring, at 25, 45 and 80 cm depths, by suction cups. DON was ^{15}N-enriched at all depths under both pure stand and mixture. Under the white clover pure stand there was high level of DIN, considerably exceeding that of the mixture. DIN was highly ^{15}N enriched under white clover pure stand, whereas label was almost absent under the mixture. The findings confirmed our hypothesis that white clover affects DON and DIN concentrations in soil water. Plant competition, root turnover, exudation and other factors, contributing to leaching of DON are discussed.

Keywords: DON, DIN, ^{15}N-enrichment, leaching, white clover

Introduction

Dissolved organic N is a significant part of total dissolved N (TDN) leached from legume-based grassland soils (Farrell *et al.,* 2011) but the origin of DON is largely unknown. N_2 fixing legumes in grassland mixtures have been widely used in grassland ecosystems for both economic and environmental purposes. Previous studies have reported higher leaching of inorganic N from fields of white clover pure stands than from a mixture with non-leguminous species (Bouman *et al.*, 2010) but, so far, white clover contribution to DON is not clearly described. This knowledge is needed to understand how forage legumes can be used for enhancing the sustainability of agricultural systems with the purpose of reducing mineral N inputs as well as reducing N leaching. We investigated the importance of white clover for the presence of DIN and DON in soil water in the soil profile.

Materials and methods

A field experiment was established at the Foulumgård Experimental Station (Jutland, Denmark) in April 2011 by installing PVC cylinders (Ø30 cm; 25, 45, and 80 cm in length) into: (1) white clover pure stands, and (2) perennial ryegrass-white clover mixtures (initial clover proportion of total biomass was between 17 and 34%). White clover was leaf labelled with ^{15}N urea (99.6 atom% ^{15}N, 0.5% w/v) during the period July-September 2011. Soil water was collected in September, November, December, January and March by Teflon suction cups inserted 5 cm below the cylinders. Samples for analysis of ^{15}N-enrichment of DIN and DON were prepared by liquid diffusion by using the Teflon floating method (Sørensen and

Jensen, 1991). Effect of sward types were analysed by repeated measures analysis of variance using the PROC MIXED procedure of the SAS software.

Results and discussion

The results of this experiment show that white clover had an effect on the concentration of DIN and DON in soil water down the profile. DIN concentration under clover pure stand was significantly higher ($P<0.001$) that under mixture, whereas DON concentrations under both sward types were more constant (Figure 1).

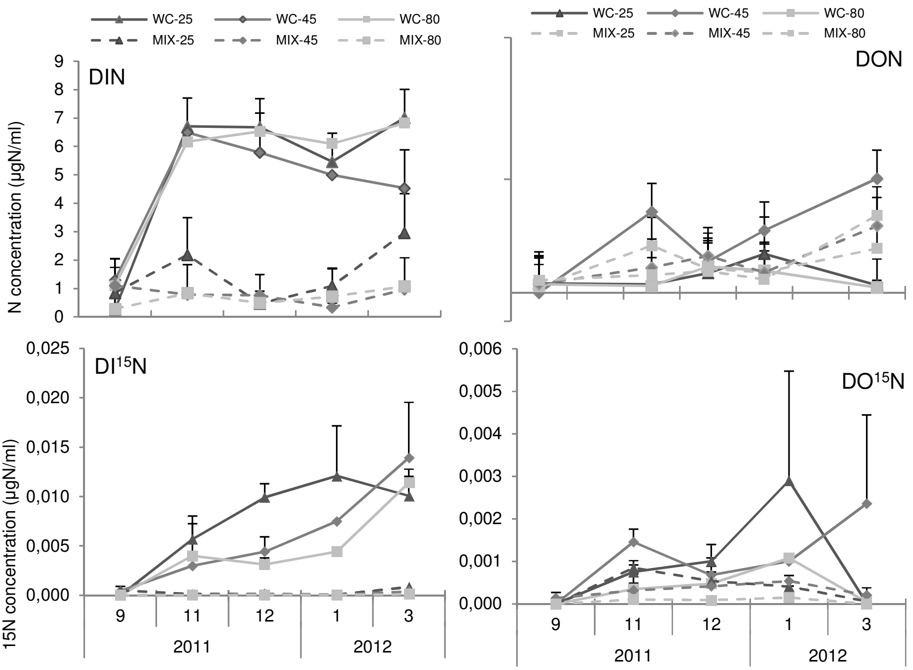

Figure 1. DON and DIN concentration and ^{15}N enrichment in time scale in the soil profile below clover pure stand and clover-grass mixture. Data are means +1 SE.

Table 1. DON and $DO^{15}N$ (µg/ml) proportion of TDN (%) in soil water under white clover pure stand and clover-grass mixture in the 2011-12 leaching season.

Treatment	Depth, cm	DON of TDN (%)					$DO^{15}N$ of $TD^{15}N$ (%)				
		Sep.	Nov.	Dec.	Jan.	Mar.	Sep.	Nov.	Dec.	Jan.	Mar.
WC pure stand	25	57	4	10	20	4	0	12	9	19	0
	45	0	31	16	NA	47	0	33	13	NA	15
	80	22	4	12	11	3	0	9	13	20	0
Mixture	25	21	43	71	40	35	0	87	80	85	7
	45	23	53	63	68	74	100	100	71	90	50
	80	61	43	61	40	72	0	100	67	78	0

The $DON/DO^{15}N$ proportion of $TDN/TD^{15}N$ varied during the leaching season and was considerably higher under the grass-clover mixture (Table 1). DON constituted the largest part of total N under the clover pure stand in September at 25 cm, but a low proportion of $DO^{15}N$ of $TD^{15}N$ indicated higher white clover contribution to the DIN pool. The $DO^{15}N$

proportion of $TD^{15}N$ indicated that white clover contributed mostly to the DON pool under the mixture.

The presence of ^{15}N in soil water indicated that white clover contributed to N leaching at all samplings, and that grassland composition had a significant effect on ^{15}N enrichment. $DI^{15}N$ under the clover pure stand increased with a delay compared with DIN, and both $DI^{15}N$ ($P<0.001$) and $DO^{15}N$ ($P<0.05$) pools were significantly higher under clover pure stand than under the mixture. This delay could be due to delays in ^{15}N release from clover shoots and roots relative to the general N turnover from SOM resulting in N leaching, or it could be explained by delays in clover ^{15}N release caused by internal N allocation and redistribution before deposition to the rhizosphere (Rasmussen *et al*., 2013).

Rapid root turnover, rhizodeposition (Unkovich and Pate, 2000), lower demand for soil N (Armstrong *et al.,* 1996), leaf decay (Dahlin and Stenberg, 2010) of white clover and the absence of companion species likely caused significantly higher DIN concentration and ^{15}N enrichment of both DIN and DON pools under the white clover pure stand. The presence of ^{15}N in ryegrass leaves (data are not presented) indicated that symbiotically fixed nitrogen was transferred and, to some extent taken up by, ryegrass in the mixture. Furthermore, low clover-grass biomass ratios caused a quantitatively lower release of ^{15}N into the system that caused lower ^{15}N enrichment of N pools under the mixture. Thus, N uptake by ryegrass (Rasmussen *et al*., 2008), N immobilization and differences in clover biomass probably gave lower DIN concentrations and ^{15}N enrichment accordingly changing DON/$DO^{15}N$ proportion of TDN/$TD^{15}N$ under the legume-grass based sward.

Conclusion

The present experiment showed that white clover has an effect on leaching of both DIN and DON. The presence of ^{15}N label in both DIN and DON pools at all depths in both treatments supports the findings of Rasmussen (2008) that the main direct source of DON and DIN in the soil water is decay of recently growing white clover. Studies are needed on the indirect white clover contribution such as activation of microbial biomass and SOM decomposition.

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Nitrate-N residue in the soil under a perennial ryegrass sward with and without white clover under cutting conditions

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Abstract

In this 5-year experiment (2007-2011) on a sandy loam soil (Merelbeke), we compared perennial ryegrass (*Lolium perenne* L.) (PRG) with and without white clover (WC) under cutting conditions and 4 nitrogen fertilization levels (200-330 kg N ha^{-1}). Potential leachable N residue in the soil, measured end of October-November, was very low for PRG-WC (14 kg NO_3-N ha^{-1}) in comparison with the upper limit of 90 kg NO_3-N ha^{-1} (Flemish Manure Decree, 2007), but was significantly higher in comparison with PRG (8 kg NO_3-N ha^{-1}). There was no significant difference in residual NO_3-N between the N fertilization levels. The low residual nitrate concentrations in the soil can be explained by the high N export with the harvested biomass, exceeding the N-fertilization. White clover produced on average 23% of the annual DM and stimulated the N export, in comparison with that of PRG.

Keywords: perennial ryegrass, white clover, N export, residual NO_3-N

Introduction

Farmers in Flanders see potential in introducing white clover (WC) into their grassland, because chemical N fertilization is expensive and restricted by the Flemish Government in accordance with the EU Nitrate Directive. The effect on yield and quality is the farmer's main interest, but farmers, advisors and authorities are uncertain about the consequences of white clover in the sward for the leachable N residue in the soil at the end of the growing season.
In this experiment, we compared perennial ryegrass (PRG) - the most important grass species in grassland seed mixtures - with and without WC under cutting conditions and under different fertilizer application rates stated in the Flemish Manure Decree.

Materials and methods

In May 2006, PRG (40 kg ha^{-1}) and PRG-WC (40 kg ha^{-1} incl. 4 kg WC) was sown on a sandy loam soil in Merelbeke. The experimental design was a split-plot design with four replicates, with sward type as a split plot. The experiment started in March 2007 and ran until 2011. The following total-N fertilization rates (kg ha^{-1}) for grass and grass-clover were imposed:

(1) 170 N_{slurry} + 100 $N_{mineral}$ = 200$N_{available}$
(2) 250 N_{slurry} + 100 $N_{mineral}$ = 250 $N_{available}$
(3) 170 N_{slurry} + 180 $N_{mineral}$ = 280 $N_{available}$
(4) 250 N_{slurry} + 180 $N_{mineral}$ = 330 $N_{available}$

Treatment 3 corresponded with the maximum level of cattle slurry and total N on grassland allowed by the Flemish Government (Manure Action Plan 2007). When derogation is allowed more $N_{animal\ origin}$ can be applied, so 250 N_{slurry} + 100 $N_{mineral}$ (Treatment 2) was legitimate. Today, 310 $N_{available}$.ha^{-1} is allowed on grassland under cutting conditions on non-sandy soils in Flanders. Slurry was applied by shallow injection in March (1st cut) and May (2nd cut). The actual N input (= slurry in t ha^{-1} × N-content slurry, sampled during application) corresponded very well with the assumed N input. $N_{mineral}$ was applied as ammonium nitrate (27% N). The plots (56.3 m^2 gross area, 8.4 m^2 harvested for yield determination) were cut 5 times per year. At each cut the dry matter (DM) yield was measured and a grab-subsample per plot was separated into PRG, WC and unsown species. Samples were analysed by NIRS to determine the N-content of the forage. N export (kg ha^{-1}) was calculated as DM yield × N-content. The NO_3-N content of the 0-90 cm topsoil was determined each year (29 October - 27 November) for 3 horizons of 30 cm, sampled and analysed separately according to ISO 14256-2:2005.

Table 1. Fertilization regime: application time and dose of slurry and mineral N fertilizers.

		Application for		
		1st cut	2nd cut	3rd cut
170 N_{slurry}	Litres ha^{-1}	25000	25000	
250 N_{slurry}	Litres ha^{-1}	35000	35000	
100 $N_{mineral}$	kg ha^{-1}	45	30	25
180 $N_{mineral}$	kg ha^{-1}	60	60	60

Results and discussion

To avoid leaching, the NO_3-N content in the 0-90 cm soil profile should not exceed 90 kg ha^{-1} at the end of the growing season in Flanders (Manure Action Plan, 2007). The residual NO_3-N content in the soil at the end of the growing season (November) was significantly higher over the years for PRG-WC (14 kg ha^{-1} NO_3-N) in comparison with PRG (8 kg ha^{-1} NO_3-N) but the absolute level was far below this limit of 90 kg ha^{-1} for PRG-WC and PRG (Table 2). The surplus of 6 kg NO_3-N was divided within the 0-90 cm layer as follows: 4 kg in the upper layer 0-30 cm, 1 kg in the layer 30-60 cm, and 1 kg in the deepest layer 60-90 cm. The amount of residual NO_3-N was always very low during the experimental period of 5 years, but every year it was significantly higher for PRG-WC in comparison with PRG (Table 2). There was no significant difference in residual NO_3-N between the N-fertilization levels.

Table 2. Residual NO_3-N at the end of the growing season and N-export by harvest of forage.

Fertilization ($N_{available}$)	Sward	Residual N (kg NO_3-N ha^{-1}) 2007	2008	2009	2010	2011	Average	N-output by harvest of forage (kg N ha^{-1}) 2007	2008	2009	2010	2011	Average
N 200	PRG	13	5	5	6	8	7	278	337	285	259	294	291
	PRG/WC	22	9	11	6	12	12	278	397	384	442	482	397
N 250	PRG	13	6	7	7	8	8	300	358	326	303	333	324
	PRG/WC	26	14	20	8	11	16	336	426	393	478	455	418
N 280	PRG	9	6	7	6	8	7	339	391	342	320	359	350
	PRG/WC	22	9	9	6	11	11	331	435	391	461	515	426
N 330	PRG	25	9	8	7	9	11	342	392	346	357	397	367
	PRG/WC	24	14	18	6	12	15	349	458	403	496	515	444
Average	PRG	15a	6a	7a	6a	8a	8a	315a	369a	325a	310a	346a	333a
	PRG/WC	24b	11b	14b	7a	12b	14b	323a	429b	393b	469b	492b	421b

Treatments with the same letter in the same column are not significantly different (Duncan test, $P<0.05$)

The low residual NO_3-N content is favourable from the environmental point of view, but rather a normal situation under cutting conditions and confirmed in other studies (Verbruggen *et al*., 2003; De Vliegher and Carlier, 2008). The low residual nitrate N concentrations in the soil for the PRG treatments can be explained by the high N export during harvest, exceeding the N-fertilization in terms of available N for each N fertilization level: 291 kg N (200N), 324 kg N (250N), 350 kg N (280N) and 367 kg N (330N).

Each year, white clover was a substantial component of the sward during the whole growing season in this experiment, fertilized with 200-330 kg N ha^{-1}: the clover content in the annual dry matter yield of the PRG-WC treatments varied between 20% and 26% and in the last cut between 22% and 28%, without significant differences between the N levels (Table 3). A medium-high N fertilization level in combination with a significant white clover content under cutting conditions resulted in a significant increase of the N export ha^{-1} by harvest in comparison with that of PRG: + 107 kg N (200N), + 94 kg N (250N), +76 kg N (280 N), +77 kg N (330N) and an average of 88 kg N ($P<0.05$) (Table 2). PRG-WC stimulated the production of crude protein (= N export × 6.25) even in growing conditions with a considerable N-fertilization. This is in agreement with results in other studies (De Vliegher *et al.,* 2006; Smit and Elgersma, 2006; De Vliegher and Carlier, 2008). A study of more than 400 fields over several years confirmed that productivity of mixed swards is directly related to the contribution of white clover in the sward (Pfimlin *et al*., 1993).

Table 3. Clover content in annual production and last cut.

Treatment	Clover content (% of DM yield)											
	Annual						Last cut					
	2007	2008	2009	2010	2011	Average	2007	2008	2009	2010	2011	Average
N 200	9	22	20	45	34	26a	9	21	37	43	28	28a
N 250	21	24	21	41	21	26a	27	21	40	32	14	27a
N 280	10	19	14	31	27	20a	18	12	27	26	29	22a
N 330	11	19	20	41	21	22a	13	17	39	26	18	22a
Average	13	21	19	39	26	23	17	18	36	32	22	25

Treatments with the same letter in the same column are not significantly different ($P<0.05$)

Conclusion

Under cutting conditions and a medium-to-high N fertilization level, white clover was a substantial component of the sward: on average it contributed 20% of the dry matter yield.
The residual nitrate-N content in the soil was significantly higher for the grass-clover mixture but it was very low in absolute terms in all years of the study, and there was no risk of nitrate leaching during the winter period.

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Species mixtures – Nitrous oxide emissions after application of synthetic urine

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Abstract

Nitrous oxide (N_2O) emissions were recorded for 70 days on patches treated with synthetic urine (500 kg N ha^{-1}) on a field experiment with perennial ryegrass, tall fescue, white clover and red clover, in pure stands or in mixtures (either 25% seed weight of each or 67% of one species and 11% of the others), fertilized with low nitrogen application (100 kg N $ha^{-1}yr^{-1}$).

Introduction

Mixtures of grass and legume species in grassland forage production can contribute to higher yields and reduced needs for N fertilizer (Kirwan *et al.*, 2007). In a pasture, however, urine patches can provide the substrate for high N_2O emission in spite of low N fertilization. As part of the EU-FP7 project Multisward, the effect of legume/grass on N_2O emission was studied. This gas is undesired because of its global warming effect.

Materials and methods

Pure stands of each species as well as mixtures with either 25% seed weight of each species (centroid) or 67% of one and 11% of the other three species were sown in 2010 on a loamy soil at Ås (59°4'0N, 10°47'E; 75 m a.s.l.; annual precipitation 785 mm and average temperature 5.3°C). In 2011 a low level of nitrogen fertilization was applied (100 kg N ha^{-1}) and plots were harvested 5 times. N_2O emissions were measured using 30 static chambers (6 on the centroid, 3 on the other treatments), from the middle of August, shortly after the fourth harvest and one week before application of artificial urine (Ambus *et al.*, 2007) supplying 500 kg N ha^{-1}, and continued until the emissions decreased to background level at the end of October. Flux sampling frequency was about twice a week, reduced to once a week at the end of the period. Sampling procedures and gas analysis were as described in Nadeem *et al.* (2012). Herbage was harvested on 13 September at a cutting height of 4-5 cm, and analysed for species composition.

Results and discussion

Initial N_2O emissions before urine application were low (1.3 μmol m^{-2} h^{-1}; average of all treatments and three dates). After urine application, emissions increased rapidly, on average up to 15 μmol m^{-2} h^{-1} (Figure 1). Ammonium in the soil increased from 0.6 to 24 g NH_4-N m^{-2} on average of all treatments, while nitrate increased from 0.3 to 2.6 g NO_3-N m^{-2} (20 cm depth, data not shown). Thus at this stage, nitrification might have been the major source of N_2O. On 29 August, and in the preceding days, abundant precipitation raised the water content close to saturation point (Figure 1, lower part). Some nitrate was probably leached as the average soil content decreased to 0.6 g NO_3-N m^{-2}. However, since nitrification (which builds up nitrate) and leaching, if present, occurred simultaneously the available data cannot lead to conclusive estimates of leaching. At the end of this precipitation period N_2O emissions decreased, then increased again and peaked before the fifth harvest, and finally decreased to below the initial level by the beginning of October. Precipitation was frequent during the whole recording period. Although high water content in the soil usually leads to anoxia, with continuous rain the oxygen content of the soil solution may remain relatively high. Soil mineral N data suggest that nitrification occurred during most of the recording period.

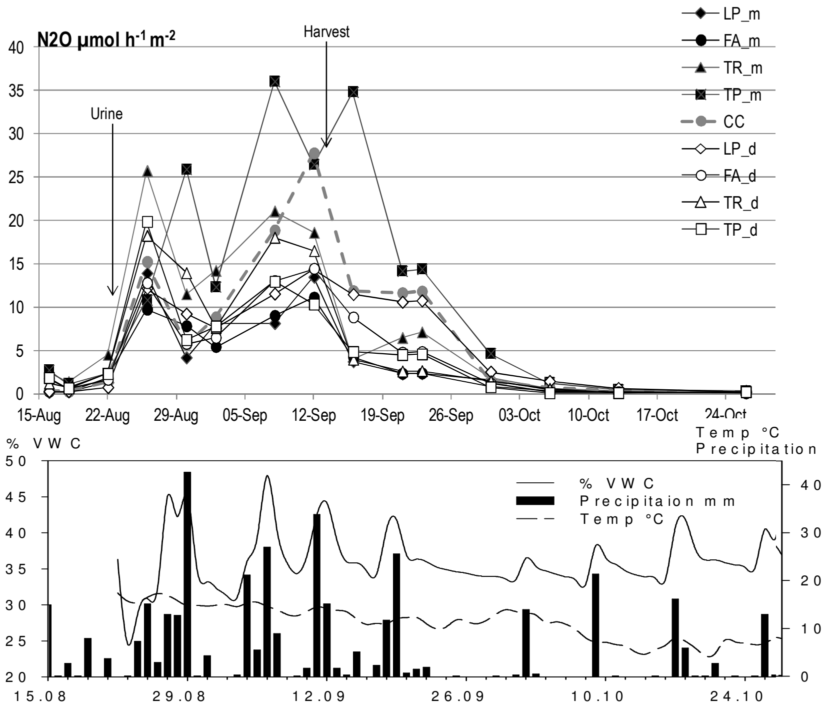

Figure 1. Above: N_2O emission rates (µmol h^{-1} m^{-2}). Treatment: Lp: *Lolium perenne,* Fa: *Festuca arundinaceae,* Tr: *Trifolium repens*, Tp: *T. pratense,* C: centroid (25% seed weight of each of the four species), m: pure stand, d: mixture with 67% seed weight of the species mentioned and 11% of each of the three other species. Below: soil temperature (Temp °C) and water content as percentage of soil volume (% VWC) monitored at 5 cm depth, and precipitation (mm).

Accumulated over the whole recording period, the red clover pure stand had the highest N_2O emission (18 mmol m^{-2}) corresponding to 0.50 g N m^{-2}; thus, 1% of applied N. The centroid had the next highest emission (about 11 mmol m^{-2}), but this was not statistically significantly different from any of the other mixtures and the white clover pure stand. Both tall fescue and perennial ryegrass pure stands had significantly lower total emissions (Figure 2A). Relative to DM herbage yield, the centroid mixture and other mixtures had significantly lower N_2O emissions (≤62 µmol g^{-1} DM) than the clover pure stands (≥85 µmol g^{-1} DM), and were comparable to the ryegrass pure stand. The exception was the tall fescue dominated mixture, which together with the pure tall fescue had the lowest N_2O emission per g DM yield (Figure 2B).

Prior to the application of urine, pure grasses, and ryegrass in particular, showed clear symptoms of nitrogen deficiency. Visual inspection showed a tremendous response of pure grasses to urine; however, this was not sufficient to raise the herbage yield above the yields of the best mixture and of pure red clover. Tall fescue was able to recover more nitrogen (data not shown), probably due to deep rooting, and this is the likely reason for the large yield and low N_2O/yield ratio of the tall fescue-dominated stand (Figure 3).

A B

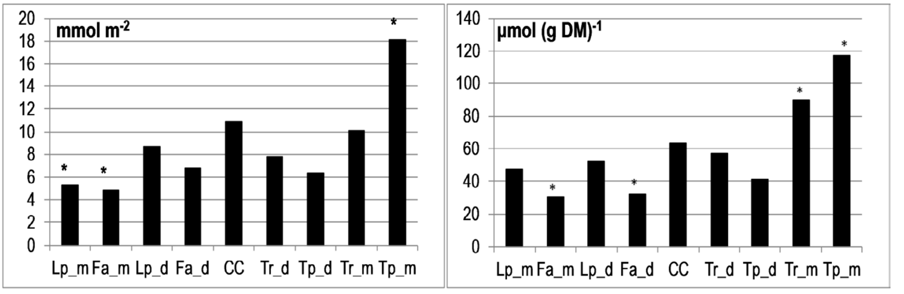

Figure 2. A: Total N_2O emission (mmol m^{-2}) during the 70-day sampling period. B: Total N_2O emission divided by the DM yield of the 5th harvest, cut during the recording period. See Figure 1 for a description of treatments. *: significantly different from CC ($P \leq 0.05$).

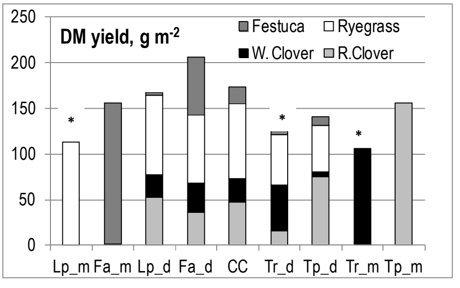

Figure 3. Herbage dry matter yield and composition of the fifth harvest taken 20 days after application of urine. See Fig. 1 for a description of treatments.
*: significantly different from CC ($P \leq 0.05$).

Conclusion

The maximum N_2O emission was 1% of the high urine N load (500 kg N ha^{-1}), that is half the default emission factor by IPCC for cattle dung and urine on pasture (IPCC 2006). As expected mixtures reduced emission compared to clover pure stands, for the most without reducing yields. As expected, when both bigger yields and lower N_2O emission are considered, mixtures performed better than pure stands of grasses or legumes.

Acknowledgements

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Effects of different sowing ratios on the persistency of chicory within ryegrass or a ryegrass-red clover mix

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Abstract

The effects of different sowing mixtures with perennial ryegrass (*Lolium perenne*) (cv. Premium) (PRG) or PRG with red clover (*Trifolium pratense*) (cv. Merviot) on the persistency of chicory (*Cichorium intybus*) (cv. Puna II) was investigated. Field plots (10×2.5 m) were sown in a randomized block design with four replicates. Treatments included chicory sown with PRG alone, or with a PRG-red clover mix, with 10 combinations of chicory at ratios of 0.9, 0.7, 0.5, 0.3 and 0.1. To determine persistency, the number of chicory plants within a 0.36 m × 0.25 m quadrat at 6 random sites within each plot was counted in spring and autumn of the first and second harvest year and the population m^{-2} determined. The highest chicory populations were found when chicory was sown at 90% of the target proportion of the sown sward. Chicory-ryegrass plots sown with red clover and not receiving artificial nitrogen were found to have fewer chicory plants by spring of the first harvest year and this effect increased with time. Overall, sowing plots with ryegrass only, and treating with artificial nitrogen, was the best approach to maintain the persistency of chicory within mixed swards.

Keywords: chicory, *Cichorium intybus*, persistency, mixed swards, sowing rates

Introduction

Including chicory in grass seed mixtures is becoming more popular, as it offers high yields of very palatable and nutritious fodder for grazing livestock with, typically, crude protein (CP) and water-soluble carbohydrates (WSC) concentrations that are higher than perennial ryegrass (PRG) (*Lolium perenne)* (Li and Kemp, 2005). Chicory persistency is limited to 3-5 years and is highly dependent on plant establishment success at sowing (Li *et al*., 1997). However, little is known about the persistency of chicory when sown in differing ratios with other forage species. This experiment determined the effects of sowing chicory in combination with PRG only or PRG with red clover (RC) on the persistency of chicory over three years.

Materials and methods

Field plots (10×2.5 m) were sown with chicory (cv. Puna II) and perennial ryegrass (cv. Premium) in differing proportions, and either with or without red clover (cv. Merviot), in a replicated randomized design with four blocks. Forages were sown on 25 June 2009 at rates corresponding to a proportion of their usual monoculture rate, not the total seed rate (i.e. chicory at 6 kg ha^{-1}, PRG at 33 kg ha^{-1} and PRG-red clover at 22 + 11 kg ha^{-1}). Treatments consisted of chicory sown with PRG alone or a ryegrass-red clover mix in a 2:1 ratio, resulting in 10 combinations with chicory included as 0.9, 0.7, 0.5, 0.3 and 0.1. The experimental area was treated with glyphosate herbicide before ploughing. Lime was applied to correct any soil deficiencies prior to cultivation. Each plot was sown using an Oyjord experimental plot drill before the area was harrowed using an Einbock harrow and rolled. Slug pellets were applied at 2.5 kg ha^{-1} on the 10 July. All plots received 60 kg ha^{-1} P_2O_5 and

60 kg ha^{-1} K_2O in the seedbed. The PRG-chicory plots received 50 kg N ha^{-1} during establishment. During the establishment year, plots were harvested using a Haldrup plot harvester on 14 September and 19 October. During the first and second harvest years, plots were harvested at 4-week intervals and the PRG-chicory plots received inorganic N on 4 occasions. To determine the persistency of the mixtures, the number of chicory plants within a 0.36×0.25 m quadrat at 6 random sites within each plot were counted at establishment (7 weeks post-sowing), in autumn 2009, in spring and autumn of the first and second harvest year, and in spring of the third harvest year, and the plant population m^{-2} determined. Data were analysed using Genstat® 11.1 by polynomial contrast analysis to determine the effects of sowing ratios.

Results

When chicory was sown with either ryegrass or a ryegrass-red clover mix, there was a positive linear effect of the sowing ratio on chicory plant numbers during the establishment year and these differences were still significant by the spring of the third harvest year (Table 1).

Table 1. Mean plant populations of chicory (plants m^{-2}) when chicory was sown in combination with perennial ryegrass, with or without red clover (RC), from establishment through to spring of the third harvest year.

	RC	Chicory Sowing Ratio (R)					sed	Prob	
		0.1	0.3	0.5	0.7	0.9		RC	R
Establish 2009	−	30	82	157	221	270	20.4	ns	***
	+	31	91	165	200	280			
Autumn 2009	−	34	76	97	134	154	12.7	ns	***
	+	26	75	117	140	174			
Spring 2010	−	33	84	116	188	222	12.7	ns	***
	+	29	68	133	170	201			
Autumn 2010	−	26	69	74	92	112	15.4	ns	***
	+	21	45	83	107	124			
Spring 2011	−	19	58	64	107	119	10.9	*	***
	+	21	34	55	82	121			
Autumn 2011	−	31	69	77	81	108	11.9	***	***
	+	8	12	21	35	69			
Spring 2012	−	25	59	72	75	85	11.0	***	***
	+	3	14	19	33	62			

Ns: P>0.05; *: P<0.05; ***: P<0.001; R: Sowing Ratio

Figures 1 and 2 show the number of chicory plants m^{-2} present in plots sown without and with red clover, respectively, in the five differing ratios and how the chicory persisted over time. The largest decline in chicory plant populations was found to relate to the highest sowing rate. Plots not receiving artificial nitrogen and sown with red clover were found to have a reduced number of chicory plants by spring of the second harvest year (P<0.05), and this effect increased further with time (P<0.001) (Table 1). Therefore, by autumn of the first harvest year the chicory population of these plots was found to be below 25 plants m^{-2} when chicory was sown at 10% of its usual monoculture sowing rate. In contrast, by spring of the third harvest year, in plots where chicory was sown with ryegrass and treated with artificial N, the chicory population was still 25 plants m^{-2} when chicory was sown at 10% of its usual monoculture sowing rate with ryegrass but without red clover.

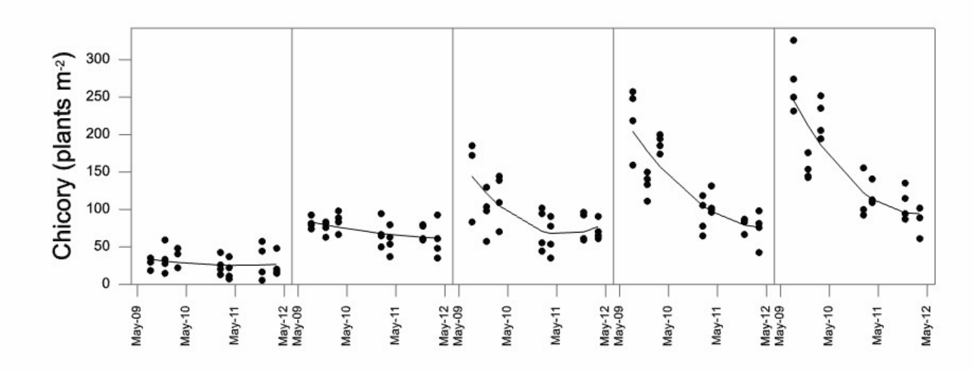

Figure 1. Plant populations of chicory (plants m^{-2}) when sown in combination with perennial ryegrass without red clover at a sowing ratio of 0.1, 0.3, 0.5, 0.7 and 0.9 (left to right) from establishment through to spring of the third harvest year.

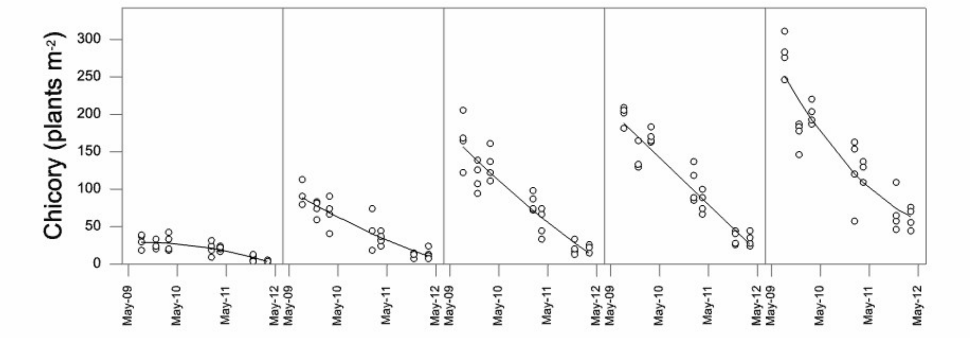

Figure 2. Plant populations of chicory (plants m^{-2}) when sown in combination with a perennial ryegrass-red clover mix at a sowing ratio of 0.1, 0.3, 0.5, 0.7 and 0.9 (left to right) from establishment through to spring of the third harvest year.

Conclusions

The highest plant populations for chicory were found when chicory was sown at 90% of the target proportion of the sown sward and this effect was found at establishment through to the spring of the third harvest year. Chicory-ryegrass plots sown with red clover and not receiving artificial nitrogen were found to have fewer chicory plants by spring of the second harvest year and this effect increased further with time. Overall, sowing plots with ryegrass only, and treating with artificial nitrogen, was the best approach to maintain the persistency of chicory within mixed swards as chicory did not compete well with red clover when sown in combination with ryegrass.

Acknowledgements

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The effect of different fodder galega-grass mixtures and nitrogen fertilization on forage yield and chemical composition

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Abstract

Fodder galega (*Galega orientalis* Lam.) is a forage legume that has been grown in Estonia for approximately forty years. Pure galega is known to be a persistent and high-yielding crop, rich in nutrients, particularly crude protein (CP). Galega is usually grown in a mixture with grass in order to optimize its nutrient concentration, increase dry matter (DM) yield and improve fermentation properties. There are certain grass species suitable for the mixture. In this study, galega-grass mixtures with timothy (cv. Tika), meadow fescue (cv. Arni) and bromegrass (cv. Lincoln) were investigated in four successive years (2008-2011). Three cuts were taken during 2008, 2009, 2011 and two in 2010. Nitrogen (N) fertilization rates were N0, N50, N100, applied in spring before the first and second cuts. Early season N applications to galega-grass swards can help N-deficiency in the spring. The total (DM) yield varied from 7.2 to 13.3 t ha^{-1}. DM yield was dependent on the year, mixture and fertilization level. The CP concentration in the DM varied from 155-208 g kg^{-1}. CP was dependent on the year, mixture and fertilization. High N fertilization favoured grass growth and reduced the role of galega in the sward. The dry and warm summer favoured the galega growth in the years 2010-2011.

Keywords: fodder galega, goat's rue, galega-grass mixtures, forage yield, fertilization

Introduction

Along with other forage legume crops (like lucerne and clovers), goat's rue or fodder galega (*Galega orientalis* Lam.) has been grown in Estonia for almost forty years. Galega is very persistent with a high-yielding ability. Results have shown that yields can possibly be 8.5 to 10.5 t of dry matter (DM) and 1.7 to 1.8 t of crude protein (CP) per hectare with a CP concentration of 200-220 g kg^{-1} DM (Raig *et al*., 2001). The nutritive value is the highest when the first cut is taken at shooting, budding or the beginning of flowering (Raig *et al.,* 2001). In order to connect the need for nitrogen fertilizer with biologically fixed nitrogen, it is most favourable to grow galega in a mixture with grass. Of plant nutrients, nitrogen has the highest effect on yield and quality of forage crops. When choosing grasses for mixtures, the species development speed, duration and the effect on nutritive value should be considered. Earlier results have shown that growing galega in mixtures with grasses improves the nutritive value and ensiling properties of the forage crop (Lättemäe *et al.,* 2005, 2010). The aim of this investigation was to study the effects of different galega-grass mixtures and N fertilization on DM yield and chemical composition of forage.

Materials and methods

The experimental field was established in 2003 in Saku (latitude 57º25'N) and the data were collected from 2004. The data in four successive years (2008-2011) were recorded in this study. The trial plots were established on a typical soddy-calcareous soil where the agrochemical indicators were as follows: pH_{KCl} 7.4 (ISO 10390); humus concentration C_{org} 4.1% and concentration of lactate soluble P and K being 97 and 166 mg kg^{-1} respectively. Three galega-grass mixtures were used. The galega variety 'Gale' was sown in binary mixtures with meadow fescue cv. 'Arni' (10 kg seed ha^{-1}), timothy cv. 'Tika' (6 kg ha^{-1}) and bromegrass cv. 'Lincoln' (15 kg ha^{-1}) respectively. The sowing rate of the seed of 'Gale' was 20 kg ha^{-1} in all mixtures. In order to increase competitivity of grasses and yield of the first cut, three N fertilization levels were used: N0, N50 and N 100 kg ha^{-1} (April, May I or II decade). The crop was cut with a scythe, then weighed and samples were taken for analyses. Prior to sampling the botanical composition of crop was determined. A three-cut system was used during harvest with three replicates of the plots of each treatment. All statistical analyses were carried out by using the GLM procedure of SAS.

Results and discussion

The results indicate that galega-grass mixtures provided high DM yields during the period since the field trial was established. In 2008-2011 the yields varied from 7.2 to 13.3 t ha^{-1} (Table 1). The average yield was higher in 2011 and variable, ranging from 11.4 to 13.3 t ha^{-1}. There were significant differences between the average yields at different N levels and mixtures.

Table 1. The DM yield of fodder galega-grass mixtures in 2008-2011.

Mixture	2008			2009			2010			2011		
	N0	N50	N100	N0	N50	N100	N0	N50	N100	N0	N50	N100
Gale/Arni	11.6	10.7	11.6	7.6	7.2	7.8	10.4	10.0	9.4	12.7	13.3	12.4
Gale/Tika	10.8	11.3	10.8	8.2	11.4	11.4	11.8	10.1	10.9	11.7	11.5	13.0
Gale/Lincoln	11.3	12.4	10.9	8.1	10.1	11.4	9.8	9.7	10.7	12.0	11.4	11.7
Average	11.2	11.5	11.1	8.0	9.6	10.2	10.7	9.9	10.3	12.1	12.0	12.4
	§LSD $_{0,05}$ = 0.39			§LSD $_{0,05}$ = 0.64			§LSD $_{0,05}$ = 0.45			§LSD $_{0,05}$ = 0.60		
	¶LSD $_{0,05}$ = 0.22			¶LSD $_{0,05}$ = 0.37			¶LSD $_{0,05}$ = 0.32			¶LSD $_{0,05}$ = 0.41		

§- Least significant difference of N treatment; ¶- $LSD_{0,05}$ of mixture treatment

There were significant differences between Gale-Arni vs. Gale-Tika and Gale-Lincoln, as well as Gale-Tika and Gale-Lincoln at N0 and N100 fertilization levels. Application of N fertilizer changed the botanical composition of the sward, with increased grasses and reduced proportions of Gale in the sward. The average Gale proportion was 59% in 2008. In the second year Gale was reduced to 27% due to frost damage during the second decade of May (temperature down to -3.9°C). When the two-cut system was applied the Gale recovered in 2010. The Gale proportion also declined considerably when fertilization increased (Figure 1.). The average Gale proportion is essential at N0 and N50 treatments. When it is higher, the CP concentration increases in the crop (r=0.53; P<0.05). At fertilization level of N0 and N50 the meadow fescue cv. Arni was less competitive. The highest competitiveness was shown by the bromegrass Lincoln at the N100 fertilization in 2009.

The nutritive value of mixtures is presented in Table 2, and in general, this mainly depended on fertilization level. Lower CP and metabolizable energy (ME) concentrations were found in treatments when no N fertilizer was used. When fertilization level increased, CP concentration and ME increased but NDF and ADF decreased. Galega usually has a faster development speed compared with grasses. Therefore, the ADF and NDF concentrations were higher in treatments where the Gale proportion was higher and in the Gale-Lincoln treatment, and this obviously due to high fibre concentration of bromegrass.

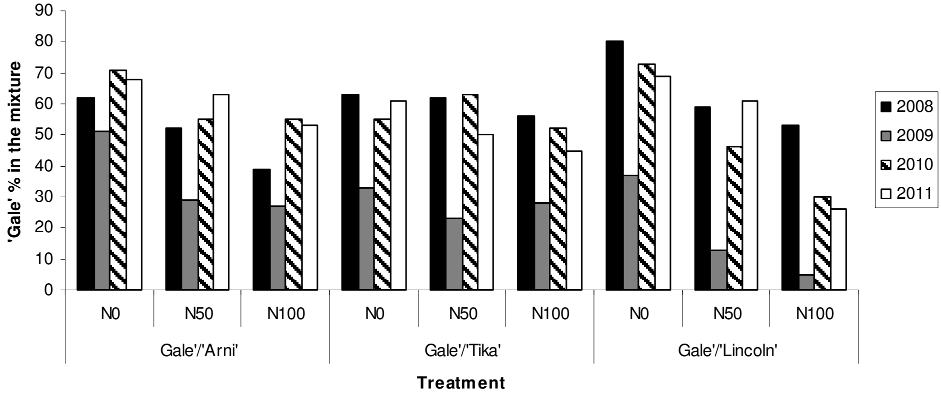

Figure 1. The botanical composition of galega-grass mixture of first cut in 2008-2011.

Table 2. The nutritive value of the fodder galega-grass mixtures of first cut in 2008-2011.

Mixture	N fertilizer	CP g kg^{-1} DM	NDF g kg^{-1} DM	ADF g kg^{-1} DM	ME MJ kg^{-1} DM
Gale-Arni	N0	186	465	316	10.1
Gale-Arni	N50	190	456	312	10.2
Gale-Arni	N100	208	414	291	10.5
Gale-Tika	N0	183	469	327	10.0
Gale-Tika	N50	206	458	314	10.1
Gale-Tika	N100	215	425	289	10.5
Gale-Lincoln	N0	155	493	343	9.7
Gale-Lincoln	N50	171	439	331	9.9
Gale-Lincoln	N100	204	482	313	10.1

Conclusions

The galega-grass mixtures maintained high yielding ability and nutritive value for many years. The nutritive value of mixtures was mainly dependent on N fertilization. High N fertilization rate favoured grass growth but reduced the role of galega in the sward. Similar metabolizable energy values were obtained in the Gale-Arni and Gale-Tika mixtures. The ME concentration was lower in the Gale-Lincoln mixture due to higher fibre concentration of bromegrass compared with other grasses. On the basis of these results, a fertilization rate of 50 kg N ha^{-1} should be recommended in order to avoid grasses being lost from the pasture, while also helping to avoid N deficiency in the spring.

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The botanical composition of grass-legume mixtures supplied with a biopreparation and varied rates of nitrogen fertilization

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Abstract

The aim of this study was to determine the changes in the floristic composition of grass-legume swards during three years of using a biopreparation and different rates of nitrogen fertilization. In April 2007 the field experiment was established in a randomized block design with three replications. The experimental factors were the three mixtures: A1 - *Festulolium* 50%, red clover 50%; A2 - *Festulolium* 50%, alfalfa 50%; and A3 - *Festulolium* 50%, red clover 25%, alfalfa 25%. In addition, a biopreparation was used: B1 - no biopreparation, or B2 - biopreparation at a dose of 0.9 l ha^{-1}. Fertilizer nitrogen levels were as follows: C1 - without nitrogen fertilization; C2 - 60 kg N ha^{-1}; C3 - 120 kg N ha^{-1}. Nitrogen was applied as ammonium nitrate (34% N) in three equal amounts for each successive regrowth. Potassium was also applied for each regrowth and phosphorus once in early spring. A detailed study included the sward floristic composition of the first cut, which was determined by botanical-weight analysis. In subsequent years the mixtures that were fertilized with nitrogen had a relatively stable share of grasses and legumes and there was a slight increase of weeds. However, the treatments supplied with the biopreparation mainly showed an increase in proportion of legumes.

Keywords: botanical composition, grass-legume mixtures, biopreparation, nitrogen dose

Introduction

Research has shown that *Festulolium braunii* can be grown not only in single-species plantings but also in mixtures with legume, especially with red clover (Staniak, 2009; Sosnowski and Jankowski, 2010). Published data also confirm the possibility of cultivating the species with alfalfa (Borowiecki, 1997a,b). However, there are no reported studies on the cultivation of the species in two and three–component mixtures with alfalfa and with red clover. The aim of this study was to determine the changes in the floristic composition of the sward of *F. braunii* mixtures with alfalfa and red clover supplied with a biopreparation and under different nitrogen fertilization treatments.

Materials and methods

A field experiment was established in April 2007 in a randomized block arrangement with 3 replications at the field site of the Grassland Department and Landscape Architecture of Siedlce University (52.169°N, 22.280°E). The experimental soil belonged to hortisole type, consisting of low-loam sand. The soil was characterized by a neutral pH, average content of humus and potassium, and high contents of available forms of phosphorus and magnesium. It also contained 0.18% total nitrogen.
The experimental factors were as follows. Mixtures (A) comprised: A1- *Festulolium braunii* (cv. Felopa) 50%, *Trifolium pratense* (cv. Tenia) 50%; A2 - *Festulolium braun* 50%, *Medicago sativa* ssp. *media* (cv. Tula) 50%; A3 - *Festulolium braunii* 50%, *Trifolium pratense* 25%, *Medicago sativa* ssp. *media* 25%. Biopreparation (B) comprised either: B1- no

biopreparation (control); or B2 – with biopreparation. Nitrogen fertilization (C) comprised: C1 - without nitrogen; C2 - 60 kg N ha^{-1}; or C3 - 120 kg N ha^{-1}. The biopreparation was used at a dose of 0.9 L ha^{-1} diluted in 350 L of water. The composition of the biopreparation (UGmax) is given in Table 1. For each successive regrowth, nitrogen in the form of ammonium nitrate (34% N) was applied in three divided doses. Potassium fertilization was used for each regrowth (120 kg K_2O ha^{-1} annually), in divided doses with the N. However, phosphorus, at 80 kg P_2O_5 ha^{-1}, was applied once in early spring. All plots were cut three times in each year, 2008-2010.

Table 1. The composition of the biopreparation.

The content of macro-and micronutrients mg L^{-1}						Microorganisms
N	P_2O_5	K_2O	Mg	Na	Mn	Lactic acid bacteria, photosynthetic bacteria, *Azotobacter*, *Pseudomonas*, yeasts, actinomycetes
1200	500	3500	100	200	0.3	

The detailed study included the sward floristic composition of the first cut, which was determined by the botanical-weight analysis. After cutting, samples (1 kg) of plant biomass were taken and than treated for botanical analysis by determination of the percentage of grasses, legumes and weeds. The obtained results were evaluated statistically by using analysis of variance for multivariante experiments. Medium differentiation was verified by Tukey's test at significance level $P \leq 0.05$.

Results and discussion

The botanical-weight analysis of the tested crops showed significant differentiation of the proportions of plant groups in the different mixture treatments (Table 2). In the mixtures supplied with biopreparation, a significant decrease of weeds was recorded. The smallest share of weed plants (average 4.3%) was obtained using the biopreparation in the three–component mixture A3 (*Festulolium* with alfalfa and clover). The greatest reduction in weed (from 14.1 to 5.3%) after applying the biopreparation was in a crop of *Festulolium* with hybrid alfalfa (mixture treatment A2). In comparison with the control, microbial soil treatment resulted in increase of grasses and legumes in each cultivated mixture. Mineral nitrogen fertilization also reduced the infestation of weeds. It should be noted, however, that this trend depended on the mixture composition. The lowest weed infestation, compared to treatments without nitrogen, formed an average of 6.4% in the plots of *Festulolium* with red clover (A1) and supplied with 120 kg N ha^{-1} (C3). It should be also noted that the dose of nitrogen applied in the mixture resulted in a decrease in share of grasses and an increase of legumes, while the three-component mixture A3 reduced the proportion of alfalfa **a**nd clover and increased the grasses. The dependence of the mixture composition and nitrogen fertilization was also described in the studies of Harasim (1995) and Szwed (1997). Jodełka *et al.* (2006) showed that in grass-legume mixtures fertilized only with phosphorus and potassium there was a decrease in the proportion of grasses. The decrease amounted about 25% with a significant increase of weeds (average 16% in relation to control). The greatest reduction of infestation was achieved by combining the biopreparation with the highest nitrogen dose (120 kg ha^{-1}). The share of weeds on these treatments ranged from 1.2% in the *Festulolium* mixture with red clover (A1), to 5.3% in a mixture of *Festulolium* with alfalfa (A2). These crops were characterized by relatively stable average 48% share of grasses and legumes. The study shows that regardless of biopreparation and nitrogen dose the highest infestation (10.4%) was for the *Festulolium* with red clover (mix A1). In addition, they were characterized by the smallest (average 39%) proportion of legumes. The addition of alfalfa to te mixtures (A2 and A3) increased the amount of legumes in the sward (average 49%) and slightly reduced the weed infestation (to less than 10%). Use of biopreparation, independently of the mixture type and nitrogen dose, resulted in a significant increase in the proportions of grasses and legumes in the sward, and a decline of weeds.

Table 2. Botanical composition (in %) of the first cut sward of *Festulolium braunii* mixtures with red clover and alfalfa depending on the type of mixture, and application of biopreparation and nitrogen dose (average of years).

Nitrogen dose (C)	Biopreparation (B)	Type of mixture (A)									Mean		
		A1			A2			A3					
		G	L	W	G	L	W	G	L	W	G	L	W
C1	B1	47.3	38.2	14.5	38.4	42.5	19.1	37.9	49.6	12.5	41.2	43.4	15.4
	B2	55.9	30.6	13.5	43.6	53.1	3.3	43.7	49.8	6.5	47.7	44.5	7.8
C2	B1	49.1	36.6	14.3	42.2	46.2	11.6	43.1	47.5	9.4	44.8	43.4	11.8
	B2	51.3	41.0	7.7	43.0	49.9	7.1	41.7	52.6	5.7	45.3	47.8	6.8
C3	B1	48.4	40.0	11.6	45.2	45.9	8.9	40.2	46.9	12.9	44.8	44.3	11.1
	B2	49.8	49.0	1.2	44.6	50.1	5.3	50.6	47.5	1.9	48.3	48.9	2.8
Biopreparation (B)													
B1		48.3	38.3	13.4	41.9	44.9	14.1	40.4	48.0	11.6	43.5	43.7	13.0
B2		52.3	40.2	7.5	43.7	51.0	5.3	45.3	50.0	4.7	47.1	47.1	5.8
Nitrogen dose (C)													
C1		51.6	34.4	14.0	41.0	47.8	11.2	40.8	49.7	9.5	44.5	43.9	11.6
C2		50.2	38.8	11.0	42.1	48.1	9.8	42.4	50.1	7.5	44.9	46.0	9.4
C3		49.1	44.5	6.4	44.9	48.0	7.8	45.4	47.2	7.4	46.5	46.6	7.2
Mean		50.3	39.3	10.4	42.8	48.0	9.2	42.9	49.0	8.1	45.3	45.5	9.4

G – grasses; L – legumes; W – weeds

A1 – *Festulolium* + red clover; A2 – *Festulolium* + alfalfa; A3 – *Festulolium* + red clover + alfalfa

C1 – without nitrogen; C2 – 60 kg N ha^{-1}; C3 – 120 kg N ha^{-1}

$LSD_{0.05}$ for: B(G) 3.3, (L) 3.2, (W) 6.7, C(G) n.s., (L) n.s., (W) 4.3, CxB(G) 3.4, (L) 4.2, (W) 4.7

Conclusion

Using a biopreparation in the cultivation of *Festulolium* with legumes reduced the occurrence of weed plants in the sward. Grasses and legumes participation were on 40-50% level. A similar trend was obtained by using mineral nitrogen fertilization. The smallest share of weeds (from 1.2 to 5.3%) was obtained by combining the biopreparation with mineral fertilization at a dose of 120 kg N ha^{-1}. The studies have also shown that the addition of alfalfa as a mixture component caused an increase in participation of legumes and reduction of weeds.

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Potential of Westerwold ryegrass as a nurse crop for grass-clover mixtures

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Abstract

The purpose of nurse crops is to improve establishment and initial yield of newly sown leys without having detrimental effects on the sward after the short-lived nurse crop has disappeared. In a field experiment 0, 1.75, 3.5, 7.0 and 14 kg of seeds ha^{-1} of Westerwold ryegrass (*Lolium multiflorum* Lam. var. *westerwoldicum* Mansh. = LW) were added as a nurse crop to grass-clover mixtures to test the effects on both dry matter (DM) yield and weed-proportion at establishment and on gaps in the sward and botanical composition after the LW plants had disappeared. The addition of LW increased DM yield in the first harvest following sowing by 22% when 1.75 kg LW seed was applied (1.38 Mg vs. 1.13 Mg DM ha^{-1}), and by up to 81% in the 14 kg-treatment. The supplement of 1.75 kg LW seed decreased the weed proportion from 32% to 25% and further to about 12% at the 7 and 14 kg LW treatments. In contrast, at the end of the first main utilization year when the short-lived LW had disappeared, the treatments with 7 and 14 kg of LW showed about 50% more gaps than the other LW sowing rates and *Lolium perenne* yield was decreased by about 20%. We conclude that LW used as a nurse crop may improve initial DM yield and suppress weeds successfully. However, due to the pronounced risk of gaps and deteriorating botanical composition at high sowing densities of LW, the seed rate should be kept well below 7 kg ha^{-1}.

Keywords: legume-grass mixtures, Westerwold ryegrass, *Lolium multiflorum* Lam. var. *westerwoldicum* Mansh., nurse crop, weeds, sward density

Introduction

The success of legume-grass mixtures depends crucially on their development after sowing. All sown species must establish properly and weeds must be successfully suppressed (Sanderson *et al.*, 2012). In addition, an early and high first yield has to be produced. Modern mixtures are composed of fast- and slow-developing species (Nyfeler *et al.*, 2009). The fast-developing species cover the soil rapidly and produce the initial yield. Since they usually are rather short lived they need to be replaced by the slow-developing, but more persistent, species throughout the first main utilization year (Suter *et al.*, 2012; Finn *et al.*, 2013). The amounts and proportions of seed of the various components are meticulously fine-tuned in order to guarantee a seamless and successful sequence of species providing high yields of good quality forage (Suter *et al.*, 2010).
Based on the success of combining fast-developing with slow-developing species, the question arises of whether adding an extremely fast-developing, but very short-lived species as a nurse crop can further increase the initial yield of the mixture and improve suppression of weeds. *Lolium multiflorum* Lam. var. *westerwoldicum* Mansh. (LW) exhibits these traits. However, later growth of the mixture must not be compromised by the use of LW. Thus, in order to test the suitability as a nurse crop in this context, a field experiment with different sowing densities and cultivars of LW was established.

Materials and methods

From 2007 to 2009, a field experiment with two legume-grass mixtures was established at four sites belonging to the ART Reckenholz-Tänikon Research Station. Mixture 'A' consisted of *Trifolium pratense* L., *Trifolium repens* L., *Lolium perenne* L., *Dactylis glomerata* L., *Festuca pratensis* Hudson and *Phleum pratense* L. In mixture 'B' *D. glomerata* and *F. pratensis* were replaced with *Festuca rubra* L. and *Poa pratensis* L. To each mixture 0, 1.75, 3.5, 7 and 14 kg seed ha^{-1} of one of the following LW cultivars were added: 'Andrea', 'Ducado', 'Grazer', 'Jivet', 'Lifloria', 'Limella', 'Lirasand' and 'Sabroso'. The experiment was a randomized complete block design with four replicates. At the first cut, taken 7-9 weeks after sowing, the herbage was harvested with a parcel-mower and dry matter (DM) yield was determined. The weed proportion was expressed as % of biomass, based on samples taken from each plot. Prior to the fifth (the final) cut of the first main utilization year, the gaps were visually assessed and expressed as % of the surface. Data were analysed by analysis of variance, testing 'sowing density of LW', 'cultivar', 'mixture' and their interaction effects.

Results and discussion

Adding LW increased DM yield in the first harvest following sowing by 22% when 1.75 kg LW was applied (1.38 Mg vs. 1.13 Mg DM ha^{-1}), and by up to 81% in the 14 kg LW treatment (Figure 1a). This increase in yield was identical for both mixtures and for all cultivars (sowing density of LW × cultivar: ns), although there was a cultivar effect in dry matter yield (P<0.05), caused by cv. 'Sabroso', which produced a significantly lower yield than the other varieties (1.36 vs. 1.62 Mg DM ha^{-1}; data not shown). The steady increase in DM yield caused by increased sowing density of LW suggests that the effect of the nurse crop on dry matter yield may also be present at higher sowing densities of LW than were used in this experiment. In an earlier study with two cultivars of LW (Opitz von Boberfeld, 1983), the use of 20 kg of LW ha^{-1} led to a 27% increase in dry matter yield in the sowing year.
In order to achieve a positive effect on quality of forage and weed suppression, the increased dry matter produced by the nurse crop has to decrease both the weed proportion in the herbage and weed growth, as expressed by weed DM ha^{-1}. In this experiment, the proportion of weeds decreased strongly from 32% at 0 kg LW to 25% at 1.75 kg LW and further to about 12% at 7 and 14 kg LW (Figure 1b). The cultivars of LW showed significantly different (P<0.001) weed-proportions, with cv. 'Sabroso' exhibiting the highest (25%) and cv. 'Andrea' the lowest (16%) value (data not shown). Not only weed proportion, but also weed DM yield, was reduced significantly (P<0.01) by addition of LW seed. While weed DM yield was 0.41 Mg DM ha^{-1} at 0 kg LW it was less (0.37 Mg DM ha^{-1}) at 1.75 kg LW and even lower (0.23 Mg DM ha^{-1}) at the 7 and 14 kg LW treatments (data not shown). This is evidence for an immediate effect on weed suppression, an important factor in avoiding establishment of perennial gap fillers (e.g. *Taraxacum officinale*) at this early phase of development of the mixture. Weed DM yield was not affected by the cultivar of LW.
The differences between cultivars found in total DM yield and weed-proportion of the mixture indicate that appropriate choice of cultivar is important. However, it remains unclear whether these differences are due to differences at the individual cultivar level or are caused by cultivar type, e.g. single-cut vs. multi-cut or diploid vs. tetraploid, as suggested by Opitz von Boberfeld (1983). Thus, further analyses at cultivar-type level need to be conducted.
At the end of the first main utilization year, when the short-lived LW has disappeared, the treatments with a sowing density of 7 and 14 kg of LW showed about 50% more gaps than the others (Figure 1c) irrespective of the cultivar or the mixture. The effect of the 7 and 14 kg LW sowing densities on gaps may be underestimated by simply looking at gap percentage: *Trifolium repens* produced more dry matter at 14 kg LW than at 0 kg LW (P<0.0531; data not shown), suggesting important ingrowth into gaps, thus lowering detected gap percentage. Most importantly, high sowing rates of LW exhibited also about 20% less DM yield of *L. perenne*: it was 0.55 Mg DM ha^{-1} at 0 kg LW and 0.45 Mg DM ha^{-1} in the 14 kg LW

treatment (P<0.05). Since *L. perenne* is a key component of yield, stability and quality of these mixtures this is a very negative effect.
For all of our results, the various mixtures did not differ in their reaction to the LW treatments (LW × mixture: ns), allowing for more general conclusions. It is of great importance that, irrespective of mixture and cultivar of LW, a sowing density of 7 kg LW ha^{-1} and above had detrimental effects on botanical composition and also increased gap percentage. Thus, our results show that the threshold of 20 kg seeds ha^{-1} of LW, the value mentioned by Opitz von Boberfeld (1983) should be greatly reduced.

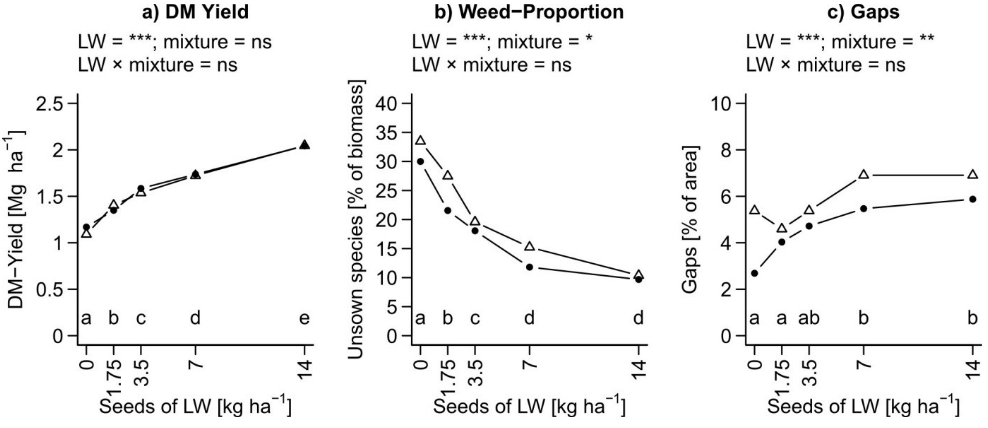

Figure 1. Effects of including Westerwolds ryegrass as a nurse crop at different seed rates and mixture (dots, Mixture A; triangles, Mixture B) on DM yield and % of unsown species in the first harvest after sowing and on gaps in the sward at the end of the first year of utilization.

Conclusions

We conclude that use of LW as a nurse crop may improve initial dry matter yield of newly sown swards and suppress weeds successfully. Due to the pronounced risk of gaps at high sowing densities of LW, its seed rate should be kept well below 7 kg ha^{-1}.

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Effects of mixing species on light interception of intensively managed swards

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Abstract

Increased resource capture due to niche complementarity might explain the positive diversity-productivity relationship often observed in experimental grasslands. In heavily fertilized grasslands, the high leaf area index achieved by monocultures seems unfavourable to niche complementarity for light. However, under moderate fertilization and frequent defoliations, differences in light interception between sward types might be important. This study aimed at assessing the effects of combining forage species with widely differing leaf traits on light interception of agricultural grasslands as compared with grass monocultures. The percentage of Photosynthetically Active Radiation (PAR) intercepted by the swards was measured after four weeks of regrowth in monocultures, two- and four-species mixtures of *Lolium perenne*, *Cichorium intybus*, *Trifolium pratense* and *Trifolium repens* fertilized at 145 kg N ha^{-1} yr^{-1}, as well as in *L. perenne* monocultures fertilized at 350 kg N ha^{-1} yr^{-1}. The moderately fertilized *L. perenne* monocultures intercepted significantly less PAR (73%) than all other monocultures, which intercepted more than 95% of PAR. In the *L. perenne-C. intybus* mixture and in the *C. intybus* monoculture, PAR interception was higher than in the *L. perenne* monoculture, although biomass production was similar. As evidenced by its monoculture, *C. intybus* thus captured a high fraction of PAR at low level of biomass but no diversity effect between this species and *L. perenne* was detected. Grass-clover mixtures intercepted more PAR than the *L. perenne-C. intybus* mixture and as much as the heavily fertilized *L. perenne* monoculture. Combining *L. perenne* with *C. intybus* or especially a clover species therefore improved light interception of ryegrass-based swards at moderate levels of N fertilization.

Keywords: multi-species grasslands, Photosynthetically Active Radiation, light interception

Introduction

The need for increasing productivity while becoming more sustainable (sustainable intensification) poses new challenges to agriculture. Nowadays, many productive grasslands in Europe consist of grass monocultures with high nitrogen fertilization (Rochon *et al.*, 2004). On the other hand, studies have shown that combining only a few plant species with complementary traits can significantly increase productivity of intensively managed grasslands compared with that of grass monocultures (e.g., Nyfeler *et al.*, 2009). A more complete capture of resources is thought to form the basis for the observed diversity-productivity relationship. Spehn *et al.* (2005) showed that mixing species with different functional traits improved PAR interception in extensively managed grasslands. However, intensively managed grasslands reach a high biomass density within a few weeks after mowing (Suter *et al.,* 2001). In these conditions, because of the unidirectional nature of light (PAR), the vertical gradient for light within the canopy is steep. Moreover, PAR capture is limited by the amount of PAR reaching the top of the canopy, without possibility for the plants to acquire PAR from another source. Thus, complimentary effects between plants with different sizes and leaf angles probably differ in extensive and intensive systems. This study aimed at assessing the effects of combining two or four forage species with widely differing leaf traits on light interception of agricultural grasslands, as compared with moderately or heavily fertilized grass monocultures.

Material and methods

Fifty-five swards of 15 m^2 each (3×5 m) were sown in August 2010 in a completely randomized design. The composition of the swards was based on four species with different leaf traits (leaf angle and height): *Lolium perenne*, *Cichorium intybus*, *Trifolium pratense* and *Trifolium repens*. Sixteen monocultures (4 plots of each species), 24 two-species mixtures (4 plots of each combination) and 12 four-species mixtures were fertilized at the same moderate level of nitrogen (N) fertilization (145 kg N ha^{-1} yr^{-1}), and 3 monocultures of *L. perenne* were heavily fertilized (350 kg N ha^{-1} yr^{-1}). All swards were cut seven times in 2011. The following measurements were performed in August 2011 after four weeks of regrowth: The relative abundance of the species was assessed in each plot by sorting community biomass from a 50×50 cm sub-plot and measuring the dry weight of each species. The Photosynthetically Active Radiation (PAR) was measured above the canopy (PAR_{above}) and at the soil level (PAR_{below}) in 5 different locations in each plot with a ceptometer (AccuPAR LP80, Decagon Devices, USA). The proportion of PAR intercepted by the swards was calculated as: 100(1 - PAR_{below} / PAR_{above}). ANOVA and Tukey's test were performed to assess the difference in PAR interception among the swards.

Results and discussion

The proportion of PAR intercepted by the swards varied from 68 to 99%. The lowest values were observed in the moderately fertilized monocultures of *L. perenne* (73 ± 2.5%; mean ± SE), while the monocultures of the three other species and of the heavily fertilized *L. perenne* intercepted all available PAR (>95%; Figure 1a). Thus, the 2- or 4-species mixtures could not further increase PAR interception compared to these monocultures (ANOVA, logit transformation, P=0.70), making a positive diversity-resource capture relationship impossible. In contrast, some monocultures of grassland species studied by Spehn *et al.* (2005) had a vegetation cover as low as 50% at the Swiss site and, correspondingly, intercepted less than 50% of the PAR. Our swards reached biomass levels of up to 288 g DW m^{-2}, while a biomass of 150 g DW m^{-2} was sufficient to intercept more than 95% of PAR (Figure 1a). Thus, in our intensively managed plots (fertilized and frequently defoliated), 10 of the 12 swards reached biomass values that were higher than needed to intercept all available PAR. The only two exceptions were *L. perenne* monoculture and *L. perenne*-*C. intybus* mixture.

C. intybus monoculture intercepted more than 95% of PAR with a biomass level of 153 g DW m^{-2} while *L. perenne* was only able to intercept 73% with nearly the same amount of biomass (123 g DW m^{-2}; Figure 1a). These results indicate that the leaf traits of *C. intybus* allowed interception of more PAR per g DW m^{-2} than those of *L. perenne*. Such interspecific differences (identity effects) originate from biomass allocation to leaves and stems and/or from leaf traits, e.g. leaf angle and specific leaf area. As a consequence, the mixture of *L. perenne* and *C. intybus* intercepted more PAR than the *L. perenne* monoculture, although the amount of standing biomass was the same in both sward types (115 g DW m^{-2}; Figure 1a). Nevertheless, the *L. perenne*-*C. intybus* mixture showed no significant difference in PAR interception compared with the average of the monocultures of its components (Figure 1b, solid line, P=0.11). Thus, no significant diversity effect for PAR interception was detected on a dry weight relative abundance basis when combining *C.intybus* with *L. perenne*. The effect seems to be dominated by the higher light interception of *C. inybus* at low biomass.

Conclusion

This study shows that monocultures and mixtures of, and respectively with, the productive forage species *T. pratense* or *T. repens*, as well as monocultures of *C. intybus* achieve a complete interception of the PAR (>95%) after 4 weeks of regrowth with a fertilization of 145 kg N ha^{-1} yr^{-1}, therefore not allowing mixtures to intercept more PAR than monocultures. However, at the same level of N fertilization, pure *L. perenne* stands intercept significantly less PAR than the mixtures tested in this study. Thus combining *L. perenne* with *C. intybus* or especially *T. pratense* and/or *T. repens*, improves light interception of ryegrass-based swards at moderate levels of N fertilization.

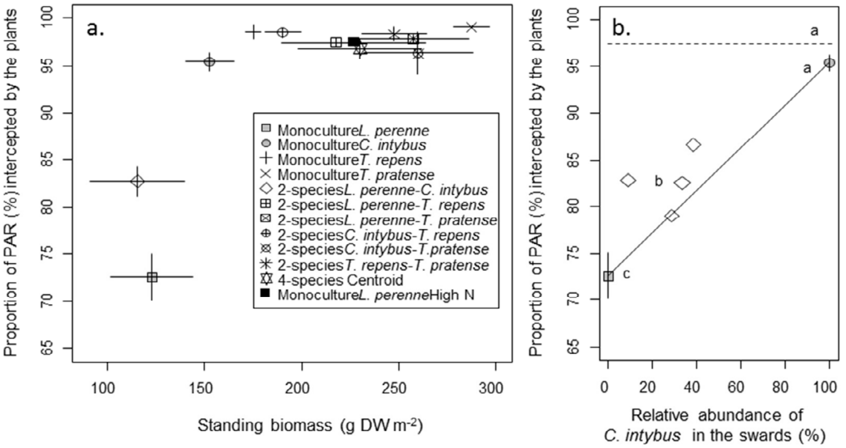

Figure 1. a. Proportion of PAR intercepted by different swards as a function of the standing biomass; error bars: standard error of the mean. b. Proportion of PAR intercepted by the swards as a function of the relative abundance of *C. intybus*; solid line: PAR interception in the mixtures as expected from the monocultures of their components on a dry weight relative abundance basis, doted line: PAR interception in the heavily fertilized monoculture of *L. perenne*. a, b, c: a common letter means that PAR interception is not significantly different at the 5% level.

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Diverse grassland mixtures for higher yields and more stable sward quality

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Abstract

In the last decades of agricultural intensification, species diversity has been largely ignored. However, in nutrient-poor grasslands, plant diversity has been shown to correlate with increased primary production. If such positive productivity effects also apply to agricultural grasslands, resource-use efficiency could be improved. To investigate whether other grassland species and diverse grassland mixtures could be more productive and nutrient efficient, a grassland on a shallow sandy soil in the south of the Netherlands was sown in April 2009 with seven non-leguminous grassland mixtures at three levels of N-fertilization (total N = 80, 200 and 320 kg ha^{-1}). Overyielding was significant, but unrelated to fertilization level: 8.0% overyielding for mixtures with two functional groups of grassland species and 14.4% for mixtures with three functional groups. Transgressive overyielding was not apparent; the DP mixture, containing only one functional group and dominated by the tall growing *Dactylis glomerata,* was equally productive as the more diverse mixtures. The results suggest that more digestible species can be included in the mixture without reducing maximum biomass production. Sward quality of diverse grassland mixtures remained better, having fewer invading species and more surface covered by sown species.

Keywords: grassland, *Dactylis glomerata*, overyielding, species diversity, species identity

Introduction

In the last decades of agricultural intensification, species diversity has been largely ignored. In the Netherlands the main focus was on favouring and improving the most suitable economic species, *Lolium perenne*. Conventionally fertilized grasslands of the Netherlands are dominated by sown cultivars of *L. perenne*, with invading unsown species like *Poa trivialis, Poa annua, Stellaria media* and *Taraxacum officinalis*. Most grasslands on sandy soils are resown every 4-10 years due to botanical degradation often linked to drought periods; having negative effects on soil biodiversity, soil organic matter content and water retention (van Eekeren *et al.*, 2008). Fertilization rates have been reduced in conventional agriculture due to legislation, while the area of grassland that has restricted fertilization rates due to nature considerations and organic production rules is growing. Under these circumstances other grassland species might become more suitable. Moreover, in nutrient-poor grasslands plant diversity has been shown to correlate with increased primary production (Hooper *et al.*, 2005). If such positive productivity effects also apply to agricultural grasslands, grassland production could be improved using mixtures with a diversity of functional types.

Materials and methods

In April 2009 an experiment was started with seven grassland mixtures (Table 1), three levels of N-fertilization and 3 replications (63 plots). Plot size was 7×4 m. The trial was sown on commercially used grassland with a shallow sandy soil in the middle south of the Netherlands. In the year of sowing, all the plots received equal fertilization (138 kg N ha^{-1}) by mixed artificial fertilizer at the beginning of the experiment and two applications of cattle slurry at 20 m^{3} ha^{-1}. In 2010 all treatments received cattle slurry in early spring (80 kg total N ha^{-1}),

and the N2 and N3 fertilization treatments received an additional 120 or 240 kg N ha^{-1} (N2 and N3, respectively) of artificial fertilizer (CAN) in 3 equal parts before the first, second and third cut. The plots were harvested four times in 2010. Dry matter yield was determined by cutting a strip of 0.81×5 m, using a two-wheel tractor. After weighing the fresh biomass, a sub-sample was dried for 48h at 70°C and analysed for dry matter. In May 2011, all sown and invading species were listed per plot and the percentage of cover of each species in the upper surface of the biomass was assessed visually. This assessment of cover percentage is used as an indicator for the contribution of each species to the biomass production of the (fairly homogeneous) grass sward, though it may overestimate tall growing species slightly.
For the analysis of the diversity effect, the mixtures were clustered according to the number of functional groups. L (sod grass), DP (tussock grasses) and PC (non-leguminous herbs) were categorized as having one functional group, LDP and LPC as having two functional groups, and LDPPC and RICH as having three functional groups. Overyield was defined as the difference between the yield of the plot and the average yield of the constituent single functional groups (L, DP and/or PC), while transgressive overyield was defined as the difference between the yield of the plot and the highest yield of the constituent single functional group. The effects of number of grassland functional groups and fertilization level on production and species presence were tested by an ANOVA using GENSTAT software.

Table 1. Composition of tested grassland mixtures.

Mixture	L	DP	PC	LDP	LPC	LDPPC	Rich
Species			--- seeding rate (kg ha^{-1}) ----				
Lolium perenne	40			20	35	19	15
Dactylis glomerata		10		5		5	4
Phleum pratense		30		15		14	8
Cichorium intybus			5		1.5	1.5	1
Plantago lanceolata			5		1.5	1.5	1
Festuca rubra							4
Festuca arundinacea							4
Festuca pratensis							4
Daucus carota							1

Results and discussion

In autumn 2009, the newly sown grass mixtures were well established, though the establishment of *P. pratense* and *F. arundinacea* was disappointing, while *F. pratensis* was virtually not present. In spring 2011 the presence of the sown species was only slightly changed, except for a clear reduction of *Plantago lanceolata* (a known autumn grower) while *P. pratense* had almost disappeared. Thus, the DP mixture was completely dominated by *D. glomerata* with only 2% of ground covered with *P. pratense*, while in the mixtures LDP, LDPPC and RICH its presence could only be noted but no percentage of ground cover could be assigned. Botanical composition was not affected by fertilization level, except for the type of invading species: *T. repens* was the main invader at fertilization level N1, while *P. annua* and *S. media* were the main invaders at levels N2 and N3. However, the number of invading species and the percentage of cover with sown species were affected by the number of functional groups sown (Table 2; $P<0.05$). The major part of this effect was caused by mixture PC, which had a very open sward, but also the cover with sown species of L and DP was 7-9% lower than of the mixtures LDPPC and RICH. Thus, this result does illustrate the effect of species diversity on resistance to weed invasion, while simultaneously indicating the importance of specific species in a mixture (‘species identity’) as was suggested by Huyghe *et al*. (2012).

Average aboveground biomass production was limited to only 7.8 t DM per hectare, and significantly affected by fertilization rate and the diversity of the mixture sown (P<0.001; Table 3). Besides a general diversity effect, there was one functional group with a large additional effect: mixtures that included tussock grasses, dominated by *D. glomerata,* were more productive than the other mixtures (+1.4 ton DM ha^{-1}; P<0.001).

Table 2. Presence of species with more than 1% of cover (average of all N-levels). Values with different letters are significantly different (P<0.05) within each column.

Number of functional groups in mixture	% of ground cover		
	Sown species	Invading species	Number of invading species
1	70^{a}	19	10.1^{b}
2	88^{b}	3	9.1^{b}
3	91^{b}	3	7.2^{a}

Table 3. Yields of mixtures at different fertilization levels (ton DM ha^{-1}). Values with different letters are significantly different (P<0.05) within each column or row.

	Number of functional groups in mixture	1	2	3	Average
Fertilization level	N1	5.5	6.0	6.5	5.9^{a}
	N2	7.9	8.3	8.9	8.3^{b}
	N3	8.6	9.3	10.0	9.2^{c}
	Average	7.3^{A}	7.9^{B}	9.2^{C}	7.8

There was significant (P<0.001) overyielding: 8.0% for mixtures with two functional groups and 14.4% for mixtures with three functional groups. This was relatively small compared Nyfeler *et al.* (2009) and Finn *et al.* (2012) and was possibly because here we deliberately did not include N-fixing herbs. The level of overyielding was not affected by fertilization level, contradicting the assumption that positive diversity effects are more important under resource-poor conditions. Transgressive overyielding was not apparent; the mixture DP was as productive as LDPPC and RICH. As these more diverse mixtures had only ± 40% of cover with *D. glomerata*, it indicates the possibilities to increase digestibility by adding more palatable species like *L. perenne,* without decreasing the yield of the highest yielding species. Again, the large effect of *D. glomerata* on yields stresses the importance of species identity.

Conclusion

In this experiment, diversity effects on productivity and resistance to weed invasion were modest and unrelated to the level of N-fertilization. However, results indicate that possibilities exist to improve agricultural production using grassland mixtures with a diversity of functional types adapted to specific agro-ecological conditions.

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Red clover inclusion in ryegrass-white clover swards for grazing and cutting

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Abstract

Increasing plant species diversity in grasslands may improve productivity and stability of yields. In a field experiment we investigated the herbage dry matter (DM) yield of two-species swards of perennial ryegrass-white clover (*Lolium perenne* L.-*Trifolium repens* L.) and three-species swards of ryegrass-white clover with red clover (*Trifolium pratense* L.). Five different managements represented cutting, grazing, and combinations thereof, with different slurry-fertilization treatments in 1- to 4-year-old swards. The three-species mixture over-yielded the two-species mixture in years 1 and 2. Across all four years the yields were 8-10% higher in cut swards. Inclusion of red clover increased yields of clover across the four years by 51% without fertilizer and by 90% when fertilized. Responses to slurry fertilization were similar in both mixtures and were mainly independent of sward age. There was a complementary effect over the season and across managements. Red clover dominated in the first and third cuts; white clover dominated in the second and fourth cuts. Red clover dominated in cut swards and white clover in grazed swards. The prospects of inclusion of red clover in sown swards include higher nitrogen-use efficiency in ruminants, increased provision of home-grown protein, increased soil fertility and improved sward flexibility.

Keywords: multi-species mixtures, cutting, grazing, grass-clover

Introduction

Increasing plant species diversity in grasslands has been shown to improve productivity, resilience to environmental stress and nutrient use. In Danish agriculture, white clover has been widely used in mixture with grasses due to its good persistence, moderate to high yields and its resistance to grazing. Red clover is now also becoming more commonly used because of its high forage yield potential, despite its poor persistence and poor resistance to grazing. The complementary growth of red clover and white clover could maximize yields and increase the persistence of the clover component under conditions that are not optimal due to the effects of grazing, cutting or fertilization. This study focuses on two-species swards of perennial ryegrass-white clover and three-species swards of perennial ryegrass with white and red clovers, with five different management regimes representing cutting, grazing and combinations, and different fertilizer treatments.

Materials and methods

During 2006-2010 we investigated yield and botanical composition of two- and three-species 1 to 4-year-old swards (Eriksen *et al.* 2013). The seed mixtures (26 kg ha^{-1}) were: 1) 15% white clover and 85% perennial ryegrass, and 2) 4% red clover, 14% white clover and 82% perennial ryegrass. The grass-clover leys were subjected to five treatments during 2006-2010: (1) Grazing regime with cattle slurry application in spring (100 kg total-N ha^{-1}); (2) Grazing regime without slurry application; (3) One spring cut with cattle slurry application in spring (100 kg total-N ha^{-1}) followed by grazing; (4) Cutting regime (four cuts) with cattle slurry application (200 kg total-N ha^{-1}, half in spring and half after first cut); and (5) Cutting regime (four cuts) without slurry application. Herbage production was estimated under the grazing regime in temporarily fenced-off parts of the grazed plots in spring and August.

Results

The three-species mixture produced higher yields than the two-species mixture when the swards were young, especially in year 1, and lower yields when swards were four years old. The annual yields under the cutting regime showed very different developments with increasing age of the sward (Figure 1). The two-species mixture gave constant yields over time, whereas the three-species mixture showed a linear yield decrease over the four years. This was the net effect of a decrease in ryegrass yield that was larger than the increase in clover production followed by decreasing clover contents in three and four-year-old swards.

Fertilizer responses were similar in both mixtures and independent of sward age. The overall mean annual yield increase, as a result of slurry application, was 1.34 t DM, corresponding to 11 kg DM per kg plant-available N.

Compared to the cutting regime, the botanical composition in grazed plots differed in two aspects: 1) where red clover dominated in the two first years under the cutting regime, this dominance was already broken after the first year under grazing, and 2) under grazing, the clover content – especially white clover – was less influenced by fertilization than under the cutting regime (Figure 2). In the combination of cutting and grazing (spring cut followed by grazing) the red clover was more persistent, and in the August cut in the two-year-old sward the red and white clovers were present in almost equal proportions.

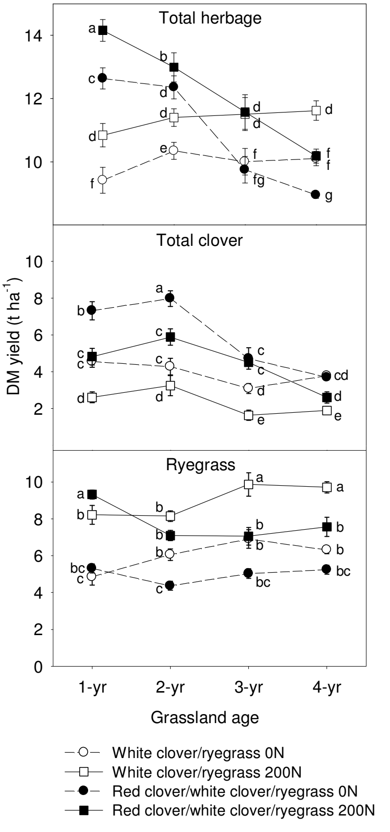

Figure 1. Annual total, clover and ryegrass yields of two- and three-species swards under cutting regime.

Discussion

Under the cutting regime the average annual DM yield across all sward ages increased with red clover inclusion – by 19% without fertilizer and by 16% when fertilized; the corresponding clover yield increased by 51% without fertilizer and 90% when fertilized. Frankow-Lindberg *et al.* (2009) also found a positive yield effect of red clover inclusion in the first production year and also partly in the second year, but not in the third year. In the spring harvest of subplots with a rest period in the grazed plots the inclusion of red clover increased the average production by 26% without fertilizer and by 18% with fertilizer, and in August the figures were 46 and 40%. However, the most pronounced effect of the inclusion of red clover in grazed plots compared to the two-species mixture was in spring total clover content, with a 145% increase without fertilizer and 175% when fertilized. The corresponding values for August were 20% and 41%. Thus, the strongest effect on clover content of red clover inclusion was under fertilized conditions.

The persistence of the depression in ryegrass yield in the three-species mixture suggests a decrease in ryegrass plant density due to the vigorous growth of the red clover in spring and, to some extent, in August. The depression of ryegrass occurred despite a very low content of red clover in the seed mixture (1 kg ha^{-1}) compared to more commonly used seed rates of 2-6 kg ha^{-1}. Avoiding this assumed reduction in ryegrass plant density would require even lower seed rates of red clover with the probable consequence of lower first-year yields in the three-species mixture.

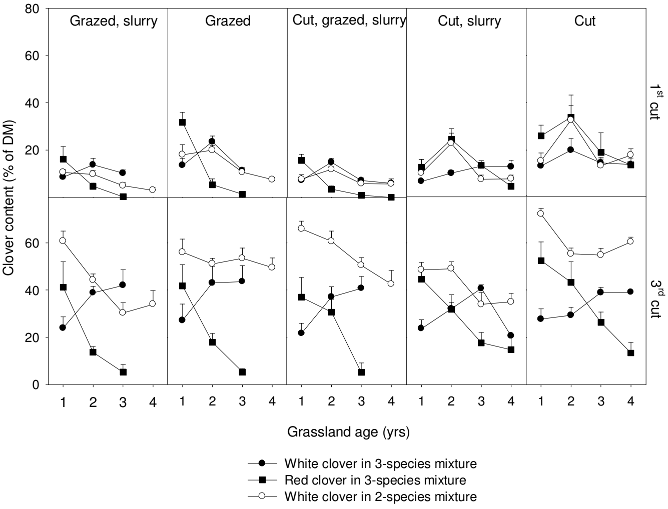

Figure 2. Clover dynamics in two- and three-species swards under different managements.

Grazing imposes recycling of N in animal excreta, trampling and more frequent defoliation, and different clover species responded differently under grazing conditions. Grazing had depressed the red clover content by the second production year, although it did not eliminate it. The combination of cutting and grazing delayed this depression from the second until the third year. White clover performed well, and was almost the same under grazing and cutting regimes, as previously demonstrated by Søegaard (2009). Thus, the three-species mixture with red clover has potential as a flexible multi-purpose mixture in grasslands used for grazing and cutting.

Conclusions

This study has shown advantages for including red clover in the traditional ryegrass-white clover mixtures that are widely used in north-western Europe. The three-species mixture that included red clover gave higher yields than the two-species mixture in years 1 and 2, and, under the cutting regime, across all four years the yield was still 8-10% higher. More spectacularly, under the cutting regime, the effect of red clover inclusion on total clover yield across the four years led to a 51% increase in the absence of slurry fertilizer, and a 90% increase when fertilized with slurry. The practical prospects of this may be increased soil fertility from N fixation and a lower fertilizer requirement in the arable phase of the mixed crop rotation, and a more flexible sward that is able to perform well under changing managements.

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The impact of contrasting management conditions on clover performance

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Abstract

The current focus on sustainable agricultural production has increased interest in using forage legumes to reduce inorganic-N inputs in Norwegian agriculture. This study quantified the impact of tractor traffic, fertilization level and clover inclusion rate on grassland yield, clover content in herbage and N_2 fixation in field experiments on mineral soils in northern, western and mountainous regions of Norway. Three seed mixes (0, 15, 30% clover in total seed weight), two N fertilizer rates (110, 170 kg N ha^{-1}) and three levels of post-cut tractor traffic were examined. Preliminary results suggest that tractor traffic exerts site-specific effects on grassland yield, clover content and N_2 fixation. Tractor traffic also reduced fertilizer-use efficiency. Increased N fertilizer level strongly reduced the clover content in the grass-clover swards, thereby decreasing N_2 fixation. Increased clover inclusion rate had small positive effects on the parameters studied.

Keywords: grass-clover leys, fertilization, low input agriculture, N_2 fixation, soil compaction, tractor traffic

Introduction

In Norway, red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) are widely used in seed mixes. However, clovers are more vulnerable to waterlogging and soil compaction than grass species (Pulli, 1988). Previous studies have shown that in compacted soil, root and shoot production and N_2 fixation are limited (Cook *et al.*, 1996). The objective of the present study was to quantify the impact of tractor traffic, nitrogen (N) fertilization level and clover inclusion rate on forage crop yield, clover content in herbage and N_2 fixation.

Materials and methods

In spring 2010, field trials were established on mineral soils at three sites with different climates: Tjøtta in northern Norway (65°49'N, 12°25'E), Fureneset in western Norway (61°22'N, 5°24'E) and Løken in the east-central Norwegian mountains (61°04'N, 09°04'E; 500 m a.s.l.). Three seed mixes (0, 15 or 30% clover in total seed weight) containing red and white clover and a mixture of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and smooth meadow-grass (*Poa pratensis* L.) as grass components were tested. The pure grass mixture was included to assess the contribution of clover to yield and quality and to estimate N_2 fixation by the total nitrogen difference method (Danso, 1995). The total seed rate was 30 kg ha^{-1} in all treatments. Two levels of fertilizer were used in 2011 and 2012: 110 kg total N ha^{-1} as cattle slurry applied in spring and 170 kg N ha^{-1} which included 60 kg N ha^{-1} from mineral fertilizer applied after the first cut. Three levels of two wheel-by-wheel passes with tractor traffic were introduced after each cut: no traffic, traffic with a light tractor (3-4 Mg) and traffic with a heavy tractor (7 Mg). The experiment had a split-plot design with three replicates. The field trials were cut twice a year, dry matter yield (DMY) was recorded and botanical composition estimated visually just before each cut. Crude protein content in herbage was estimated by NIRS analysis. Analysis of variance was applied separately to data from each site using a split-split plot model at Fureneset and Tjøtta to evaluate the significance of tractor traffic (medium plots), N fertilization level (main plots) and seed mix (small plots) on DMY and clover content. At Løken a split plot model with tractor traffic on main plots and a combination of fertilization and seed mix on small plots was used.

Results and discussion

In 2012 there was no statistically significant reduction (P<0.05) in total DMY by tractor traffic at any site. However, there was a clear tendency of yield reduction at Løken (P=0.06) and to some extent at Fureneset and Tjøtta at the high-N fertilization level (Table 1). The higher content of silt in the soil at Løken (81%) compared with Fureneset (35%) and Tjøtta (11%) may explain these results. Håkansson (1973) also found considerable yield reductions related to tractor traffic on a silty loam and silty sand in northern Sweden. DMY increased significantly more with higher N fertilization level without tractor traffic than with traffic at Løken (P<0.01) and Tjøtta (P<0.05), and the same tendency was found at Fureneset (Table 1). When soil was compacted, nutrients appeared to be less available due to reduced root growth as reported previously by Cook *et al.* (1996). The DMY increase from increased N fertilization was significantly higher in pure grass stands than in grass-clover stands at Fureneset (P<0.01) and Tjøtta (P<0.05) and tended to be higher at Løken (Table 1). Inclusion of clover in the seed mix had more impact on yield than increased N fertilization.

Table 1. Impact of tractor traffic and clover inclusion at two fertilization levels (N-low = 110 kg N ha^{-1}, N-high = 170 kg N ha^{-1}) on mean DMY (t ha^{-1}y^{-1}) and DMY increase between N-high and N-low in 2012 at Fureneset, Tjøtta and Løken.

	DMY (t ha^{-1}yr^{-1}) 2012								
	Fureneset			Tjøtta			Løken		
	N-low	N-high	Increase	N-low	N-high	Increase	N-low	N-high	Increase
Tractor traffic									
No traffic	7.87	9.41	1.54	6.99	8.98	1.99	4.83	6.75	1.91
Light tractor	7.74	9.20	1.47	7.22	8.88	1.67	3.31	4.34	1.04
Heavy tractor	7.56	8.68	1.12	7.41	8.54	1.14	3.91	5.05	1.14
Seed mix									
Pure grass	5.44	8.20	2.76	5.58	7.74	2.16	2.94	4.50	1.57
15% clover	8.51	9.23	0.73	8.07	9.32	1.26	4.28	5.80	1.52
30% clover	9.22	9.86	0.64	7.97	9.34	1.37	4.83	5.83	1.00

Table 2. Impact of tractor traffic, N fertilization level and clover inclusion rate on clover content before the second cut as percentage of DMY in 2011 and 2012 at Fureneset, Tjøtta and Løken.

	% clover in DMY at second cut					
	2011			2012		
	Fureneset	Tjøtta	Løken	Fureneset	Tjøtta	Løken
Tractor traffic						
No traffic	21	43	20	34	43	26
Light tractor	19	39	28	35	34	37
Heavy tractor	15	44	22	35	37	32
Fertilization						
110 kg N	28	55	29	57	54	45
170 kg N	9	30	17	12	21	19
Seed mix						
15% clover	19	40	21	35	37	31
30% clover	18	45	26	34	38	33

Tractor traffic had only minor effects on the clover content in clover-grass stands (Table 2). At Løken, tractor traffic significantly favoured the growth of clovers in 2011 and a similar tendency was found in 2012. However, in these treatments the crude protein content decreased in 2012 (results not shown). This indicates that tractor traffic limited the uptake of soil N, depressed growth of grass species and made clovers more competitive. Increased N fertilizer rate significantly decreased clover growth at all three sites (Table 2), confirming that grass species compete better than clovers for soil N and consequently display greater growth (Huss-Danell *et al.*, 2007). The difference between clover inclusion rates decreased over time and in 2012 there was no difference in clover content at any site (Table 2). Vegetative propagation of white clover can explain this diminishing effect of inclusion rate. Simple calculations showed that N_2 fixation by clovers contributed a considerable amount of N to the system. However, the amount varied widely between sites, years and treatments (range 17.4-118 kg ha^{-1} yr^{-1}). Tractor traffic seemed to reduce N_2 fixation from the first to second cut, an effect that was strongest at Løken. Fertilization after the first cut dramatically decreased N_2 fixation, e.g. by 58% at Fureneset in 2012. Increased clover inclusion in the seed mix promoted N_2 fixation to some extent, particularly early in the growing season.

Conclusions

Tractor traffic had site-specific effects on grassland yield, clover content and N_2 fixation. Traffic also reduced the use-efficiency of applied fertilizer. Increased N fertilization strongly reduced the proportion of clover in the grass-clover swards and thereby N_2 fixation. Including clovers in the seed mix had more impact on yield than increased N fertilization. Increased inclusion of clover in the seed mix had negligible effects on yield, clover content and N_2 fixation.

Acknowledgements

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The relationship between functional group interactions and multifunctionality in grasslands

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Abstract

Species composition and biodiversity of managed grassland ecosystems deliver multiple ecosystem processes. A major challenge is to identify community compositions that optimize the simultaneous supply of several ecosystem functions. Here, we quantify the impact of species interactions on multifunctionality in a grassland ecosystem, using a statistical approach that separately estimates the impact of species identity and interaction effects. We apply a multivariate extension of this approach to experimental data on multiple grassland functions, including aboveground biomass, forage quality measurements and soil measurements. We found that differing species-interaction relationships are associated with the multiple functions. This approach can help facilitate the selection of a community composition that optimizes species interactions for multiple functions.

Keywords: multifunctionality, ecosystem functioning, species identity effects, species interaction, diversity effects

Introduction

The biodiversity and ecological processes within ecosystems combine to provide a range of services that contribute to human well-being (Naeem *et al.*, 2009). Species composition and biodiversity of managed grassland ecosystems have been shown to affect multiple ecosystem services, including forage production, resistance to weed invasion, soil nutrient cycling and waste decomposition. These services are of economic importance. There is methodological challenge to determine the composition(s) of multispecies mixtures that optimize the supply of multiple processes. Increasing the number of species in a grassland mixture increases the potential for interspecific interactions such as complementarity and facilitation, which have been proposed as mechanisms in diversity-function relationships. However, effects of species interactions on ecosystem functions can be negative as well as positive; a pair of species that interact positively for one ecosystem function may interact negatively for another.

Biodiversity affects ecosystem functioning through a combination of component species-identity effects and interactions among the species (Kirwan *et al.*, 2009). In managed ecosystems, mixtures can be constructed to exploit the species-identity effects and interactions among species. It is therefore important to understand how species-identity and interaction effects impact on multiple ecosystem functions. Kirwan *et al.* (2007, 2009) developed a modelling methodology for assessing the impact of species-identity and interaction effects on an ecosystem function. Here, we apply the models to multiple ecosystem processes from the BIODEPTH experiment. We examine the patterns of identity and interaction effects across different ecosystem functions.

Materials and methods

To assess the impact of functional group identity and interaction effects on ecosystem multifunctionality, we used published data on nine ecosystem functions measured as part of the BIODEPTH experiment (Hector *et al.*, 2005). BIODEPTH experimentally manipulated plant species diversity at eight European grassland sites and measured the response of ecosystem processes, including above-ground biomass in years 1, 2 and 3, root biomass production by year 3, nitrogen % and nitrogen yield in above-ground vegetation, soil mineral nitrogen, decomposition of wooden sticks (lignin) and decomposition of cotton strips (cellulose). To facilitate general comparisons of identity and interaction effects across sites, the diversity-interaction (D-I) models (Kirwan *et al.*, 2007, 2009) were fitted at the functional group level. For each of the nine ecosystem functions (Y_i), the following model was fitted at each site:

$$\mathrm{Y_i} = \beta_G \mathrm{G} + \beta_L \mathrm{L} + \beta_H \mathrm{H} + \beta_{GL} \mathrm{G} * \mathrm{L} + \beta_{GH} \mathrm{G} * \mathrm{H} + \beta_{LH} \mathrm{L} * \mathrm{H}$$

where G, L and H are the proportions in the community of each of the grass, legume and herb functional groups respectively, β_G, β_L, and β_H are the functional group identity effects and β_{GL}, β_{GH}, and β_{LH} are the pairwise functional group interactions. To facilitate a comparison of effects across ecosystem functions that were measured on different scales, effects are presented as percentages.

Results and discussion

The identity of the best performing functional group differed across the ecosystem processes (Figure 1). Grass was the best performing functional group for aboveground biomass in year 1 and for root biomass. Legume was the best performing functional group for soil N and lignin and cellulose decomposition. Patterns of species interaction also differed across ecosystem processes, but were in general positive (Figure 2). All three functional group interactions had positive effects on aboveground biomass in years 1, 2 and 3 and plant-N yield (Figure 1). The grass-herb interaction was associated most strongly with aboveground biomass production in year 1 (Figure 1), while the grass-legume and herb-legume interactions were most strongly associated with aboveground biomass production in years 2 and 3 and plant-N yield.

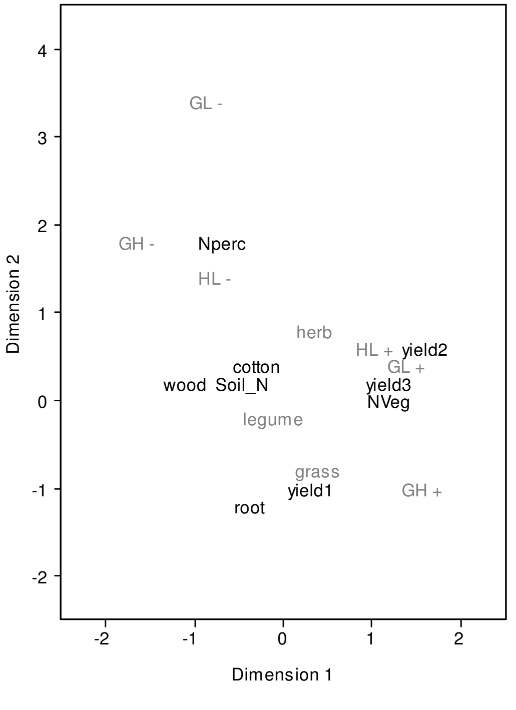

Figure 1. Associations between functional identity and interaction effects and multiple ecosystem processes. The diversity-interaction models were fitted to the data from each site for each of the nine responses, resulting in 81 sets of model coefficients. Detrended correspondence analysis was applied in order to identify associations between the multiple ecosystem processes, functional group identity effects (grass, legume, herb, indicating which is the best performing for an ecosystem function) and species interaction effects (GL, GH, HL, which can be positive, negative). Ecosystem processes that are similar in terms of the functional group identity and interaction effects appear closer together on the plane.

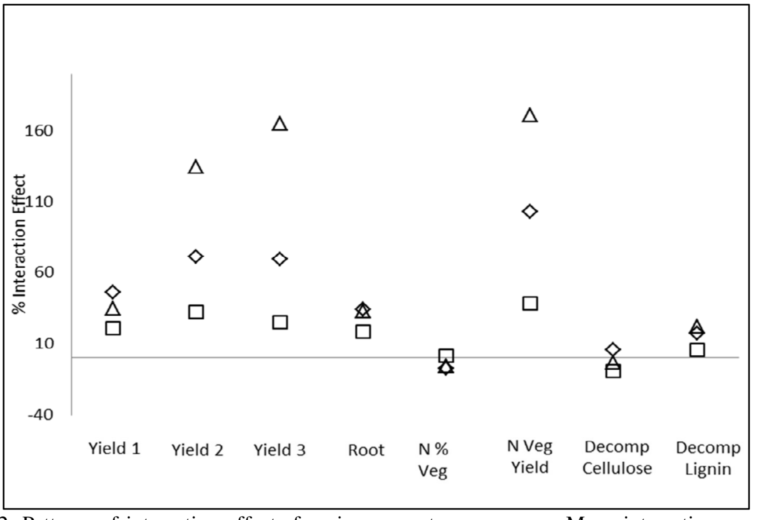

Figure 2. Patterns of interaction effects for nine ecosystem processes. Mean interactions across the nine sites are given for Grass × Legume (◇), Grass × Herb (□) and Legume × Herb (△) interactions.

Conclusions

The multiple ecosystem processes were related to both individual functional group performances (identity effects) and interactions among functional groups. However, there was no single biodiversity-multifunctionality relationship. The comparative performances of the functional groups differed depending on the ecosystem function. The scale of the contributions of interactions among the functional groups also varied across the ecosystem functions. This implies that if a mixture community was selected to optimise diversity effects for one particular ecosystem function, then the benefits of potential interactions might not be achieved for other functions where different species interactions are important.

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Forage yield, weed suppression and fertilizer nitrogen replacement value (FNRV) of alfalfa-tall fescue mixtures

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Abstract

Adding plant diversity to forage systems may help growers deal with increasing fertilizer costs and a more variable climate. Maintaining highly diverse forage mixtures in forage-livestock production is difficult and may warrant a closer reexamination of simpler grass-legume mixtures to achieve similar objectives. This study evaluated the performance of alfalfa (*Medicago sativa* L.) and tall fescue (*Schedonorus phoenix* (Scop.) Holub) mixtures against tall fescue monocultures fertilized with nitrogen across six different sites in the United States. Experimental treatments included three alfalfa-fescue mixtures, an alfalfa monoculture, and six tall fescue monocultures that received 0, 50, 100, 150, 200 and 300 kg N ha^{-1}, respectively. Total forage yield, grass N yield, weed biomass, within-season yield stability and fertilizer nitrogen replacement values (FNRV) were measured during the 2011 growing season. Mixtures yielded an average of 6860 kg ha^{-1}, which was similar to fescue monocultures fertilized with 100 kg N ha^{-1}. Alfalfa-dominated plots exhibited the highest seasonal yield stability and mixtures effectively suppressed weeds. Most FNRVs for alfalfa were lower than reported in other studies (mean 116 kg N ha^{-1}). Overall, good mixture yield mainly depended upon the alfalfa component as tall fescue growth did not benefit greatly from the association. Although replacing N fertilizer with alfalfa has environmental benefits, forage yield in mixture might be improved with cultivars that better complement each other when grown together.

Keywords: alfalfa, grass mixtures, Fertilizer Nitrogen Replacement Value, weeds

Introduction

The relationship between plant diversity and forage productivity in grasslands has received much attention in recent years. Conclusions from these studies have been mixed but show few agronomic benefits to sowing highly diverse mixtures (e.g., Tracy and Faulkner, 2006). Some level of plant diversity should be beneficial to various aspects of forage production, however. For example, sufficient plant diversity should enable grassland communities to resist environmental stress and produce more stable yield. The practicality of maintaining highly diverse forage communities in forage-livestock production is questionable though, so it may warrant a closer reexamination of simpler grass-legume mixtures to achieve similar objectives. The objective of this study was to evaluate performance of alfalfa-grass mixtures against grass monocultures fertilized with N. Performance was evaluated by measuring forage yield, grass N yield, seasonal yield stability and weed biomass during the 2011growing season. Fertilizer nitrogen replacement values (FNRV) of alfalfa in mixture were estimated as well.

Materials and methods

The experiment was established at six locations in five US states (Virginia, Wyoming, Utah, Wisconsin, Pennsylvania and Maryland). Experimental treatments included three alfalfa-tall fescue mixtures, one alfalfa monoculture, and five N-fertilizer rates applied to tall fescue monocultures, plus an unfertilized control. Three mixtures were sown at the same rate but with alfalfa:fescue seed ratios of 75:25, 50:50 and 25:75. The ten treatments were randomly assigned to 30 plots at each site yielding three replications per treatment. Plot size varied slightly across sites, but none were smaller than 2×3 m. Five nitrogen application rates were applied to tall fescue monoculture plots at rates of 50, 100, 150, 200 and 300 kg N ha^{-1}. Plots were mechanically harvested when alfalfa plots had reached approximately 10% bloom. In 2011, total dry matter yield was quantified at each harvest by cutting a strip from the centre of each plot. A subsample of plant material was sorted to tall fescue, alfalfa and weed components. The tall fescue component from each sample was ground to 1 mm and analysed for %N content by combustion. The experiment was analysed as a randomized complete block design with subsampling. The six sites were treated as blocks with each block containing 30 plots. Yield data, N yield of tall fescue, within season yield CV (coefficient of variation), and weed biomass were analysed using Proc GLM procedure in SAS. The fertilizer nitrogen replacement value (FNRV) of alfalfa in mixture was estimated using methods similar to Zemenchik *et al.* (2001).

Results and discussion

Total yield ranged from 4994 kg ha^{-1} at the Wyoming site to 8633 kg ha^{-1} at the Pennsylvania site. Alfalfa-fescue mixture yields were similar to grass monocultures that received 100 kg N ha^{-1} (Table 1). Most yield contribution in alfalfa-grass mixtures came from the legume

Table 1. Treatment means from the 2011 growing season. N = N fertilization rate, ALF = % of alfalfa in mix.

Treatment	Forage		Weed		Yield CV		Grass N Yield	
	Kg ha^{-1}		Kg ha^{-1}		%		Kg N ha^{-1}	
N0	4038	c†	398	b	84	a	68	d
N50	5551	dc	215	b	76	abc	121	bcd
N100	6586	bc	153	b	78	ab	146	bc
N150	7498	abc	255	b	76	abc	169	abc
N200	8085	ab	110	b	73	abc	193	ab
N300	8812	a	100	b	65	abc	242	a
ALF100	6139	acd	1002	a	46	c	-	
ALF75	7230	abc	163	b	48	bc	156	bc
ALF50	7002	abc	148	b	52	bc	143	bcd
ALF25	6348	bc	121	b	60	abc	117	cd

†Within columns, means followed by the same letter are not significantly different according to Bonferoni t test (P<0.05)

component, as has been reported elsewhere (Jones *et al.*, 1988). Weed biomass was much higher in alfalfa monocultures compared with other treatments (Table 1). The highest weed biomass occurred in the early spring and in plots with heavy alfalfa weevil infestations, which were common in some monoculture stands. Although mixtures suppressed weeds better than alfalfa monoculture, N fertilization of monoculture fescue plots was also effective. Grass inclusion in mixture and N fertilization probably made stands more competitive against weed invasion. Within-season yield variation was lowest for swards sown with 100% alfalfa and highest for unfertilized fescue (Table 1).

The higher stability of alfalfa-dominated plots was likely the result of more even production over the growing season as deep-rooted alfalfa compensated for slower grass growth in summer (Sleugh *et al.*, 2000). Evidence for this growth compensation can be seen in seasonal yield at the Virginia site (Figure 1). As expected, nitrogen yield in forage increased with N application rate in monocultures and averaged 156 kg N ha $^{-1}$. Mean nitrogen yield of tall fescue in mixtures was lower (138 kg ha^{-1}) and similar to monocultures that received between 50 and 100 kg N ha^{-1} (Table 1). The FNRV of alfalfa averaged 140 kg N ha^{-1} for alfalfa sown at 75% of mixture and 86 kg N ha^{-1} for mixtures with 25% alfalfa. The 50:50 mixtures were intermediate at 123 kg N ha^{-1}. These values were lower than other studies of other comparably aged legume stands. For example, Zemenchik *et al.* (2001) reported FNRVs between 200-250 kg N ha^{-1}. Given the rising costs of N fertilizer and a possible renewed interest in grass-legume plantings, standardization of FNRV calculations may be warranted to facilitate comparisons among studies.

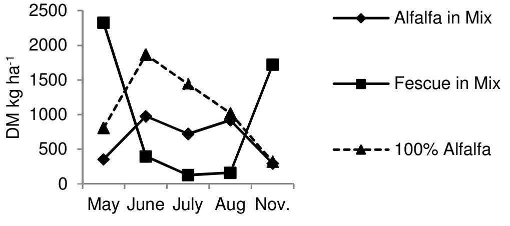

Figure 1. Seasonal yield at the Virginia site.

Conclusion

Alfalfa-grass mixtures yielded similarly to monocultures fertilized with 100 kg N ha^{-1}. Alfalfa was estimated to replace about 116 kg N ha^{-1} in mixtures. Mixtures also helped suppress weeds compared with monoculture alfalfa. Alfalfa inclusion was critical in stabilizing seasonal yields, mainly due to the better growth of alfalfa during summer months. While the benefits of alfalfa-grass mixtures were substantial, we believe they could be greater. One direction of future research could involve more breeding or selection efforts for grass and legume cultivars that possess better growth complementarity in mixtures.

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Plant diversity effects are robust to cutting severity and nitrogen application in productive grasslands

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Abstract

The replacement of nitrogen fertilizer with nitrogen derived from N_2 fixation offers important economic and environmental advantages. We investigated the effects of plant diversity of grasses and legumes, nutrient application and cutting on ecosystem function (aboveground biomass) in a productive grassland system. We varied resource levels (two levels of nitrogen fertilizer) and biomass removal intensity (two levels of cutting height) across a gradient of plant diversity (four species: *Lolium perenne, Phleum pratense*, *Trifolium pratense* and *Trifolium repens*) and relative abundance (varying species proportions) over a period of three years. Aboveground biomass was on average 16.5% greater in mixtures than in monocultures. On average, the yield benefits due to increasing evenness of four species mixtures were at least as big as the effects of increasing nitrogen by 150 kg ha^{-1} yr^{-1}. These results indicate the potential of grass-legume mixtures to provide yield benefits at moderate levels of nitrogen application.

Keywords: mixtures, monocultures, evenness, yield, legume

Introduction

There is increasing pressure on agricultural systems to become more efficient in resource use, thereby reducing inputs while maintaining or increasing productivity (Gruber and Galloway, 2008; Finn *et al.* 2013). The addition of nitrogen-fixing species to grass-dominated or monoculture swards has long been recognized for its potential to increase yields. In intensively managed grasslands, mixture systems have rarely gone beyond two species, and associations other than legume-grass have rarely been considered. The conditions associated with intensive pasture management such as grazing intensity and high soil nitrogen levels may prevent the realization of yield benefits of multi-species swards. Here, we investigated whether diverse communities were more productive than their constituent monocultures under different regimes of environmental pulse (cutting) and press (nitrogen-fertilization) perturbation.

Materials and methods

Main plots were laid out (8.1×4 m) and sown in mid-September 2006 with communities of four commonly used agronomic grassland species (*Lolium perenne*, *Phleum pratense*, *Trifolium pratense*, *Trifolium repens*). Sowing proportions of these four species were varied according to a simplex design giving 25 distinct communities with different levels of evenness (E): four monocultures (E = 0), six binary mixtures (E = 0.67) and 15 different four-species mixture communities (E = 0.29, 0.64, 0.88, 1) (Kirwan *et al.*, 2007). All communities were sown at two levels of initial overall community biomass based on seed weight. Sowing densities were defined according to the sowing rates for each species as monocultures. Main plots were divided into four equally sized sub-plots. Split-plot treatments consisted of two levels of nitrogen (approximately 50 and 200 kg ha^{-1} yr^{-1} of nitrogen) and two levels of cutting severity at harvest (7 cm and 2 cm) in factorial combination. Wet conditions over all three years limited harvesting to three occasions in 2007 and 2009 (spring, summer and autumn) and two in 2008 (late spring and late summer).

Results

Significant overyielding occurred over the three years, and across each of the nitrogen levels and cutting treatments (see Figure 1 for comparison of average monoculture yields and average mixture yields at high evenness). This diversity effect was robust to the effects of nitrogen addition and cutting and exceeded the average effect of adding 150 kg ha^{-1} yr^{-1} of nitrogen to monocultures in the first two years and overall. The diversity effect was maintained across the first two years, but was reduced in year three.

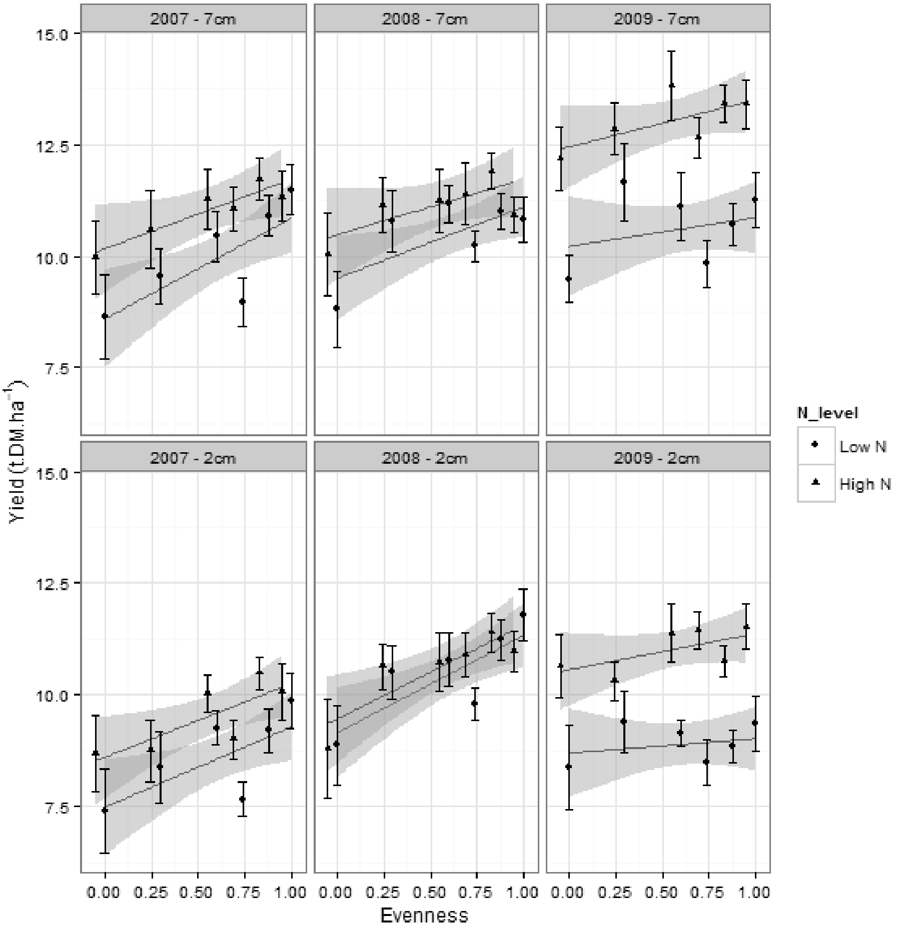

Figure 1. Effect of nitrogen level and sward evenness on biomass across cutting treatments and year. Regression lines show the evenness effect at high (upper line) and low (lower line) levels of nitrogen. The mean and standard deviation of all replicates at a given evenness level is illustrated. Confidence intervals are presented in grey shading. The y-axis intercept corresponds to the average monoculture performance; the slope of each line corresponds to the magnitude of overyielding.

Discussion

In this study, the observed effects of sown plant diversity on total yield show that some agronomic mixtures under relatively low nitrogen levels may perform as well as (or better than) grass monocultures under high nitrogen levels and at different levels of cutting severity. Indeed, mixture yields at the low level of nitrogen fertilization were comparable to the yields of the best-performing monoculture at the high nitrogen level (*L. perenne* in 2007 and *P. pratense* in 2008 and 2009), and were consistently higher than average monoculture yields. See also Nyfeler *et al*. (2009) for similar results. Although overyielding was not as large in magnitude as that found in other studies, it was unaffected by both nitrogen and cutting and it was realized from the first year after establishment. The diversity effect was substantially reduced in year 3, most probably due to lowered levels of legumes in the sward. This also likely explains the large yield difference between fertilizer levels in the third year, whereby the lower amounts of legumes resulted in reduced nitrogen fixation and transfer to the sward.

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Persistence and yield of *Lolium perenne* in different harvest systems

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Abstract

The effects of cut date, cut interval and number of cuts on dry matter (DM) yield and persistence were studied in two field trials with pure stands of perennial ryegrass (*Lolium perenne* L.) in Sweden (57-59°N). The aftermath effect of the harvest system was studied one year after treatment. There was a site-specific impact of harvest system, with the most critical parameter being time of first and last cut. Delaying the first cut increased DM yield, but the aftermath effect depended on site. Early first harvest increased ley DM yield in the next year at northern sites, but decreased it at southern sites.

Keywords: *Lolium*, harvest, defoliation, system, yield, persistence

Introduction

Timing of cuts in a forage harvesting system affects the growth and persistence of grass leys. Delaying the first cut of perennial ryegrass increases the yield and proportion of stems, but has the opposite effect on aftermath growth (Gilliland, 1997). The cultivation of perennial ryegrass in Sweden extends up to 60°N latitude and the species are not fully adapted to the Swedish climate. A successive decline in biomass production over time has been reported for perennial ryegrass in Sweden (Gutmane and Adamovich, 2008; Halling, 2012). Therefore any improvement in harvest management that maintains yield will be important for farmers. This study explored the effects of different timing of cuts, growth intervals and numbers of cuts on the yield and persistence of perennial ryegrass.

Materials and methods

Field trials were conducted at two sites using a single-factor, randomized block design with four replicates. Pure stands of perennial ryegrass variety SW Birger were established in spring 2007, undersown in a cover crop at a seed rate of 32 kg ha^{-1}, at Uppsala (59°50'N, 17°42'E) and Rådde (57°61'N, 13°26'E). Uppsala is close to sea level and Rådde is located in the southern Swedish highlands at about 185 m a.s.l. The trials had eight different harvesting systems (Table 1). The normal time for first cut in Table 1 refers to booting stage (50% of heads more than half visible). The 'early' time is one week before and the 'late' one week after normal. At Uppsala, the third cut was one week later than planned in Table 1, but the fourth cut was as planned. At Rådde, the first cut in treatment D was two days earlier than in A and E. At Uppsala, all early first cuts were taken at the same time. In the second ley year, all treatments were harvested twice, to record the aftermath effects. Plots were fertilized with 100, 80, 60 and 60 kg N ha^{-1} before cuts 1, 2, 3 and 4 respectively, and P and K were applied according to plant-available levels in the soil. The average plot size was 12 m^2 and all plots were harvested using a Haldrup plot harvester with 1.5 m working width. The herbage removed was weighed, sub-sampled and dried for 3 hours at 105°C for dry matter (DM) determination. In the first ley year (2008), the growing season started on 20 April at Uppsala and 16 April at Rådde and ended on 27 October at Uppsala and 26 October at Rådde (season start and end defined as five days with mean daily temperature above or below 5°C, respectively). Uppsala had warmer weather, with a total temperature sum (threshold 5°C) of 1534°C for the whole growing season, compared with 1415°C for Rådde.

Table 1. Harvesting systems with planned dates and length of growing season (wk.=weeks).

Code	First cut	Second cut	Third cut	Fourth cut
A	Early (30/5)	6 wk. (11/7)	8 wk. (5/9)	
B	Normal (6/6)	6 wk. (18/7)	8 wk. (12/9)	
C	Late (13/6)	6 wk. (25/7)	8 wk. (19/9)	
D	Early (29/5)	6 wk. (11/7)	8 wk. (5/9)	6 wk. (17/10)
E	Early (30/5)	7 wk. (18/7)	7 wk. (5/9)	
F	Normal (6/6)	6 wk. (18/7)	7 wk. (5/9)	
G	Late (13/6)	5 wk. (18/7)	7 wk. (5/9)	
H	Late (13/6)	6 wk. (25/7)	6 wk. (9/9)	

Each cut was statistically analysed using variety and site as fixed factors and replicate as random factor. Least squares means were calculated from the statistical model and are presented in the results. The Mixed procedure SAS Version 9.3 was used for these analyses (Littell *et al.*, 2006).

Results

The DM yields in different cuts at each site were analysed separately (Table 2).

Table 2. Least square means of dry matter yields (kg ha^{-1}) in treatment year and aftermath year at the two sites.

	First ley year					Second ley year			
Harvest system	1st cut	2nd cut	3rd cut	4th cut	Total yield	1st cut	2nd cut	Total yield	Yield ley 1 + 2
Uppsala									
A	5 358	4 800	5 809		15 967	4 399	3 128	7 527	23 494
B	6 824	4 854	5 033		16 711	3 952	3 108	7 060	23 771
C	7 596	4 972	5 667		18 234	2 910	3 008	5 918	24 152
D	5 321	4 838	5 583	893	16 636	1 453	2 871	4 325	20 961
E	5 364	6 292	5 687		17 343	4 630	3 106	7 735	25 078
F	6 663	4 697	5 390		16 750	3 762	3 050	6 812	23 562
G	7 747	3 802	5 554		17 102	4 167	3 109	7 276	24 378
H	7 778	4 720	4 941		17 439	3 868	3 005	6 873	24 312
Rådde									
A	5 655	2 791	3 935		12 381	3 987	2 990	6 978	19 359
B	6 879	2 694	3 374		12 947	4 348	2 979	7 327	20 274
C	7 358	3 005	3 383		13 746	4 207	2 903	7 111	20 857
D	4 993	2 529	3 933	1 388	12 844	3 543	3 404	6 947	19 791
E	5 354	3 466	3 324		12 143	3 903	2 802	6 705	18 848
F	6 570	2 737	3 135		12 441	4 111	2 601	6 712	19 153
G	7 251	2 201	3 596		13 048	4 117	2 883	7 000	20 048
H	7 343	2 974	2 716		13 033	4 398	2 671	7 069	20 102
LSD	282	300	379		513	582	205	699	-
P HS	0.001				0.001				
P HS*S	NS	0.001	0.041		NS	0.001	0.001	0.001	-

LSD = Least significant difference at *P*<0.05, HS = harvest system, S = site, NS = not significant; for explanation of treatments A-H see Table 1

A significant interaction was found between treatment and site (P<0.041-0.001), except for the first cut and total DM yield in the first ley year at both sites. DM yield was significantly greater (by 16-27%) when the first cut was delayed by one week from early to normal time at both sites, but not when it was delayed by one week from normal to late. Delaying the third cut by two weeks resulted in a significant DM yield increase of about 5% of total seasonal DM yield at both sites when the two late systems C and H were compared. However, this delay decreased total DM yield in next year by 12% at Uppsala. The highest yielding systems were those with late first and third cuts. At Rådde, there was no significant effect of this delay in the next year. The largest negative aftermath effect was found in the four-cut system at Uppsala (treatment D), which decreased the total DM yield in the next year by 43%. The strongest aftermath effect on DM yield was in the first cut of the second year at Uppsala, but this pattern was not seen at Rådde. The aftermath effect at Rådde was first seen at the second cut. A delay from early to normal first cut had a negative effect (treatment F to E), but a delay from normal to late first cut had a positive effect (treatment G to F). A delayed second cut at Rådde decreased DM yield in the second cut in the next year (treatments E to A and H to G). A delayed third cut at Rådde decreased DM yield in the second cut in the next year (treatments E to A and H to G). However, in the total DM yield the aftermath effect was counterbalanced. More generative heads were observed in the first regrowth after an early first cut (data not shown).

Discussion

There was a strong site impact on the effect of different harvest systems, which is difficult to explain. At the more northern site (Uppsala), a delayed third cut or a late fourth cut had a much larger negative influence on the persistence of perennial ryegrass, expressed as DM yield next year, than at the more southern site (Rådde). Length of the growing season after last cut was almost the same at both sites. The effect of delaying the first cut from early to normal time on DM yield in the next year was also site-specific, giving a positive aftermath effect at the northern site, but a negative effect at the southern site. This study focused on DM yield, but nutritive quality is also an important aspect when choosing the optimal harvest system.

Conclusions

Harvesting system had a site-specific impact on yield of perennial ryegrass. The most critical parameter was time of first and last cut.

Acknowledgements

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Adaptation and evaluation of a mechanistic grass growth simulation model for grass-based systems

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Abstract

An accurate grass growth model would be a valuable tool in anticipating grass growth and grass utilization at farm level. Ideally, a grass growth simulation model must be accurate, dynamic, use realistic input parameters and incorporate meteorological data. The objective of this study was to parameterize the grass growth model developed by Jouven *et al.* (2006) to increase its accuracy of grass growth simulation in the south of Ireland. The model was parameterized using an optimization technique where a number of the parameters in the model were optimized with the objective function of minimizing the root mean square error (RMSE). Both meteorological and grass growth data for the period 2005 to 2009 were included in the optimization process. During validation the Jouven Model was compared to the Adapted Model. RMSE was reduced from 20.45 kg DM ha^{-1} day^{-1} with the Jouven Model to 14.62 kg DM ha^{-1} day^{-1} with the Adapted Model. MSPE was reduced from 476 to 183. The proposed changes to the Jouven Model improved grass growth simulation in the south of Ireland. The adapted version of the Jouven Model can be used for grass growth simulation; albeit without perfect simulation.

Keywords: grass growth, model, adaptation, simulation

Introduction

Budgeting grass supply allows farmers to minimize the quantity of purchased feed required in the diet of grazing livestock. Forecasting grass growth and hence reducing volatility around grass supply will be a key feature of profitable milk and meat production systems in the future. An accurate model to simulate grass growth would be a valuable tool in anticipating and planning grass growth and grass utilization at farm level. Three grass growth models were evaluated by Hurtado-Uria *et al.* (2013). The model developed by Jouven *et al.* (2006) (hereafter referred to as the Jouven Model) was shown to have the greatest potential to simulate grass growth in Ireland; however, Hurtado-Uria *et al.* (2013) concluded that the model would require adaptation and parameterization to improve its grass growth simulation. The objective of this study was to parameterize the Jouven Model to increase its grass growth simulation accuracy in Ireland using data from 2005 to 2009.

Materials and methods

The Jouven Model is described in detail by Jouven *et al.* (2006). The model combines functional and structural components. In the model a site is described by its nitrogen (N) index (Bélanger *et al.*, 1994), water holding capacity (WHC) and grassland community. Only above-ground grass growth is modelled. To parameterize the Jouven Model for Irish conditions, the Solver Tool pack for Microsoft Excel was used in association with Moorepark meteorological and grass growth data from the 2005-2009 period (Hurtado-Uria *et al*., 2013). The optimization procedure was applied with the objective function set to minimize the root mean square error (RMSE) of observed versus predicted grass growth, by adjusting a number of parameters. A number of constraints were included to ensure both inputs and outputs were sensible in the optimization process. Herbage production was modelled for the period 2005 to 2009 using climatic data from Teagasc, Moorepark (hereafter referred to as Moorepark). The grazing season was divided into three periods: spring (January to April), mid-season (April to August) and autumn (August to November). Modelled data were compared with grass growth measured at Moorepark for 2005-2009 (Hurtado-Uria *et al*., 2013) using RMSE (Jin *et al*., 2005) and mean square prediction error (MSPE) (Rook *et al*., 1990) as measures of fit.

Results and discussion

The Adapted Model simulated total herbage production for the years 2005 to 2009 with more accuracy than the Jouven Model (Figure 1). The random variation of the MSPE was 0.905 for the Adapted Model compared to 0.482 for the Jouven model, and R^2 (0.87) was improved for the Adapted Model compared to the Jouven model (R^2=0.66). The Adapted Model simulated the seasonal herbage production in the spring for the years 2005 to 2009 with more accuracy than the Jouven Model. The Jouven Model undersimulated grass growth in all years (data not shown). The best fit for the Adapted Model was achieved in spring with a MSPE of 25 kg DM ha^{-1} day^{-1} (R^2=0.89) with the random variation making up the greatest component of the MSPE and the mean bias was zero (Table 1), and was poorest in mid-season. The Adapted Model reduced the RMSE from 16.36 kg DM ha^{-1} for the Jouven Model to 12.84 for the Adapted Model).

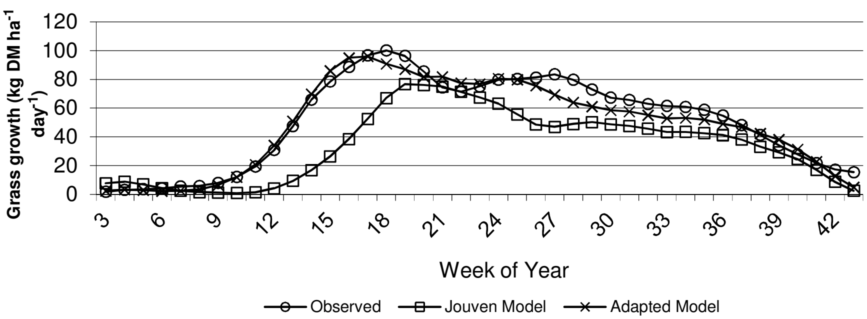

Figure 1. Average grass growth (kg DM ha^{-1} day^{-1}) simulated by the Jouven Model, the Adapted Model and measured grass growth data for the average of 2005-2009 at Moorepark.

Table 1 Mean square prediction error (MSPE) and R^2 of the Adapted Model for five years (2005-09) and for spring (January-April), mid-season (April-August) and autumn (August-November).

Period	Model version	Proportion of MSPE			MSPE	R^2
		Mean bias	Line bias	Random		
Total	Adapted	0.034	0.060	0.905	152	0.87
Spring	Adapted	0.000	0.106	0.894	25	0.89
Mid-season	Adapted	0.034	0.177	0.789	221	0.43
Autumn	Adapted	0.072	0.120	0.808	166	0.72

Several parameters were changed, including the initial biomass of green vegetative material (reduced from 650 to 300 kg DM ha^{-1}) and initial biomass of dead vegetative (from 560 to 300 kg DM ha^{-1}); bulk density of green vegetative material was increased from 850 to 1200 g DM m^{-3}; minimum temperature threshold (reduced from 4°C to 0°C), and maximum temperature threshold (reduced from 20°C to 18°C); the initial and end of reproductive growth temperatures changed from 600°C d to 725°C d and from 1200°C d to 975°C d, respectively.

Conclusion

The Adapted Model improved grass growth simulation in the south of Ireland. This improvement was achieved with changes on the parameters used by the model, thus making the adaptation possible for other locations. The adapted version of the Jouven Model can be used for grass growth simulation; however, further changes to the model could be made around the functions of the model.

Acknowledgements

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Species mixtures – dry matter yield and herbage quality as affected by harvesting frequency under low N supply

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Abstract

A factorial field experiment with perennial ryegrass, tall fescue, white clover and red clover was established at Ås, Norway in 2010. Monocultures of each species and mixtures with either 25% seed weight of each species or 67% of one species and 11% of the other three species in all combinations were sown at two seed rates. In 2011 and 2012 the plots were harvested either three or five times a year. A low level of fertilization was applied. Mixtures yielded more than monocultures; in the second year they yielded almost as much as the high N control of perennial ryegrass. On average, monocultures had better herbage quality (NE_L, digestibility) than mixtures, but not enough to compensate for the lower DM yields in terms of harvested NE_L or digestible DM ha^{-1}.

Keywords: mixtures, herbage quality, perennial ryegrass, tall fescue, white clover, red clover

Introduction

Mixtures of grass and legume species in forage production can contribute to higher yields and reduced needs for fertilizers (see review by Sturludottir *et al*., 2013). The experiment reported here is part of the EU-FP7 project Multisward, and aims to describe how mixtures of perennial ryegrass, tall fescue, white clover and red clover perform in terms of dry matter (DM) yield and herbage quality under low nitrogen input and two harvesting frequencies.

Materials and methods

Monocultures of each species, as well as mixtures with either 25% seed weight of each species or 67% of one species and 11% of the other three species in all combinations, were sown at two total seed rates (normal and half, i.e. 20 and 10 kg ha^{-1}, respectively) in a randomized split-plot design with harvesting regime as the main plot factor, and species composition and seed rate as subplot factors. In 2011 and 2012 the plots were harvested either three or five times a year (3H and 5H). A low level of N fertilization (100 kg $ha^{-1}yr^{-1}$) was applied together with P (33 kg ha^{-1} yr^{-1}) and K (150 kg ha^{-1} yr^{-1}). Control plots of perennial ryegrass monocultures receiving an additional 200 kg N $ha^{-1}yr^{-1}$ were included in the experiment (3H only, 2 replicate subplots per seed rate). DM yield was recorded at each harvest. Herbage quality variables (crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), indigestible NDF (iNDF), ash, potassium, digestibility and net energy for lactation (NE_L)) were estimated by NIRS (Fystro and Lunnan, 2006) in samples from all plots with the normal seed rate from all harvests in 2011. Digestible NDF (dNDF) was calculated as NDF-iNDF. For each quality variable an average value across harvests was calculated by weighting according to the yield of each harvest. The data were analysed by analysis of variance.

Results and discussion

The average total DM yields in 2011 were 11.1 and 9.7 t ha^{-1} in the 3H and 5H harvesting regimes, respectively. In 2012 the equivalent figures had decreased to 10.9 and 7.9 t ha^{-1}. All mixtures yielded significantly (P<0.05) more than all monocultures in both harvesting regimes in both years, except that the yield of the red clover monoculture in the 3H regime in 2012 was similar to that of the lowest yielding mixtures (Figure 1). The high-N perennial ryegrass monoculture yielded 18% more than the best-performing mixture in 2011. In 2012 this yield difference was reduced (and not significant). In the 3H regime, grass monocultures had significantly lower yields in 2012 than in 2011, while the white clover monoculture and red clover-dominated mixture had higher yields in 2012 than 2011. In the 5H regime the yield in 2012 was significantly lower than the yield in 2011 in perennial ryegrass and red clover monocultures as well as in mixtures dominated by these two species. The yield decrease in grass monocultures may be due to a lack of N, which appears to have been more severe in perennial ryegrass. An increase in yield in some clover-dominated treatments may be due to slower establishment of these species. A limited tolerance to frequent cutting in red clover may explain the yield reduction in the 5H red clover-dominated plots from 2011 to 2012.

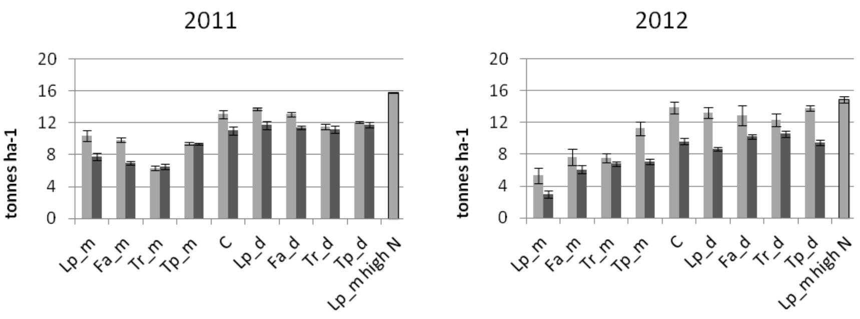

Figure 1. Total dry matter yield in 2011 and 2012. Lp: *Lolium perenne,* Fa: *Festuca arundinaceae,* Tr: *Trifolium repens*, Tp: *T. pratense,* C: centroid (25% seed weight of each of the four species), m: monoculture, d: mixture with 67% seed weight of the species mentioned and 11% of each of the three other species; light grey: three harvests per year, dark grey: five harvests per year. Averages of four replicate subplots +/- SE. The two seed densities were combined here for simplicity as the effect of this factor on total DM yield was small.

Grass monocultures were significantly higher in WSC and dNDF, and significantly lower in CP, iNDF and ash, than clover monocultures, which had the opposite characteristics (Table 1). Further, perennial ryegrass had more WSC and less iNDF than tall fescue, and white clover had more CP and less dNDF than red clover. There was no significant interaction between species and harvesting regime for these five quality variables, and the mixtures were intermediate between grass and clover monocultures. For NE_L, digestibility and potassium content the picture was more complex (Table 2). Perennial ryegrass and white clover from both harvesting regimes and tall fescue from the 5H regime had significantly higher NE_L and digestibility than red clover from the 3H regime, while red clover from the 5H regime and tall fescue from the 3H regime were intermediate. On average, monocultures (low N) had significantly higher digestibility and NE_L than the mixtures (Table 3), indicating that these quality variables were affected by species interactions. The high N perennial ryegrass monocultures had significantly higher CP, dNDF and iNDF, and significantly lower WSC, NE_L and digestibility, than the low N perennial ryegrass monocultures (Table 1 and 2). The shift of carbohydrates from WSC to fibers in perennial ryegrass (and possibly tall fescue) upon higher nitrogen levels may explain the reduced quality of mixtures. The difference in quality between monocultures and mixtures was not large enough to compensate for the lower

DM yields of monocultures; the variation in total yield measured as NE_L ha^{-1} or digestible DM ha^{-1} resembled variation in DM ha^{-1}.

Table 1. Monoculture herbage quality. Average values for variables for which there was no significant effect of harvesting regime and no significant interaction between harvesting regime and species. Lp: *Lolium perenne,* Fa: *Festuca arundinaceae,* Tr: *Trifolium repens*, Tp: *T. pratense,* CP: crude protein, WSC: water soluble carbohydrates, dNDF: digestible neutral detergent fibre, iNDF: indigestible NDF, all expressed as % of DM. Values not followed by the same letter are significantly different. * indicates a significant effect of additional nitrogen supply when comparing the Lp monocultures with the Lp high N controls. N = 4. *P*<0.05 for all tests.

	Lp	Fa	Tr	Tp	Lp–high N control
CP	10.0 c	10.2 c	23.3 a	20.8 b	13.26 *
WSC	28.7 a	24.1 b	12.2 c	12.1 c	20.6 *
dNDF	43.2 a	44.1 a	22.9 c	26.1 b	44.7 *
iNDF	6.55 c	8.20 b	9.62 a	9.81 a	7.95 *
Ash	5.8 b	6.2 b	8.9 a	8.4 a	7.0

Table 2. Monoculture herbage quality. Average values for variables for which there was a significant interaction between harvesting regime and species. Lp: *Lolium perenne,* Fa: *Festuca arundinaceae,* Tr: *Trifolium repens*, Tp: *T. pratense,* 3H, 5H: three or five harvests per year, NE_L: net energy for lactation, Dig: digestibility, Pot: potassium, both expressed as % of DM. Values not followed by the same letter are significantly different. * indicates a significant effect of additional nitrogen supply when comparing the Lp monocultures with the Lp high N controls. N = 2. *P*<0.05 for all tests.

	Lp		Fa		Tr		Tp		Lp-high N control
	3H	5H	3H	5H	3H	5H	3H	5H	3H
NE_L	6.42 a	6.26 ab	5.98 c	6.26 ab	6.43 a	6.41 a	6.02 c	6.13 bc	6.12 *
Dig	77.1 a	75.2 ab	73.0 cd	75.3 ab	74.3 bc	73.7 bc	70.3 e	71.6 de	72.7 *
Pot	2.22 d	2.42 cd	2.58 bc	2.68 b	2.79 ab	2.76 b	3.02 a	2.62 bc	2.54

Table 3. Average values for herbage quality variables for which there was a significant difference between the average monoculture and the average mixture. NE_L: net energy for lactation. Values not followed by the same letter are significantly different. *P*<0.05 for all tests.

	Monocultures (n = 16)	Mixtures (n = 20)
NE_L	6.24 a	6.01 b
Digestibility	73.8 a	71.5 b

Conclusion

Mixtures yielded more than monocultures; in the second year they yielded almost as much as the high-N control of perennial ryegrass. On average, monocultures had better herbage quality (NE_L, digestibility) than mixtures, but not enough to compensate for the lower DM yields in terms of harvested NE_L or digestible DM ha^{-1}.

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Forage yield in intercropping vetches with other annual legumes

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Abstract

The concept of mutual intercropping of annual legumes for forage production is based on four main principles: (1) same time of sowing, (2) similar growing habit, (3) similar time of harvest and (4) that one component should have good standing ability and another has a poor one. A small-plot trial was conducted at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi in 2009/2010 and 2010/2011. It included eight intercrops of vetches (*Vicia* spp.) with other annual legumes and the pure stands of each. Among the autumn-sown intercrops were common (*V. sativa* L.), Hungarian (*V. pannonica* Crantz) and hairy (*V. villosa* Roth) vetches with faba bean (*V. faba* L.), and bitter vetch (*V. ervilia* (L.) Willd.) with pea (*Pisum sativum* L.), while the spring-sown ones comprised common vetch and Narbonne vetch (*V. narbonensis* L.) with faba bean and white lupin (*Lupinus albus* L.). The highest two-year average forage dry matter yield (10.8 t ha^{-1}) was obtained from the intercrop of hairy vetch with faba bean, while the highest two-year average value of Land Equivalent Ratio (LER) was in the intercrop of Narbonne vetch with white lupin (1.16).

Keywords: forage dry matter, intercropping, Land Equivalent Ratio, vetches, *Vicia*

Introduction

In most European temperate regions, pea (*Pisum sativum* L.), common vetch (*Vicia sativa* L.) and other annual legumes are traditionally intercropped with cereals for both forage and grain production. Little is known on intercropping annual legumes with each other. An initial research project on this topic was launched during the last decade, managed jointly by the Faculty of Agriculture of the University of Novi Sad and the Institute of Field and Vegetable Crops. The primary target of this concerted action was the role of annual legumes in establishing perennial ones, where the annual legume acts as a 'bioherbicide' (Ćupina *et al.*, 2009a), and with mutual intercrops and varietal mixtures of annual legumes, with a basic idea of retaining high crude protein content in forage dry matter, unlike intercropping with cereals (Mikić *et al.*, 2012). The aim of this research was to assess the forage yield potential in intercropping various vetch species with other annual legumes.

Materials and methods

A small-plot trial was conducted at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi in 2009/2010 and 2010/2011, including eight intercrops of vetches with other annual legumes and the pure stands of each. Among the autumn-sown intercrops, there were common, Hungarian (*V. pannonica* Crantz) and hairy (*V. villosa* Roth) vetches with faba bean (*V. faba* L.), and bitter vetch (*V. ervilia* (L.) Willd.) with pea, while the spring-sown ones comprised common vetch and Narbonne vetch (*V. narbonensis* L.) with faba bean and white lupin (*Lupinus albus* L.). All the intercrops were designed according to the four basic principles of the mutual annual legume intercropping (Ćupina *et al.*, 2011): (1) same time of sowing, (2) similar growing habit, (3) similar time of harvest and (4) that one component has good standing ability and another has a poor one.

All autumn intercrops and pure stands were sown on 8 October 2009 and 15 October 2010, and the spring ones were sown on 2 March 2010 and 6 March 2011. The plot size was 5 m^2. There was a randomized block design with three replicates. The sowing rates in pure stands were 180 viable seeds m^{-2} for all vetches, 75 viable seeds m^{-2} for faba bean and white lupin, and 120 viable seeds m^{-2} for pea, with all the sowing rates reduced by 50% in all eight intercrops. The plots with cut when the pure stands or one intercrop component were in full bloom.

The forage dry matter yield (t ha^{-1}) was determined on the basis of fresh forage yield and forage dry matter proportion in the green forage samples taken after the cutting and dried to a constant mass at a room temperature. The reliability of forage dry matter yield of each intercrop was determined by calculating its Land Equivalent Ratio (LER):

$$LER = FDMY(v)_{IC} / FDMY(v)_{PS} + FDMY(oal)_{IC} / FDMY(oal)_{PS},$$

where $FDMY(v)_{IC}$ and $FDMY(v)_{PS}$ are the forage dry matter yields of the vetch component in each intercrop and each pure stand, respectively, while $FDMY(oal)_{IC}$ and $FDMY(oal)_{PS}$ are the forage dry matter yields of the other annual legume component in each intercrop and each pure stand, respectively.

The results were analysed using Statistica 8.0 software. Analysis of variance (ANOVA) was performed and means separated by Fisher's Least Significant Difference (*LSD*) test at 0.05.

Results and discussion

The highest two-year average forage dry matter yield among the pure stands (Table 1) was in autumn-sown faba bean (10.4 t ha^{-1}), and the lowest two-year average forage dry matter yield among the pure stands was in Narbonne vetch (6.5 t ha^{-1}). The two-year average forage dry matter yield in the intercrops of hairy vetch with faba bean (10.8 t ha^{-1}) and common vetch with faba bean (10.3 t ha^{-1}) were significantly higher in comparison with all other six intercrops (Table 2). The two-year average forage dry matter yield in the intercrop of bitter vetch with pea (8.1 t ha^{-1}) was significantly lower in comparison to all other intercrops. This intercrop of bitter vetch with pea had better agronomic performance in other research in the same agroecological conditions, where it produced 11.5 t ha^{-1} of forage dry matter (Krstić *et al.*, 2011). Overall, the intercrops of vetches with other annual legumes had higher forage dry matter yield in comparison with the varietal mixtures of pea with different leaf types, with a range between 6.5 and 8.1 t ha^{-1} (Ćupina *et al.*, 2010).

Table 1. Two-year average forage dry matter yield (t ha^{-1}) of pure stands of vetches and other annual legumes at Rimski Šančevi in 2010 and 2011.

Sowing time	Crop	Pure stand	Forage dry matter yield
Autumn	Vetches	Bitter vetch	7.8
		Common vetch	8.6
		Hairy vetch	9.9
		Hungarian vetch	7.3
	Other annual legumes	Faba bean	10.4
		Pea	7.4
Spring	Vetches	Common vetch	7.6
		Narbonne vetch	6.5
	Other annual legumes	Faba bean	9.4
		White lupin	8.8
$LSD_{0.05}$			1.2

In comparison with the all other tested intercrops (Table 2), those of Narbonne vetch with white lupin (1.16) and common vetch with white lupin (1.14) had significantly higher two-year average values of LER. The intercrops of vetches with other annual legumes had narrower variation of LER than intercropping grass pea with faba bean and white lupin, with from 1.04 to 1.22, in the same agroecological conditions (Ćupina *et al.*, 2009b).

Table 2. Two-year average forage dry matter yield (t ha^{-1}) and its land equivalent ratio (LER) in intercropping vetches with other annual legumes at Rimski Šančevi in 2010 and 2011

Sowing time	Intercrop	Forage dry matter yield	LER
Autumn	Bitter vetch + pea	8.1	1.07
	Common vetch + faba bean	10.3	1.09
	Hairy vetch + faba bean	10.8	1.07
	Hungarian vetch + faba bean	9.6	1.07
Spring	Common vetch + faba bean	9.2	1.08
	Common vetch + white lupin	9.3	1.14
	Narbonne vetch + faba bean	8.9	1.11
	Narbonne vetch + white lupin	8.8	1.16
$LSD_{0.05}$		0.6	0.03

Conclusions

Intercropping various vetch species with other annual legumes has a considerable potential for forage production and has preliminarily proved economically reliable. The future research will focus on yield components, quality aspects, underground biomass and symbiosis.

Acknowledgements

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Changes in species proportion over time in grass-legume mixtures under northern conditions

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Abstract

Strong benefits of sward diversity have been demonstrated in agricultural grasslands. A key factor in achieving significant productivity effects may be for mixtures to maintain relatively even species proportions over time. Here we focus on the changes in species proportion over four harvest years in mixtures of four agronomic species: timothy (*Phleum pratense*) (fast establishing), meadow fescue (*Festuca pratensis*) (persistent), red clover (*Trifolium pratense*) (fast establishing) and white clover (*Trifolium repens*) (persistent), receiving three levels of N fertilizer (20, 70 and 220 kg N ha^{-1}). Mixtures were more productive and showed greater yield stability over time than their individual components in monoculture irrespective of fertilizer treatment. The proportion of timothy fell after the second year, as expected, but increased for red clover in mixtures at 20N. The contribution of white clover was generally low and fluctuated little over time, whereas meadow fescue became more prominent. An *a priori* selection of four species possessing different functional traits has resulted in mixtures which surpassed monocultures both in total yield and yield stability over four years.

Keywords: grass-legume mixtures, yield stability, nitrogen fertilizer, nitrogen fertilizer, species composition

Introduction

In the light of dwindling resources for agriculture, ways must be found to reduce inputs but at the same time maintain or increase production. For intensive fodder production, the current practice is to apply high levels of mineral fertilizer to grass monocultures. It has been suggested that the targeted use of functional biodiversity, in particular grass-legume mixtures, could play a role here as it has the potential to increase productivity, forage quality, resource efficiency and environmental-friendliness (Peyraud *et al*., 2009). Recent results, using two grasses and two forage legumes at 31 sites across Europe that were harvested for three years, have indeed demonstrated strong positive mixing effects (Finn *et al*., 2013). Yield benefits were generally robust to changes in the relative abundance of the four component species.
In addition to the benefits from symbiotic N_2 fixation, positive effects of mixing grasses and legumes may derive from niche differentiation, such as effects resulting from differences in their seasonal growth pattern or across years (Nyfeler *et al*., 2009).
But yield stability is no less important than overall dry matter yield, and the targeted use of legumes in grassland systems may be hampered by insufficient persistence of legumes in the sward due to competitive exclusion from their grass companions. This problem may be further accentuated under marginal growth conditions in northern areas where winter survival of legumes becomes an additional stress factor (Bélanger *et al*., 2006). Within forage mixtures it has been difficult to demonstrate a clear relationship between yield stability and measures of species richness and it has been pointed out that species identity and the selection of particular species in the mixture may play a more dominant role than richness *per se* (Sanderson, 2010). The aim of the present study was to analyse the effects of species diversity on productivity and stability of grass-clover mixtures under marginal conditions. We have already demonstrated that mixtures consistently out-yielded the highest yielding mixture

component in monoculture (Helgadóttir *et al.*, 2012). Here we focus on the changes in species proportion over four harvest years.

Materials and methods

Four monocultures and grass-legume mixtures of timothy (*Phleum pratense*) (fast establishing), meadow fescue (*Festuca pratensis*) (persistent), red clover (*Trifolium pratense*) (fast establishing) and white clover (*Trifolium repens*) (persistent) were established in a completely randomized simplex design (Kirwan *et al.*, 2007) in spring 2008 at Korpa Experimental Station, Iceland. All mixtures contained all four species differing widely in sowing proportions. Both mixtures and monocultures were established at two different sowing densities. There were three different N fertilizer treatments: 20, 70 or 220 kg N ha^{-1} yr^{-1}. Total number of plots was 66. All plots received 40 kg P and 60 kg K ha^{-1} yr^{-1} in early spring. The plots were harvested twice a year for four years (2009-2012) and sown and unsown species proportions were determined by manually separating plant samples from permanent sub-plots.

Results and discussion

The mean total biomass of mixtures over the four years was in all cases higher than the mean of the mixture components in monoculture, the yield advantage being 54, 51 and 25% for 20, 70 and 220 N, respectively (Figure 1). Interestingly, the mean yield of mixtures at 70N surpassed the monoculture yield at 220N, being 7.0 and 6.9 t ha^{-1}, respectively. Monocultures generally also showed greater variation in yield than mixtures. The yield distribution was particularly skewed at 220N resulting in differences between the median and mean values.

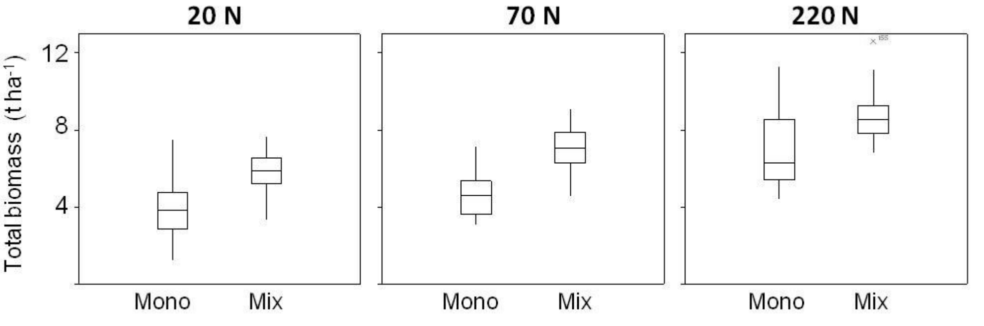

Figure 1. Boxplots of total dry matter yield, presented for monocultures (Mono) and mixtures (Mix), for 20, 70 and 220 kg N ha^{-1}, over four harvest years. The middle 50% of the data lie within the box, with a line indicating the median. Whiskers extend beyond the ends of the box as far as the minimum and maximum value.

Timothy persisted relatively well in monoculture at all N levels in the early half of the experiment but declined from then on, whereas meadow fescue established slowly but became more competitive against weeds with time (Figure 2). The clover species, on the other hand, persisted poorly in monoculture, particularly at 70 N. Similarly, timothy dominated the mixtures for the first two years, but after four years red clover made up around 40% of the herbage at 20 N, whereas meadow fescue and timothy contributed around 40% each at 220 N with weeds making up the remainder. All four sown species, on the other hand, contributed approximately equally to the herbage at 70 N after four years. This fertilizer level should therefore be sufficient under northern conditions to obtain a favourable balance between grasses and legumes, thus benefitting from N fixation of the legume component and also for optimum forage quality (Sanderson, 2010). The legume components were virtually excluded at the highest N level, as expected. Interestingly, red clover was more persistent than white clover both in monocultures and mixtures, particularly at the lower N levels, possibly reflecting its stronger competitive ability for light (Nyfeler *et al*., 2009).

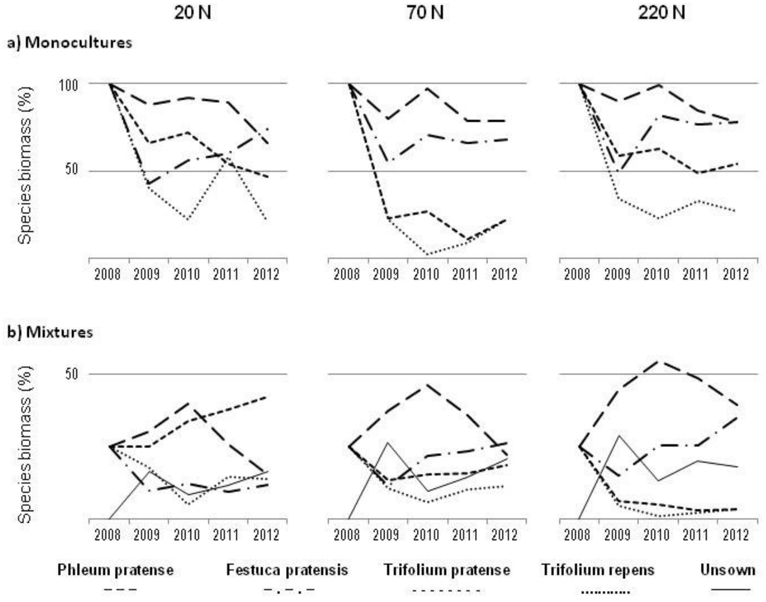

Figure 2. Changes in species proportion over time for a) monocultures, starting from sowing proportion 100%, and b) mean over all mixtures, starting from 25% for each species on average, grown at 20, 70 and 220 N ha^{-1}.

Conclusions

These results confirm that an *a priori* selection of four species possessing different functional traits has resulted in mixtures which surpassed monocultures, both in total yield and yield stability over a period of four years. These results demonstrate that species composition of mixtures is highly dependent on the N level applied. From an agronomic viewpoint, application of 70 kg N ha^{-1} can be recommended as it results in well balanced mixtures that show persistent herbage yield and should give good quality fodder.

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Undersowing a permanent meadow with red clover and its effects

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Abstract

Studies were carried out in 2006-2009 at the Experimental Farm Falenty on a permanent meadow on mineral soil (dry meadow). The aim was to assess the effect of undersowing the meadow with the red clover (*Trifolium pratense* L.). The effects were evaluated in terms of the proportion of red clover in the sward, by herbage yield and total protein content and its increase in the sward of undersown fields. The selected fields of 0.6 ha were fertilized annually with mineral fertilizers (PK, P in the form of phosphate rock meal K as potassium sulphate) or with natural fertilizers – solid manure and liquid manure. In spring 2006 some plots were undersown with tetraploid red clover (cv. Bona) at 8 kg ha^{-1}. Thus, six experimental treatments were established. On selected plots of 25 m^2 in five replicates, botanical composition, herbage yield and total protein content in the sward were recorded every year. Significant increase of red clover on undersown fields caused significantly higher yields in the three years following undersowing. There was increased total protein content in the sward and increased herbage yield on treatments with red clover.

Keywords: effects, permanent meadow, protein, red clover, sward yields, undersowing

Introduction

Legumes and their mixtures with grasses play a role in both integrated and organic production systems (Duer, 1999). Due to their high nutritive value, legumes are grown in mixtures with various grass species and their ability to fix nitrogen is particularly important in economic terms (Bartmański and Mikołajczak, 1997; Mikołajczak, Bartmański, Wolski 1999) and in view of the national obligations to reduce greenhouse gas emissions. Undersowing is a low-input method of introducing these plants into the sward which improves botanical composition of grasslands (Bartmański, Mikołajczak, 2001; Goliński, 1998). Its efficiency is determined by plant competition, fertilization and habitat conditions (Warda, 1999). The aim of the study was to assess the amount and quality of the yield of permanent meadow undersown with red clover and fertilized with various mineral and organic fertilizers.

Materials and methods

Studies were carried out in 2006-2009 on a permanent meadow. Three experimental plots of 0.6 ha each were delineated in spring 2006. Mineral (PK) fertilization was applied to one and natural (manure and liquid manure) to the other two plots (Table 1). The doses of organic fertilizers were calculated based on their N content (evaluated according to Kjeldahl method) and on the equivalent availability, which was assumed 0.5 for manure and 0.8 for liquid manure when applied to soil. At an annual fertilization rate of 60 kg N ha^{-1}, manure dose was 18-22 t ha^{-1} and that of liquid manure was 20-25 m^3 ha^{-1}. A half of each plot was undersown with tetraploid red clover (*Trifolium pratense* L.) cv. Bona. Floristic composition of meadow sward (determined before the first cut (method of Klapp, 1962) and annual dry matter yields (from 5 subplots of an area of 25 m^2 in each plot) were analysed in the study. Based on yield and the content of total protein in meadow sward, the 'protein effect' (i.e. the increment of protein production in particular treatments with undersowing relative to not-undersown treatments [PK, M and LM]) was calculated. Data concerning the DM yields were analysed using analysis of variance in Anova 1 in Statistica.

Table 1. Detailed scheme of experiment.

Plot	Fertilization treatment	Fertilization rates	Form of fertilization
PK	PK	30 kg P ha^{-1} + 60 kg K ha^{-1}	Ground phosphate rock and potassium phosphate
	PK+C	as above + symbiotically fixed N	P, K – as above with undersown red clover
Manure	M	60 kg N ha^{-1}, 30 kg P ha^{-1}, 60 kg K ha^{-1}	Manure covered the requirements for N, P and K
	M+C	as above + symbiotically fixed N	Manure as above with undersown red clover
Liquid manure	LM	60 kg N ha^{-1}, 30 kg P ha^{-1}, 60 kg K ha^{-1}	Liquid manure covered the requirements for N and K, P was supplemented with ground phosphate rock
	LM+C	as above + symbiotically fixed N	Liquid manure as above with undersown red clover

Results

Botanical composition of the sward showed dynamic changes (Table 2) in all treatments. Undersowing with red clover increased legume content. The species was competitive with respect to grasses or to herbs and weeds. Red clover had the lowest share in the year of undersowing, but this share increased to achieve maximum in the third year. Manure was more favourable than PK for the growth of red clover, and liquid manure was the least favourable. In the liquid-manure treatment the share of red clover was two times smaller than in other treatments.

Table 2. Botanical composition of the sward in the years 2006-2009.

Plant-species groups	Percent in particular treatments in years 2006 and 2009											
	PK		PK+C		M		M+C		LM		LM+C	
	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009
Grasses	74.7	86.0	79.7	72.0	82.0	83.0	88.3	65.0	71.0	90.0	79.7	78.0
Legumes	4.0	10.0	9.0	26.0	1.7	4.0	0.7	30.0	4.0	5.0	1.3	14.0
Herbs and weeds	21.3	4.0	11.3	2.0	16.3	13.0	11.0	5.0	25.0	5.0	19.0	8.0
Total	100	100	100	100	100	100	100	100	100	100	100	100

In the PK-fertilized treatment, despite lack of N fertilization, large yields (6.44 to 7.85 t ha^{-1}) were obtained (Figure 1). Undersowing with red clover (PK+C versus PK) increased the yield slightly in 2006 but yield increment was more substantial in the years 2007-2009.

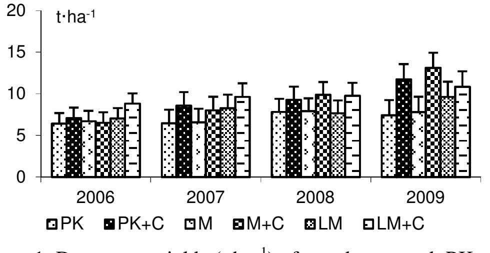

Figure 1. Dry matter yields (t ha^{-1}) of meadow sward, PK, PK+C, M, M+C, LM, LM+C – fertilization treatments; vertical bars indicate LSD value (P=0.05).

Dry matter yield of 6.72 t ha^{-1} obtained in the manure-fertilized treatment in 2006 was slightly higher than that from the PK treatment. Differences in the yield from the two treatments were also small in other years. Undersowing of the manure-fertilized treatment with red clover resulted in significant yield increments in 2008 and 2009.

Fertilization with liquid manure appeared quite effective and significantly increased yields in 2007 and 2009 compared with treatments fertilized with PK and manure. Undersowing the liquid-manure fertilized treatment with red clover increased its share in the sward and a systematic significant yield increase in subsequent years. In all treatments undersown with red clover a trend of increasing protein content in herbage was observed (Table 3).

Table 3. The average protein content (%) in the study years.

Years	Fertilization treatments						LSD 0.05
	PK	PK+C	M	M+C	LM	LM+C	
2006	14.75	14.05	15.06	15.59	16.14	17.13	2.64
2007	15.45	15.89	16.63	16.75	14.66	15.59	1.73
2008	16.81	17.41	17.39	18.08	14.47	15.52	2.87
2009	14.81	16.45	16.21	17.65	12.34	14.28	2.03

Table 4. The increment of total protein production (t ha^{-1}) in relation to PK, M and LM in particular treatments attributed to undersowing with red clover.

Years	Treatments		
	PK+C	M+C	LM+C
2006	0.09	0.01	0.30
2007	0.34	0.24	0.21
2008	0.25	0.35	0.32
2009	0.71	0.97	0.17

The increment of total protein production from treatments undersown with red clover as an outcome of the increase in yields and in protein content (i.e., the so called 'protein effect', varied among treatments (Table 4).
In the PK+C and M+C treatments the increment of protein was small in the year of undersowing but increased in subsequent years. Undersowing with red clover appeared most effective in the manure-fertilized treatment where the protein effect amounted to 0.97 t ha^{-1} in the third year after treatment.

Conclusions

Undersowing was most effective in the manure-fertilized treatment and the least effective in liquid manure fertilized treatment: the proportion of red clover in the meadow sward was 33.7% in the former and only 14% in the latter.
Undersowing with red clover was a highly effective measure and its results were manifested in the increase of yields by 20% on average, and in protein increments in subsequent years, particularly in the PK- and manure-fertilized treatments.

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Cutting strategy as a tool for manipulating the seasonal nutritive value and production in grass-clover

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Abstract

To improve the utilization of annual grass-clover a higher focus on the nutritive value during the whole growing season is needed. In a field experiment seven different cutting strategies with different regrowth lengths and cut numbers were examined in a sward composed of perennial ryegrass (*Lolium perenne* L.), festulolium (*Festulolium braunii* K.A.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.). The cutting strategies highly influenced seasonal growth and nutritive value and only slightly influenced the proportion of red and white clover and the annual yield. Strategies with an early spring cut gave the best yearly mean of digestibility of organic matter, but the strategies also showed different potential to achieve different goals for use of the harvested herbage. The results showed the necessity of a decision support system which includes the higher intake when feeding with clover.

Keyword: cutting strategy, red clover, white clover, nutritive value, digestibility

Introduction

Often there is a great focus on the nutritive value of the spring cut and only little focus on the rest of the growing season. However, in a farm situation, the whole year's complete herbage production is used for feeding different cattle groups that have different demands. The challenge is to optimize the proportion of the annual production with a satisfactory nutritive value for high producing dairy cows. More cuts are often co-ensiled in horizontal silos, which is one of the reasons for why a planning tool for cutting time that also indicates which cuts should be co-ensiled, could be useful. By comparing different feeding trials, Weisbjerg *et al.* (2011) showed that milk yield did not increase further when the *in vitro* organic matter digestibility (IVOMD) in grass-clover increased above 78%, and that point was independent of time in the growing season. This gives a possibility to use IVOMD as a goal for planning the seasonal cutting strategy. Here results are shown from an experiment with different cut number and regrowth length in 2012. The aim was to collect data as a part of the background for developing a decision support system for the seasonal cutting strategy.

Materials and methods

Seven different cutting strategies shown in Figure 1 were examined on a sandy loam at Foulum in Denmark in a sward (26 kg seed per ha; % of seed in brackets) composed of red clover (9%), white clover (9%), festulolium (50%) and perennial ryegrass (32%). The swards were fertilized with 100 kg N ha^{-1} in spring and 60 kg N ha^{-1} after the second cut. There were four replicates. The grass-clover was undersown in a spring barley (*Hordeum vulgare*) crop in 2011. Plots were harvested with a Haldrup plot harvester, nutritive value was determined by NIR, including IVOMD calibrated to the method of Tilley and Terry, and botanical composition of dry matter (DM) was determined by hand separation of subsamples.

Results and discussion

There were five 5-cut strategies and two 4-cut strategies for the growing season; see Figure 1. The growth started in April and the number of weeks in Figure 1 is for the period from 1 May to 15 October. Seasonal dry matter yield was very much affected by the cutting strategy. The most even production was found in strategy 1, with the earliest spring cut, and the most uneven was in strategy 5, with the latest spring cut (Figure 1). The highest annual dry matter yield was found in strategy 7 and the lowest in strategy 2; 16.0 and 13.9 t DM ha^{-1}, respectively (Table 1).
The proportion of clover showed the same seasonal development as is normally found; the lowest clover proportion in spring and the highest in late summer (Figure 1). Harvest time of spring cut had an effect on white/red clover during the whole season. Strategy 1-3 with the first cut 2-3 weeks after 1 May had a higher white clover and lower red clover content than strategy 4-7 with the first cut 4-5 weeks after 1 May. The yearly weighted average was 15 and 8% white clover, and 33 and 39% red clover for strategy 1-3 and strategy 4-7 respectively.

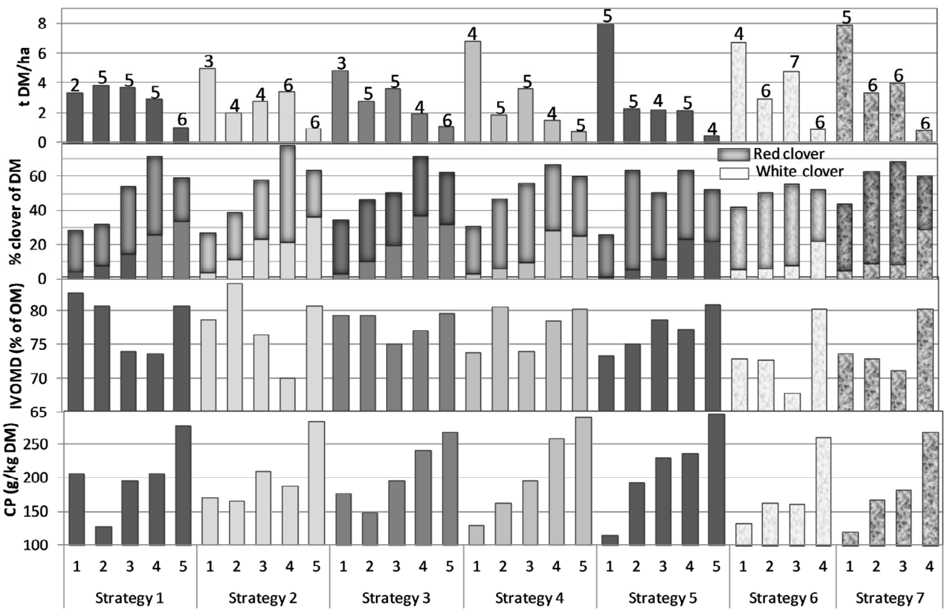

Figure 1. Dry matter yield (t DM ha^{-1}), proportion of red and white clover (% of DM), IVOMD (% of OM) and crude protein (g kg^{-1} DM) in the single cuts in different cutting strategies. Above the yield columns the number of growth weeks are shown; for spring cut the week number from 1 May and for the regrowth the number of weeks between cuts.

Cutting strategy had a high influence on the trend of IVOMD (Figure 1). The strategies were planned to reach different goals. Strategy 3 was planned to have an even nutritive value throughout the season, and therefore the third regrowth in late summer was short, because in that period the temperature is normally the highest, which means that the digestibility of organic matter is low. Opposite, in strategy 2 the cut length in late summer was long and the length of regrowth 1 and 2 were short, with the goal to optimize the nutritive value before the warm period and then in the warm period to produce herbage with a lower nutritive value, which is not expected to be sufficient for dairy cows. In strategies 4 and 5, first cut was harvested late with the aim to maximize the herbage production in spring with the best weather for high IVOMD.

The seasonal profile of crude protein was also highly affected by the strategy. In spring, with a relatively low clover proportion, the crude protein content decreased highly with later harvest time, even though the clover proportion at the same time increased. In general the crude protein content was highly affected by clover proportion and yield level.

Table 1. The annual dry matter yield and weighted mean of IVOMD and crude protein (CP). Further the best cut combination is shown for pooling cuts in two parts with the most equal IVOMD.

Strategy	Annual results			Annual herbage pooled in two parts (1 / 2)		
	DM yield ($t\ ha^{-1}$)	IVOMD (% of OM)	CP ($g\ kg^{-1}$ DM)	Cuts (number in part 1 / 2)	IVOMD (% of OM)	CP ($g\ kg^{-1}$ DM)
1	14.6^{bcd}	78.1^{a}	187^{a}	1+3 / 2+4+5	78.0 / 78.1	200 / 176
2	13.9^{e}	77.0^{b}	188^{a}	1+2+4 / 3+5	76.8 / 77.4	175 / 227
3	14.1^{de}	77.9^{a}	191^{a}	1+3+5 / 2+4	77.7 / 78.4	193 / 186
4	14.4^{cde}	75.5^{c}	170^{b}	1+2+5 / 3+4	75.7 / 75.2	147 / 213
5	14.9^{bc}	75.1^{c}	165^{b}	1+3+4 / 2+5	75.0 / 76.0	156 / 208
6	15.3^{b}	71.8^{e}	154^{c}	1 / 2+3+4	73.0 / 70.8	133 / 172
7	16.0^{a}	73.2^{d}	152^{c}	1+2 / 3+4	73.4 / 72.8	134 / 196

The goal for the grass-clover production varies very much from farm to farm. One possible goal could be to make two horizontal silos of all cuts with as equal nutritive value as possible to avoid difficulties under feeding, when switching from one silo to the other, and with a minimum 78% IVOMD in order to meet the requirement for high milk production. Results from the experiment are examined in relation to this goal example, and in Table 1 the best combination for the single strategy is shown. Only strategy 1 succeeded. By later harvest of spring cut, it was difficult to obtain 78% IVOMD, especially with 4-cut strategies, where it was not possible even by omitting a cut.

However, besides the digestibility the 'clover-effect' is also an important quality parameter for dairy cows. A higher proportion of white or red clover often increases the intake and milk yield (Peters *et al.*, 2006; Kuoppala, 2010). In addition, red clover often has a lower IVOMD than grass (Kuoppala, 2010). The situation in spring with a high digestibility and a low clover proportion is quite opposite to the summer situation, where the digestibility is low and the clover proportion is high. Therefore, it should be quantified how to balance the digestibility and clover proportion. It seems to be necessary to include the 'clover-effect' in an assessment system for making a true comparison between cuts.

Conclusion

The seasonal profile of nutritive value and production is highly affected by the cutting strategy. To optimize the herbage production to its planned use on the farm, a decision support system is needed. For that to be achieved, well defined goals of nutritive value dependent on the proportion of clover in the herbage are necessary.

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Appreciation of current and future functions of grassland by international stakeholders in Europe

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Abstract

Grasslands are the main focus of the EU-project MultiSward. Identifying stakeholder requirements and expectations with respect to multi-functionality of grasslands within Europe is part of MultiSward. A questionnaire among different stakeholders and different regions in Europe provided insight into the appreciation of the current and future functions of grasslands in Europe. The 2011 and 2012 results showed that the different functions of grasslands are highly recognized and appreciated. The large European grassland area is essential for economy, environment and people. The results of the questionnaire provided insight in the value of the different ecosystem services that grasslands provide and of different components of these services for different stakeholders in different regions. Generally, the provisioning services were considered especially valuable, followed by the regulating and supporting services, and finally the cultural services, although rankings did vary somewhat between countries and regions. We conclude that grasslands are considered by all stakeholders to be a valuable resource in Europe. Maintaining or increasing the importance of the different functions and services of grassland in Europe is a challenge for the coming years.

Keywords: functions, grassland, multi-functionality, stakeholder

Introduction

Sustainability (profit, planet, people) is high on the societal, political and economic agendas. The EU-project MultiSward aims to increase reliance on grasslands and on multi-species swards for ruminant production contributing to increased sustainability and competitiveness of livestock production systems, increased food security and enhanced environmental goods while securing optimal European grassland acreage. In order to achieve this, MultiSward wants the active participation of stakeholders. An international team of representatives of countries throughout Europe has therefore been established, representing the different regions in Europe. The countries are Ireland, the Netherlands, France, Italy and Poland representing Atlantic, mountainous, Mediterranean and Continental regions. This study aimed to identify the appreciation of the current and future functions of grasslands by international stakeholders in Europe.

Materials and methods

A questionnaire on multi-functionality of grasslands was developed in 2011 in five languages: Polish, Dutch, Italian, French and English. The questionnaire was available in a paper version in a few countries and on-line (www.multisward.eu), and it has questions on sustainability and ecosystem services. Sustainability covers economic, environmental and social issues (profit, planet, people). Respondents to the questionnaire were asked to allocate10 points across these three aspects of sustainability, giving most points to the one they considered the most important aspect (e.g. 4, 3, 3 if they consider that ecological and social aspects are of equal interest and that economy is slightly more important). Ecosystem services can be divided into four groups: provisioning services (e.g. production of food, water), regulating services (e.g. control of climate and disease), supporting services (e.g. nutrient cycles, crop pollination) and cultural services (e.g. spiritual, recreational). Respondents to the questionnaire were again asked to allocate 10 points over the four groups of ecosystem services.

Responses on the questionnaire were analysed in spring 2012. At that time there were 160 valid responses. Five regional groups were identified based on the number of responses available and on similarities between regions: Poland (31%), France (37%), The Netherlands + Belgium (11%), Ireland + UK (14%) and Italy (7%). A stakeholder analysis within MultiSward (Pinxterhuis, 2011; van den Pol-van Dasselaar *et al*., 2012) revealed that the traditional foursome of primary producer, policy maker, research and advisory are the most important stakeholders with respect to the multifunctionality of grasslands in Europe, followed by NGOs (nature, environment), education and industry. All these seven stakeholder groups responded to the questionnaire: advisory (38%), research (24%), primary producers (14%), education (9%), industry (8%), NGO's (4%) and policy makers (4%).

Results and discussion

At the start of the MultiSward project, an international stakeholder consultation (Pinxterhuis, 2011; van den Pol-van Dasselaar *et al*., 2012) showed that economic functions of grasslands, mainly feed for herbivores, were considered the most important. Stakeholders expected this to remain unchanged in future. Environmental functions of grasslands were placed second: water quality and quantity, adaptation to climate change, mitigation and biodiversity. Finally, social services were mentioned.

In this study, further information was obtained for different stakeholders and for different regions (Figure 1). In general, profit was considered to be the most important aspect, followed by planet and then followed by people. Some observations:

- Primary producers, and to a lesser extent industry parties, gave a higher value to profit than the other stakeholders and a lower value to the other components, especially people.
- Both France and Italy valued people aspects relatively higher and profit aspects relatively lower.

Grasslands can provide several services, the so-called ecosystem services. Figure 2 shows that provisioning services were considered to be the most important services. However, there were clear differences between stakeholders and between regions. The differences between regions reflect the differences in the farming structure throughout Europe.

There were almost no differences in stakeholders' appreciation of today and future functions of grasslands (not shown). The only remark from the stakeholders was that they expect increasing importance of both feed protein supply at farm level and environmentally friendly systems. Reasons for the lack of large differences between appreciation of today and future

functions may be that there is no difference or that stakeholders cannot yet imagine differences in appreciation between today and the future.
The accuracy of results will increase with increasing number of responses. In the years 2012 and 2013 a simplified version of the questionnaire will be developed with the aim of obtaining further information.

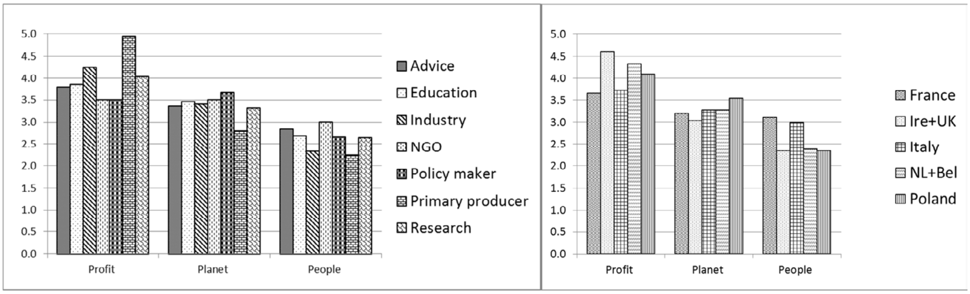

Figure 1. Importance of aspects of sustainability for different stakeholders and different regions (total of people, planet and profit equals 10 for each stakeholder group or region).

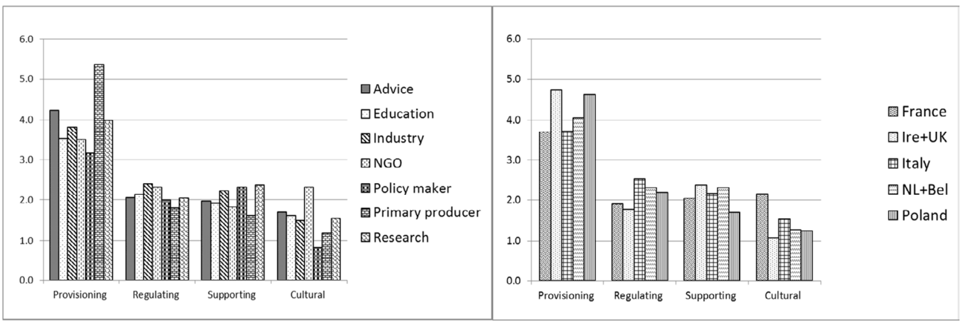

Figure 2. Importance of ecosystem services for different stakeholders and for different regions (total equals 10 for each stakeholder group or region).

Conclusion

We conclude that all stakeholders consider grasslands to be a valuable resource in Europe. Maintaining or increasing the importance of the different functions and services of grasslands in Europe is a challenge for the coming years.

Acknowledgements

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Seasonal dynamics of legume-grass herbage production and quality in a long-term pasture

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Abstract

The potential and variability of herbage output and quality under grazing were examined over a 10-year period. Species mixtures of white clover and lucerne together with perennial ryegrass, smooth-stalked meadow grass and *Festulolium* hybrid were examined on a gleyic loamy *Cambisol*. Within ten years, changes occurred in the botanical composition of pasture swards; however, in lucerne-based swards legumes predominated up to the eighth year. Legumes, especially lucerne, stimulated dry matter (DM) and crude protein (CP) production. During each grazing period, the CP content fluctuated from spring to autumn and the changes reflected differences in sward composition. Legumes had a positive effect on providing a more even annual DM and CP yield and its distribution over the grazing period.

Keywords: legume, lucerne, grazing swards, crude protein, perennial ryegrass

Introduction

Successful management of legumes influences the herbage production, nutritional quality and the sustainability of grazing systems, and their possible role is very important in the ecosystem and livestock production systems. The performance of legumes can be affected also by the companion species of the sward and environmental conditions, especially within a grazing period (Michaud *et al*., 2012). Legume performance is highly variable in its herbage production and nutritive value, though legume-grass mixtures usually exhibit less variation of nutritive value as compared with grasses. The grass-legume system confers benefits to the grass, especially in the early life of mixtures (Rochon *et al*., 2004; Nyfeler *et al*., 2011). Plant species diversity, their relative abundance, and stability of yields are highly dependent on soil nutrient status, management intensity and natural environmental conditions. At the same time it is very important to optimize functional diversity, combining species with opposite qualities and well adapted to the local environments (Solter *et al*., 2007; Huyghe *et al*., 2012). The objective of this study was to examine the potential and variability of herbage output and quality during a grazing period of 10 years.

Materials and methods

A field experiment was conducted in a randomized block design with four replicates on a gleyic loamy *Endocalcari-Epihypogleyic Cambisol* near Dotnuva, Lithuania (55°24'N, 23°50'E). The soil pH varied from 6.5 to 7.0, humus content was 2.5-3.2%, available P was 50-80 mg kg^{-1} and K was 100-150 mg kg^{-1} of soil. The following mixtures were sown: white clover and perennial ryegrass (*Tr*/*Lp*); white clover, perennial ryegrass and smooth-stalked meadow grass (*Tr*/*Lp*/*Pp*); lucerne, perennial ryegrass and smooth-stalked meadow grass (*Ms*/*Lp*/*Pp*); white clover, lucerne and perennial ryegrass (*Tr*/*Ms*/*Lp*); perennial ryegrass without nitrogen fertilization (*Lp*/N0); perennial ryegrass fertilized with 240 kg N ha^{-1} yr^{-1} (*Lp*/N240); white clover and *Festulolium* hybrid (*Tr*/*F* hybrid). All swards received 26 kg P ha^{-1} and 50 kg K ha^{-1} at the begining of spring. The perennial ryegrass received 60 kg N ha^{-1}

in spring and after the first, second and third grazing. The grazing period lasted from the beginning of May until middle of October with four-five grazings at 25-40 day intervals. The weather conditions differed considerably between the seasons: 2000, 2001, 2004 and 2005 were similar to the long-term avererage for rainfall and temperature, 1999 and 2007 were wet, 2003 dry, and 2002, 2006 and 2008 were very dry and warm. Crude protein (CP) concentration was calculated from the total N concentration in the herbage (N × 6.25), determined by the Kjeldahl procedure. The experiment data were statistically analysed using ANOVA.

Results and discussion

DM yield of all swards decreased markedly between the first year of use and the last year and significant differences between swards were obtained from the first to the tenth year (Table 1). The proportion of legumes in the legume-grass mixtures fluctuated both between years and within individual growth periods, and was very strongly influenced by environmental stress, especially in dry seasons. The sward composition and total yield were affected by legume species and its persistence. Lucerne-based swards performed best results (Table 2).

Table 1. Total annual yield of swards and its stability over ten years, t DM ha^{-1}.

Swards	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Tr/*Lp*	6.12	5.49	5.02	2.58	2.69	5.26	4.74	2.74	6.69	3.52
Tr/*Lp*/*Pp*	6.56	5.36	5.16	2.47	2.20	4.73	5.25	2.93	5.89	3.39
Ms/*Lp*/*Pp*	7.55	8.87	7.19	3.04	5.59	8.63	8.24	4.52	7.49	5.14
Tr/*Ms*/*Lp*	6.96	8.11	6.56	3.02	4.76	7.04	7.03	4.77	7.92	5.19
Lp/N0	3.23	4.45	4.73	3.12	2.31	4.38	5.21	3.13	6.80	3.94
Lp/N240	7.54	7.10	4.51	3.04	4.20	7.72	5.70	4.26	7.73	5.53
Tr/*F* hybrid	6.74	6.21	5.36	2.60	2.53	4.97	4.76	2.94	6.39	3.54
$LSD_{0.05}$	0.511	0.421	0.398	0.615	0.366	0.497	0.466	0.591	0.589	0.577

Table 2. Legume share in annual dry matter yield, %.

Swards	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Tr/*Lp*	48.8	15.5	22.8	22.5	6.9	45.6	52.7	8.7	8.8	27.0
Tr/*Lp*/*Pp*	53.1	20.6	24.0	29.6	8.4	41.0	49.1	6.1	9.7	23.3
Ms/*Lp*/*Pp*	69.3	68.2	52.6	52.2	72.5	76.0	65.8	65.1	39.4	43.5
Tr/*Ms*/*Lp*	58.1	34.6	35.5	53.9	66.8	61.3	53.2	61.1	22.2	42.1
Tr/*F* hybrid	35.6	13.4	21.0	19.1	6.9	44.3	45.9	4.6	15.2	21.6

Swards significantly affected DM yield for each grazing (Table 3). The maximum production was achieved in the first grazing for all swards. DM yield was significantly higher for the swards based on lucerne and perennial ryegrass fertilized with nitrogen compared with the other tested swards. The positive contribution of lucerne to pasture productivity has been recognized (Smith *et al*., 2000).

Table 3. DM yield in each grazing, data averaged over 1999-2008, t ha^{-1}.

	Total DM yield						DM yield of legumes					
Swards	I	II	III	IV	V	Total	I	II	III	IV	V	Total
Tr/*Lp*	1.90	0.97	0.80	0.58	0.24	4.48	0.35	0.27	0.35	0.31	0.11	1.30
Tr/*Lp*/*Pp*	1.76	0.96	0.83	0.60	0.24	4.39	0.34	0.44	0.49	0.38	0.13	1.74
Ms/*Lp*/*Pp*	2.28	1.45	1.46	1.05	0.28	6.62	0.99	0.70	0.86	0.82	0.18	3.39
Tr/*Ms*/*Lp*	2.15	1.39	1.42	0.92	0.26	6.13	0.59	0.61	0.79	0.59	0.14	2.69
Lp/N0	1.80	0.96	0.77	0.52	0.16	4.13	0.29	0.23	0.24	0.29	0.09	1.02
Lp/N240	2.19	1.28	1.15	0.83	0.31	5.73	0.07	0.06	0.06	0.05	0.02	0.22
Tr/*F* hybrid	1.89	0.99	0.84	0.63	0.24	4.60	0.30	0.25	0.31	0.34	0.10	1.23
$LSD_{0\text{-}05}$	0.112	0.072	0.082	0.071	0.099	0.233	0.069	0.086	0.097	0.075	0.113	0.286

The CP concentration in herbage significantly differed among the swards in all grazings (Table 4). During the growing period the CP content generally decreased, leading to a decline in overall nutritive value (Michaud *et al.*, 2012). In our experiment, on average over ten years of sward use, CP concentration in the herbage ranged from 167 to 207 g kg^{-1} DM over a grazing period. Differences and decreases in CP concentration among the swards were greater only from mid-June and in July. This resulted in CP concentration being lowest for the second grazing, and ranged from 133 to 184 g. CP concentration was highest in the herbage of perennial ryegrass fertilized with 240 kg N ha^{-1} yr^{-1} and also in herbage of lucerne-based swards. The lowest CP concentration was in the herbage of perennial ryegrass without nitrogen fertilization.

Table 4. CP concentration in swards, data averaged over 1999-2008.

	Grazing, CP g DM kg^{-1}				
Swards	I	II	III	IV	V
Tr/*Lp*	192	159	184	211	216
Tr/*Lp*/*Pp*	200	153	185	213	217
Ms/*Lp*/*Pp*	211	184	186	230	222
Tr/*Ms*/*Lp*	210	179	192	230	219
Lp/N0	181	133	182	198	200
Lp/N240	227	168	204	225	222
Tr/*F* hybrid	194	144	177	203	240
$LSD_{0 \cdot 05}$	11.7	12.2	11.0	9.6	12.1

Conclusions

Significant changes occurred in the composition of long-term pasture swards during the ten years of use, while in lucerne-based swards legumes dominated up to the eighth year. Legumes, especially lucerne, stimulated DM and CP production and stability over the years and provided a more even distribution over each grazing period. Although CP concentration differed among the swards, it was moderate for all swards and grazings.

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Effects of intensive management on the biomass composition of mountain meadows under recurrent drought

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Abstract

The increasing economic pressure on grassland farming in disadvantaged areas, such as in mountain regions, has often resulted in an intensification of the favourable areas and abandonment of the labour-intensive areas. In such situations, farmers often attempt to increase forage production, allocating a larger amount of dung to the easily accessible farm plots and increasing the cutting frequency, but climatic constraints such as drought hamper plant growth. In order to investigate the effect of intensive management on the botanical composition of mountain permanent meadows under recurrent drought, a seven-year field experiment was conducted in South Tyrol. Different cutting frequencies (two to four cuts per year), coupled with increasing fertilization rates (44 to 88 m³ ha^{-1} yr^{-1} of slurry, respectively), as well as a control treatment (two cuts yr^{-1}; 19.8 m³ ha^{-1} yr^{-1} of slurry), were applied. The yield share of each species was visually assessed immediately before the first cut. Depending on the treatment, a distinct vegetation dynamics was apparent after seven years. Our results show that several years of high nutrient supply, coupled to recurring drought, negatively affect the botanical composition of permanent meadows.

Keywords: mountain meadows, yield share, cutting frequency, fertilization, drought

Introduction

Mountain grassland farmers have experienced increasing economic pressure in recent years. Due to this fact, there is often an attempt at reducing the forage production costs by allocating a larger amount of dung to the easily accessible farm fields and increasing the cutting frequency. However, climatic constraints can hamper plant growth, preventing the achievement of these aims. Moreover, a detrimental vegetation dynamics may undermine the basis of a sustainable forage production. Drought has occurred repeatedly during the last decade in the southern margin of the Alps. In order to get reliable data about the effects of increasing management intensity on the botanical composition under recurring drought, a field experiment was conducted from 2006 to 2012 in South Tyrol.

Materials and methods

The study was conducted from 2006 to 2012 at the experimental farm Mair am Hof, in Dietenheim (890 m a.s.l., Bruneck, South Tyrol, I). The experimental site was a species-poor, intensively managed meadow (three to four cuts yr^{-1}). The upper soil layer (10 cm depth) was a humus-rich (6.8%) loamy sand with the following soil properties at the time of the trial establishment: pH 6.1, 48 mg kg^{-1} P, 149 mg kg^{-1} K and 175 mg kg^{-1} Mg. During the investigation period, recurrent drought was observed: a simple soil water balance was calculated for the first growth period of each treatment (from the start of the growing season to the harvest date) as the difference between the cumulative precipitation and the cumulative potential evapotranspiration according to Penman-Monteith (Allen *et al.*, 1998). Weather data were obtained from a meteorological station at the experimental farm; the start of the growing season was estimated according to the MTD method of Schaumberger (2011). Water balance values showed a water deficit of more than 100 mm in 38%, and more of 50 mm in 24%, of the total number of observations (years × treatments). No irrigation was provided during the experiment. Four treatments, corresponding to different management intensity, including three cutting frequencies (two to four cuts $year^{-1}$) coupled with increasing fertilization rates (44 to 88 m^3 ha^{-1} yr^{-1} of 2:1-dilutes slurry respectively) were applied (Table 1).

Table 1. Description of the treatments applied. Nutrient input of N, P and K is the average value of seven growing seasons ± SE.

Treatment name	Cutting frequency (cuts yr^{-1})	Fertilization			
		Slurry amount (m^3 ha^{-1} yr^{-1})	N (kg ha^{-1} yr^{-1})	P (kg ha^{-1} yr^{-1})	K (kg ha^{-1} yr^{-1})
0.9/2	2	18.9	41.4 ± 3.2	7.4 ± 0.7	66.9 ± 7.0
2/2	2	44	96.2 ± 7.3	17.3 ± 1.6	155.5 ± 16.2
3/3	3	66	144.3 ± 11.0	25.9 ± 2.4	233.2 ± 24.3
4/4	4	88	192.3 ± 14.7	34.5 ± 3.2	310.9 ± 32.5

The trial design was a randomized complete block design with three replications and plots of 4 × 4 m. The plots were mown according to the following harvest plan: 22 June and 10 October for the two-cut treatment; 28 May, 22 July and 10 October for the three-cut treatment; 22 May, 5 July, 16 August and 5 October for the four-cut treatment. A tolerance in the harvest date of ± 3 days was allowed. Starting in 2007, the yield share of each species occurring in the plots was visually assessed (as percent) immediately before the first cut. The effect of the treatments on the yield share of the functional groups grasses, legumes and forbs, of the most abundant species, and on the number of vascular species at the end of the experiment, was investigated with ANOVA. Multiple comparisons were performed using the Student-Newman-Keuls test. A probability of $P<0.05$ was considered to be significant.

Results and discussion

The treatment application resulted after seven years in clear differences concerning the yield share of several species (Table 2).

A low cutting frequency led, irrespective of the fertilization rate, to a high proportion of grasses and to the lowest proportion of legumes and forbs. *Elymus repens* became here the most abundant grass species. The most relevant difference between the two-cut treatments seems to be the share of *Alopecurus pratensis*, which had a two-fold abundance in the 2/2 treatment compared to the 0.9/2 treatment. Both species, which are similar to each other with respect to their functional traits (da Silveira Pontes *et al.*, 2010), are indeed known to be favoured by a low cutting frequency (Pavlů *et al.*, 2011). We tentatively suggest that recurrent drought further promoted the expansion of *Elymus repens*, which was already present in the plots at the beginning of the experiment (15.2% on average across all treatments in 2007). At the other extreme of the intensity gradient, the botanical composition of forage biomass of treatment 4/4 was characterized by the lowest proportion of grasses, and the highest share of both legumes (almost exclusively represented by *Trifolium repens*) and forbs, and especially of *Taraxacum officinale*. The 3/3 treatment showed intermediate features between the two-cut and the four-cut treatments and exhibited the highest proportion of *Poa angustifolia*.

All in all, all treatments seem to have been negatively affected by drought, as shown, for instance, by the overall high proportion of the drought-tolerant *Achillea millefolium*, which was scarcely represented at the beginning of the experiment (3.5% on average in 2007), and by the already-mentioned increase of *Elymus repens* in the two-cut treatments. However, the high proportion of forbs (mainly consisting of opportunistic and weed species) in treatment 4/4 indicates the appearance of vegetation gaps to a larger extent than in the other treatments.

Species richness was not affected by the treatments, failing to reflect the different nutrient supply of the treatments.

Table 2. An estimated yield share of functional groups and of selected species and number of vascular plant species at the first cut of the seventh experiment year (2012). Average values across all treatments in 2007 are shown for reference.

Year	2007	2012				*P*-value
Treatment	Mean across all treatments	0.9/2	2/2	3/3	4/4	
Grasses (%)	72.6	82.2 [a]	83.8 [a]	73.0 [a]	55.4 [b]	<0.05
Elymus repens (%) [#]	15.2	48.1 [a]	32.7 [ab]	18.5 [bc]	11.0 [c]	<0.01
Alopecurus pratensis (%)	12.2	7.3 [b]	20.0 [a]	10.3 [b]	8.0 [b]	<0.05
Arrhenatherum elatius (%) [#]	3.0	6.7 [a]	0.4 [ab]	4.8 [ab]	0.0 [b]	<0.05
Dactylis glomerata (%) [#]	14.7	4.0	10.7	5.3	7.4	0.149
Poa angustifolia (%)	13.8	8.7 [b]	14.0 [ab]	20.3 [a]	13.7 [ab]	<0.05
Lolium perenne (%) [#]	7.5	0.6 [b]	1.0 [b]	5.1 [a]	9.8 [a]	<0.01
Legumes (%)	7.4	3.0 [b]	2.3 [b]	5.7 [ab]	9.0 [a]	<0.05
Forbs (%)	20.0	14.4 [b]	13.6 [b]	21.2 [b]	36.0 [a]	<0.05
Taraxacum officinale (%)	13.2	1.7 [b]	2.0 [b]	5.3 [b]	11.3 [a]	<0.01
Achillea millefolium (%)	3.5	7.0	5.3	11.3	12.0	0.160
Heracleum sphondylium (%)	0.7	3.3	3.3	4.0	8.3	0.584
Number of vascular plant species	13.5	16.7	16.0	17.3	16.0	0.189

[#] Analysis with logarithm-transformed data ; back-transformed means are shown

Conclusions

Under recurrent drought, and depending on a gradient of nutrient input coupled with increasing cutting frequency, distinct changes in the composition of the biomass of species-poor permanent meadow have to be expected within few years. At low cutting frequency, and irrespective of the nutrient input, *Elymus repens* can become dominant, while high cutting frequency and nutrient input seem to lead to the appearance of vegetation gaps to a larger extent than at lower management intensity, as suggested by the larger proportion of opportunistic and weed species.

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Direct sowing of red clover and inter-genus hybrids - field emergence and weight of sown plants

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Abstract

At Jevíčko in the Czech Republic, a small-plot trial was established in 2011 to compare at two sowing dates (June and July) three technologies for direct sowing seeds of red clover [´Amos´ (4n) and ´Suez´ (2n)], and of two inter-genus hybrids (festucoid ´Felina´ and loloid ´Hostyn´). Direct seeding was carried out by three technologies: (1) slot seeding with a seeding drill SE-2-024, (2) strip seeding with a prototype of PP-2 drill, and (3) with a prototype of STP 300 drill. Among the direct sowing technologies the highest field emergence was in the trial with legumes and seeding drill SE-2-024 (66.1%), with seeding drill STP-300 it reached 58.0%, and with strip seeding it was 49.1%. In the trial with direct-sowing technologies the dry matter weight of 50 sown plants of grasses was 1.6 g (0.8 g root biomass and 0.8 g aboveground biomass) for SE-2-024, 0.7 g (0.3 g root biomass and 0.4 g aboveground biomass) for STP-300 and 4.5 g (1.9 g root biomass and 2.6 g aboveground biomass) for the PP–2 drill.

Keywords: red clover, grass, grassland, direct sowing, technology

Introduction

The significance of direct sowing for the improvement of permanent grassland is well demonstrated by its extent in Austria, where about 80,000 ha of permanent grassland are directly sown each year, whereas in the Czech Republic the extent of direct sowing is less than 10,000 ha (Kohoutek *et al.*, 2007). In 2011 trials were conducted to compare three different direct-sowing technologies, each at two sowing dates, for introducing legume (red clovers; 2n and 4n) and grasses (two inter-genus hybrids) into a permanent grassland sward.

Materials and methods

At Jevíčko in the Czech Republic (average annual temperature 7.4°C, annual long-term rainfall average 545 mm; altitude 342 m; geographic coordinates: 49°37'N, 16°44'E), a multifactor trial, with direct sowing into a 30-year-old permanent grassland sward (PG), was established in 2011. The existing meadow vegetation is represented by an *Arrhenatheretum* plant society. The sward was well-established meadow vegetation in a mezohygrophyte stand growing in a stream alluvium. Three direct sowing technologies were used: (1) slot seeding with SE-2-024, (2) strip seeding with a PP-2, and (3) strip seeding with a prototype of STP-300 drill (discs with coulters) with a spike-seeding mechanism. The trials were established at two different sowing-dates; 14 June (T1) and 19 July (T2). We sowed legume (L) and grass (G) seeds. The legume was red clover, cv. ´Amos´ (4n) and cv. ´Suez´ (2n) at a seed rate of 8 million germinative seeds (MGS) per ha and the grass consisted of two inter-genus hybrids, ´Felina´ (festucoid) and ´Hostyn´ (loloid) at 12 MGS per ha. The plot length was 16.11 m, with three replications.

About 3 weeks and 8 weeks after sowing, counting of germinated plants on a one-metre run (bm) was carried out with four observations on each plot, and field emergence was calculated (based on % of emerged plants out of 800 germinative seeds per m^2 for legumes, and out of 1200 germinative seeds per m^2 for grasses). The weight of 50 whole plants was also determined at the height of 100-150 mm (T1 on 4 August, 51 days after sowing, and T2 on 6 October, at 79 days after sowing). The stands were not fertilized in the sowing year 2011. The measured results were statistically evaluated with 3-factor variance analysis and the differences between averages were tested with the Tukey test ($D_{T0.05}$, $D_{T0.01}$).

Results and discussion

The strip-sown stands that were established in 2011 emerged in extraordinarily favourable weather conditions because there was regular rainfall from mid-June till mid-August; the rainfall total in June was 75.5 mm (30-year average 74.3 mm), in July 118.9 mm (30-year average 67 mm). The temperature over the evaluated period was within the long-term average. Rainfall abundance positively influenced both field emergence and later growth and development of emerged plants.

Table 1. Field emergence and dry matter weight of 50 plants of strip-sown legumes and grasses (Jevíčko, 2011).

Factor	(L) Legumes					Factor	(G) Grasses				
	Emergence		DM weight of 50 plants				Emergence		DM weight of 50 plants		
	3 weeks	8 weeks	root	aboveground	total		3 weeks	8 weeks	root	aboveground	total
	%		g				%		g		
T1	61.2	30.5	1.0	3.6	4.6	T1	40.5	36.7	0.9	1.1	2.0
T2	54.2	35.1	2.5	5.3	7.8	T2	35.1	35.1	1.1	1.4	2.5
$D_{T0.05}$	6.7	5.4	0.8	1.6	2.3	$D_{T0.05}$	6.5	7.6	0.5	0.6	1.0
$D_{T0.01}$	9.2	7.4	1.0	2.1	3.1	$D_{T0.01}$	8.9	10.3	0.7	0.8	1.3
Amos	57.6	30.5	1.8	4.6	6.4	Felina	25.0	24.6	0.7	0.8	1.5
Suez	57.9	35.1	1.7	4.4	6.1	Hostyn	50.6	47.1	1.3	1.7	3.0
$D_{T0.05}$	6.7	5.4	0.8	1.6	2.3	$D_{T0.05}$	6.5	7.6	0.5	0.6	1.0
$D_{T0.01}$	9.2	7.4	1.0	2.1	3.1	$D_{T0.01}$	8.9	10.3	0.7	0.8	1.3
SE-2-024	66.1	33.5	1.5	4.1	5.6	SE-2-024	40.4	39.3	0.8	0.8	1.6
STP-300	58.0	33.9	1.6	3.9	5.5	STP-300	33.2	32.8	0.3	0.4	0.7
PP - 2	49.1	31.0	2.1	5.4	7.5	PP - 2	39.7	34.6	1.9	2.6	4.5
$D_{T0.05}$	10.0	8.1	1.1	2.3	3.4	$D_{T0.05}$	9.7	11.2	0.7	0.8	1.4
$D_{T0.01}$	12.9	10.4	1.4	3.0	4.4	$D_{T0.01}$	12.5	14.5	0.9	1.1	1.8

In the trial with red clover, field emergence after 3 weeks reached 61.2% at T1 and 54.2% at T2 (P<0.05), and after 8 weeks the number of plants decreased to 30.5% of field emergence at T1 and 35.1% at T2. In the trial with grasses, field emergence after 3 weeks was 40.5% at T1 and 35.1% at T2, and after 8 weeks it fell to 36.7% at T1 and it remained the same at T2 (35.1%). There were no conclusive differences between the two red clover varieties in terms of field emergence, values of which were both high (57.6% for ´Amos´ and 57.9% for ´Suez´). After 8 weeks it decreased to 30.5% for ´Amos´ and to 35.1% for ´Suez´. In contrast, there were highly significant differences between the two inter-genus hybrids: field emergence of festucoid hybrid ´Felina´ reached 25.0%, whereas that of the loloid hybrid ´Hostyn´ was 50.6%. This was a conclusive increase (significant at P<0.01), and after 8 weeks these figures had decreased only slightly, to 24.6% (295 pcs plants per m^2) for ´Felina´ and 47.1% (565 pcs plants per m^2) for ´Hostyn´. Among the three tested direct-sowing technologies the highest field emergence was in the trial with legumes and the seeding drill SE-2-024 (66.1%); with the seeding drill STP-300 it reached 58.0% and with strip seeding it was 49.1% (P<0.01). After 8 weeks there was a reduction in the number of emerged plants by about half; this was likely to be due to the influence of biotic and abiotic agents (Kohoutek *et al.*, 1995).

The dry matter weight of 50 sown plants in the trial with red clover was 4.6 g (1.0 g root biomass and 3.6 g aboveground biomass) after 51 days at T1, and 7.8 g (2.5 g root biomass and 5.3 g aboveground biomass) at T2 after 79 days. The differences between T1 and T2 were highly conclusive (significant at $P<0.01$). In the trial with grasses the dry matter weight of 50 sown plants was 2.0 g (0.9 g root biomass and 1.1 g of aboveground biomass) at T1, and 2.5 g (1.1 g root biomass and 1.4 g of aboveground biomass) at T2. There were no conclusive differences between red clover varieties in the weight of 50 sown plants.

There were highly significant differences between hybrids in the dry matter weight of 50 sown plants: with festucoid hybrid ´Felina´ it was 1.5 g (0.7 g root biomass and 0.8 g aboveground biomass) and with loloid hybrid ´Hostyn´ it was 3.0 g (1.3 g root biomass and 1.7 g aboveground biomass). Fast-growing species are more suitable for direct sowing as they penetrate the sward better, and due to higher weight of a root system, they resist external influence better.

Conclusion

Among the direct-sowing technologies tested, the highest field emergence was in the trial with legumes and the seeding drill SE-2-024 (66.1%); with the seeding drill STP-300 it reached 58.0%, and with strip seeding it was 49.1%. Differences between the direct-sowing technologies showed that the dry matter weight of 50 sown plants of red clover was 5.6 g (1.5 g root biomass and 4.1 g aboveground biomass) for the SE-2-024 drill; 5.5 g (1.6 g root biomass and 3.9 g aboveground biomass) for the STP-300, and 7.5 g (2.1 g root biomass and 5.4 g aboveground biomass) for the PP-2.

The increase in biomass weight of strip-sown red clovers is due to rotational cultivation of the soil profile to a depth of 10-15 cm, and width of 15 cm, which creates ideal conditions for initial growth and development of strip-sown plants.

In the trial with direct-sowing technologies the dry matter weight of 50 sown plants of grasses was 1.6 g (0.8 g root biomass and 0.8 g aboveground biomass) for the SE-2-024 drill, 0.7 g (0.3 g root biomass and 0.4 g aboveground biomass) for the STP-300 drill, and 4.5 g (1.9 g root biomass and 2.6 g aboveground biomass) for the PP-2. The increase of weight biomass of sown plants of grasses is, in the case of strip seeding, 3-6 times greater, and is statistically significant ($P<0.01$) in comparison with shallow-surface direct sowing, and is a key factor for successful introduction of direct sowing. In arid years this effect is further intensified.

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Direct sowing of red clover and inter-genus hybrids by three technologies: forage production and quality

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Abstract

At Jevíčko in the Czech Republic, a small-plot trial was established in 2011 to compare at two sowing dates (June and July) three technologies for direct sowing seeds of red clover [´Amos´ (4n) and ´Suez´ (2n)], and of two inter-genus hybrids (festucoid ´Felina´ and loloid ´Hostyn´). Direct seeding was carried out by three technologies: (1) slot seeding with a seeding drill SE-2-024, (2) strip seeding with a prototype of PP-2 drill, and (3) with a prototype of STP 300 drill. Among the direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} [corrected DM (CDM) production 5.44 t ha^{-1}] for SE-2-024; 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP-300; and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP-2. Among the direct sowing technologies, the DM production of the inter-genus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE-2-024; 6.11 t ha^{-1} (CDM production 0.74 t ha^{-1}) for STP-300; and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP-2.

Keywords: direct sowing, red clover, intergenus hybrids, grasslands, production and quality

Introduction

Introduction of direct sowing into grasslands is influenced by a number of factors. Operational successfulness of direct sowing is still in spite of attained results insufficient and therefore the research, development and verification of new technologies continue (Kohoutek *et al.*, 2002).

Materials and methods

At Jevíčko in the Czech Republic (average annual temperature 7.4°C, annual long-term rainfall average 545 mm; altitude 342 m; geographic coordinates: 49°37'N, 16°44'E), a multifactorial trial, with direct sowing into a 30-year-old permanent grassland sward (PG), was established in 2011. The existing meadow vegetation is represented by an Arrhenatheretum plant society. The sward was well-established meadow vegetation in a mezohygrophyte stand growing in a stream alluvium. Three direct-sowing technologies were used: (1) slot seeding with a SE-2-024, (2) strip seeding with a PP-2, and (3) strip seeding with a prototype of STP 300 drill (discs with coulters) with a spike-seeding mechanism. The trials were established at two different sowing-dates; 14 June (T1) and 19 July (T2). We sowed legume (L) and grass (G) seeds. The legume was red clover, cv. ´Amos´ (4n) and cv. ´Suez´ (2n) at a seed rate of 8 million germinative seeds (MGS) per ha, and the grass consisted of two inter-genus hybrids, ´Felina´ (festucoid) and ´Hostyn´ (loloid) at 12 MGS per ha. The plot length was 16.11 m (length of harvested part was 11.11 m), with three replications.

The trial plot was not fertilized in the year of direct sowing 2011. In the first harvest year 2012 the plot was fertilized with phosphorus at the rate of 35 kg P ha^{-1} and potassium at 100 kg K ha^{-1}. Nitrogen was applied only on directly sown grasses at the rate of 180 kg ha^{-1} N (60-60-60) in the form of LAV by a seed drill HEGE-80. The trial plot was harvested in three cuts. The paper evaluates dry matter production and corrected dry matter (CDM) production [(DM × % projective dominance of a directly sown species)/100)]. The forage quality was measured using the instrument NIRSystems 6500. The observed parameters were: crude protein (CP), fibre, NEL (net energy of lactation), NEF (net energy of fattening), PDIE (ingested digestive protein allowed by energy) and PDIN (ingested digestive protein allowed by nitrogen). The measured results were statistically evaluated and differences between averages were tested with the Tukey test.

Results and discussion

Dry matter (DM) production was significantly lower in the dry year 2012 in comparison with average rainfall years by about one third, especially in the growth of the 1st and 3rd cuts. Average DM production in 2012 in the trial with red clovers was 7.14 t ha^{-1}, of which 4.29 t ha^{-1} was CDM production of directly sown red clovers. In the trial with inter-genus hybrids DM production was 6.81 t ha^{-1}, of which 1.51 t ha^{-1} was CDM production of directly sown grasses. At the trial site in long-term trials with PK fertilization the original grassland produced 3.43 t ha^{-1} DM. Total DM production in 2012 (Table 1) in the trial with red clovers (zero fertilized) was 7.50 t ha^{-1} at T1 (CDM production 4.95 t ha^{-1}) and at T2 it was 6.77 t ha^{-1} (CDM production 3.62 t ha^{-1}). In the trial with grasses the DM production (N fertilization: 180 kg ha^{-1}) 6.69 t ha^{-1} (CDM production 1.82 t ha^{-1}) at T1 and 6.92 t ha^{-1} (CDM production 1.19 t ha^{-1}) at T2. CDM of directly sown red clovers and grasses is significantly higher (P<0.01) at the first date of direct sowing in mid-June. There were no significant differences between red clover varieties in DM and CDM production: DM production of 4n variety ´Amos´ was 7.02 t ha^{-1} (CDM production 4.20 t ha^{-1}) and 2n variety ´Suez´ was 7.25 t ha^{-1} (CDM production 4.36 t ha^{-1}).

There were no differences in total DM production between directly sown inter-genus hybrids: the DM production of festucoid hybrid ´Felina´ was 6.75 t ha^{-1} (CDM production 0.77 t ha^{-1}) and of loloid hybrid ´Hostyn´ it was 6.86 t ha^{-1} (CDM production 2.25 t ha^{-1}, which is a significant increase in comparison with ´Felina´ (P<0.01)). Fast-growing species are more suitable for direct sowing as they penetrate the original sward better and resist adverse external influences.

In the trial with direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} (CDM production 5.44 t ha^{-1}) for SE-2-024, 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP-300, and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP-2. Lower CDM production of strip-sown red clovers was caused by mole damage to 30-40% of the rows after strip sowing in 2011.

In the trial with direct sowing technologies the DM production of inter-genus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE-2-024, 6.11 t ha^{-1} (CDM production 0.74 t ha^{-1}) for STP-300, and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP-2. Significantly higher DM and CDM production of strip-sown inter-genus hybrids is caused by a high proportion of loloid hybrid ´Hostyn´ on yield when CDM production was 4.15 t ha^{-1}, that is two to three times more than with other technologies and it reached the yield level of directly sown red clovers.

In the trial with red clovers the average CP concentration in 2012 was 167.2 g kg^{-1} DM, fibre 201.3 g kg^{-1}, and NEL was 5.84 MJ kg^{-1} DM. In the trial with grasses the average CP concentration in 2012 was 132.5 g kg^{-1} DM, fibre was 252.9 g kg^{-1} DM, and NEL was 5.55 MJ kg^{-1} DM.

Table 1. Dry matter production and forage quality from directly sown permanent grassland with legumes and grasses (Jevíčko, 2012).

	(L) Legumes							
			Forage quality					
	DM	CDM	CP	Fibre	NEL	NEV	PDIE	PDIN
Factor	(t ha^{-1})		(g kg^{-1})	(g kg^{-1})	(MJ kg^{-1})	(MJ kg^{-1})	(g kg^{-1})	(g kg^{-1})
T1	7.50	4.95	169.8	202.0	5.81	5.72	86.2	99.5
T2	6.77	3.62	164.5	200.5	5.86	5.78	85.3	97.2
$D_{T0.05}$	0.76	1.14	5.5	6.4	0.08	0.10	0.8	3.4
$D_{T0.01}$	1.03	1.55	7.5	8.7	0.11	0.13	1.1	4.6
Amos	7.02	4.20	167.5	198.6	5.89	5.82	86.1	99.0
Suez	7.25	4.36	166.4	203.9	5.78	5.68	85.3	97.7
$D_{T0.05}$	0.76	1.14	5.5	6.4	0.08	0.10	0.8	3.4
$D_{T0.01}$	1.03	1.55	7.5	8.7	0.11	0.13	1.1	4.6
SE-2-024	7.43	5.44	168.7	200.1	5.85	5.77	86.0	99.5
STP-300	6.74	4.20	169.7	199.2	5.91	5.83	86.5	100.7
PP-2	7.24	3.21	162.5	204.4	5.75	5.65	84.7	94.8
$D_{T0.05}$	1.12	1.69	8.2	9.5	0.12	0.15	1.2	5.1
$D_{T0.01}$	1.45	2.18	10.6	12.3	0.15	0.19	1.6	6.5

	(G) Grasses							
			Forage quality					
	DM	CDM	CP	Fibre	NEL	NEV	PDIE	PDIN
Factor	(t ha^{-1})		(g kg^{-1})	(g kg^{-1})	(MJ kg^{-1})	(MJ kg^{-1})	(g kg^{-1})	(g kg^{-1})
T1	6.69	1.82	131.5	252.7	5.53	5.35	81.4	75.9
T2	6.92	1.19	133.5	253.0	5.56	5.39	81.2	76.8
$D_{T0.05}$	0.58	0.34	5.1	5.7	0.09	0.11	0.8	3.5
$D_{T0.01}$	0.79	0.46	7.0	7.8	0.13	0.15	1.1	4.7
Felina	6.75	0.77	133.6	252.2	5.56	5.39	81.6	77.2
Hostyn	6.86	2.25	131.3	253.6	5.52	5.35	81.0	75.5
$D_{T0.05}$	0.58	0.34	5.1	5.7	0.09	0.11	0.8	3.5
$D_{T0.01}$	0.79	0.46	7.0	7.8	0.13	0.15	1.1	4.7
SE-2-024	6.21	1.08	131.6	252.4	5.47	5.29	81.3	76.1
STP-300	6.11	0.74	134.1	251.3	5.59	5.43	81.6	77.4
PP-2	8.09	2.71	131.6	254.9	5.57	5.40	81.0	75.5
$D_{T0.05}$	0.86	0.50	7.6	8.5	0.14	0.16	1.2	5.1
$D_{T0.01}$	1.11	0.65	9.9	11.0	0.18	0.21	1.6	6.6

Conclusion

In the trial with direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} (CDM production 5.44 t ha^{-1}) for SE-2-024, 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP-300 and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP-2. In the trial with direct sowing technologies the DM production of inter-genus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE-2-024, 6.11 t ha^{-1} (CDM production 0,74 t ha^{-1}) for STP-300 and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP-2.

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An evaluation of the effect of silage management practices on commercial farms in England and Wales on baled silage quality

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Abstract

Research has provided clear guidelines on how to improve baled silage quality and inhibit mould growth. The aim of this survey was to evaluate to what extent these best practice methods have been adopted on commercial farms and to evaluate if these methods improved silage quality. Details of management practices followed during the growing, ensiling and storage of round bale silage were collected, and three bales evaluated on each of 80 farms. Film-wrap seal and bale shape were evaluated and film-wrap samples were taken to determine the number of layers applied. Surface mould cover was visually assessed and silages were sampled for NIRS analysis. Bales with six layers of film-wrap had a more effective film seal compared with bales with four layers (P<0.01) and fewer bales showed surface moulding (P<0.01) and where present, less surface mould was evident (P<0.05). Bales with six layers of film applied had a higher ME and CP concentration when compared to bales wrapped with four layers of film. Bales wrapped by the stack had a better film seal and less surface mould (P<0.05) than those wrapped on the harvested field and transported to the stacking area.

Keywords: silage, bale, survey, wrap, farming practice

Introduction

Under controlled conditions, increasing the number of film-wrap layers applied to baled silage will provide a more efficient fermentation and increase dry matter recovery (Fychan *et al.,* 2006) due to the improved seal provided by additional layers. Air ingress should be minimized to avoid ensiling conditions that favour the growth of undesirable fungi. As well as affecting the nutritive value of the ensiled material, the growth of the fungi can affect animal health, especially pregnant ewes. On commercial farms, as well as being affected by number of film layers applied, baled silage preservation is affected by a range of other management and environmental factors (e.g. multiple handling, transportation and wildlife) that can cause physical damage to the film seal. The present study evaluated ensiling management practices on commercial farms and determined their effect on silage preservation and quality.

Materials and methods

During January and February 2012, eighty farms throughout England and Wales were visited. A questionnaire was completed during each visit to evaluate the management practices followed during the growing, ensiling and storage of a specific batch of round silage bales. Three bales were selected from the specified stack for evaluation, one on each layer of a three-tier stack. Film-wrap seal was evaluated by evacuating the air within each bale and measuring the time taken for air to re-enter, as described by McEniry *et al.* (2011). Bale width and height within the stack were measured to assess mis-formity during storage. The edge shape of each bale was visually assessed and categorized as good (square edged), moderate or bad (round edged with indentations). Film samples were taken from two locations on the curved side of each bale, by taking 50mm Ø plastic cores 90^o apart. Bales were then unloaded from the stack and film-wrap removed. Visible surface mould cover on all surfaces of the bale was visually assessed by calculating the number of 75×75mm grid squares covered and converting to a percentage of the total bale surface. Samples of each individual bale were taken by coring two 50mm Ø cores, 400mm deep each side of the curved surface of each bale and 180° apart. Silage samples were vacuum sealed immediately and stored at 4^{o}C prior to NIRS analysis on a fresh basis to assess nutritional and fermentation parameters. Continuous normally distributed variables were analysed by ANOVA and by Kruskal-Wallis one-way ANOVA otherwise. Counts within two-way classification tables were assessed by chi-square.

Results and discussion

The mean and range of the NIRS silage analyses are shown in Table 1. The silages evaluated were produced for feeding to a wide range of livestock - from high yielding dairy cows to dry suckler cows, as highlighted by the wide range of quality and dry matter contents observed.

Table 1. Mean, standard deviation and range of nutritional quality attributes of baled silage.

	Mean	sd	Range
Dry Matter (DM) (g kg^{-1} DM)	511	150.2	200 - 908
pH	4.96	0.444	4.08 - 6.36
Ammonia (g kg^{-1} TN)	70	28.5	50 - 210
Crude Protein (CP) (g kg^{-1} DM)	111	19.8	80 - 207
Soluble Sugars (g kg^{-1} DM)	72	18.9	17 - 108
Lactic Acid (g kg^{-1} DM)	34	15.7	1 - 80.3
Neutral Detergent Fibre (g kg^{-1} DM)	495	40.7	380 - 598
Metabolizable Energy (ME) (MJ kg^{-1} DM)	10.2	0.90	8.3 - 12.3
D-Value (g kg^{-1} DM)	638	56.6	520 - 770
Intake Prediction (g kg^{-1} W$^{0.75}$)	96	11.7	50 - 105

Table 2. Farmer responses and proportions to questionnaire.

Harvest Month	April/May (0.17)	June (0.46)	July (0.16)	Aug/Sept (0.20)
Cut Number	1st (0.75)	2nd (0.21)	3rd (0.04)	
Mower type	No Conditioner (0.22)	Swath Cond. (0.49)	Spread Cond. (0.29)	
Length of wilt	1 day (0.38)	2 days (0.49)	3+ days (0.13)	
Estimated film layers	4 layers (0.60)	6 layers (0.39)	8 layers (0.01)	
Baler operator	Farmer (0.46)	Contractor (0.54)		
Wrapper operator	Farmer (0.49)	Contractor (0.51)		
Bales fed to *:	Dairy stock (0.30)	Beef stock (0.51)	Ewes (0.30)	

* Some bales were allocated to more than one category of stock

Farmers' responses (Table 2) to the questions were assessed, and cutting month, cut number, mower type, baler and wrapper operator had no effect on either film seal or mould cover. The number of film layers present on each bale was compared with the estimated number of layers applied. Of the 48 farms that stated they had applied 4 layers of film, seven farms failed on some bales and applied 3 layers only. Of the 32 farms that stated they had applied 6 or more layers, 7 farms failed on some bales and 8 farms failed to achieve this on all bales. A more effective film seal was observed in bales with 6 layers of film than in bales with 4 layers or less (P<0.01) (Table 3a)**.** Fewer bales had mould when 6 layers had been applied (P<0.01) and the bales with mould present were affected less severely (P<0.05).

Bales wrapped in the harvested field can be at risk of physical damage to the film wrap during handling, transportation and from wildlife damage. Compared with bales wrapped in the field, bales wrapped near the stack had a more effective film seal ($P<0.05$), were affected by mould less frequently ($P<0.01$) and when affected by mould they were affected less severely ($P<0.05$) (Table 3b). ME and CP concentrations of silage from bales that had 6 layers of film were higher than silage with 4 layers of film. This has been found in previous studies, but in this case, we cannot assume that all the improvement is due to the extra film applied. It is possible that those who apply extra film were also aware of the importance of cutting grass at the correct stage of growth. Bales that were destined for feeding to ewes were better sealed ($P=0.002$) and had less surface mould ($P<0.001$) than bales for feeding other stock. Bales destined for dairy stock had lower dry matter ($P<0.001$), and higher D-value ($P<0.001$) than silage destined for feeding to other stock. Bale edge shape had no effect on mould cover although there was a trend for improved film seal ($P=0.093$) with better edge shape.

Table 3. Effect of a) film-wrap layers applied and b) wrapping site on film seal, number of bales showing visible surface mould and the percentage cover on those bales.

a)	Fewer than 4	4	6 or more	*s.e.d.*	*P*
Number of bales	15	157	62	-	
Film seal (seconds)	10.9^{a}	17.0^{a}	$38.4^{\mathbf{b}}$	$0.1554^{\#1}$	$P<0.001$
Number of mouldy bales	9	94	21	-	$P<0.01$
Surface mould (%)	3.96^{b}	2.02^{b}	0.88^{a}	$0.2324^{\#2}$	$P<0.05$

b)	In harvest field	By stack	*s.e.d.*	*P*
Number of bales	141	99	-	
Film seal (seconds)	17.2	27.0	$0.078^{\#1}$	$P<0.05$
Number of mouldy bales	84	42	-	$P<0.01$
Surface mould (%)	2.33	1.12	$0.1271^{\#2}$	$P<0.05$

$^{\#1,\#2}$; s.e.d. relates to means transformed to $\log_{10}(x+1)$ and log10(x) respectively
^{a,b}; differing superscripts indicate means differ at $P<0.01$ (film seal) and $P<0.05$ (surface mould)

Conclusions

The survey findings were in agreement with previous scientific findings, that the number of film-wrap layers applied to bales affected baled silage preservation. Increasing film layers applied reduced air ingress and minimized mould growth. Bales wrapped in the harvested field were less well sealed than bales wrapped by the stack, suggesting there was increased physical damage to the film on bales wrapped in the field. A number of farms (27%) applied fewer film-wrap layers than anticipated. This survey shows the importance of ensuring that best silage management practices are effectively communicated to the farming industry.

Acknowledgements

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Effects of combined lactic acid bacteria strains on fermentation, aerobic deterioration and mould growth in lucerne big bale silage

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Abstract

The effect of the bacterial inoculant (combined lactic acid bacteria strains *Entrococcus faecium* (DSM 22502/NCIMB 11181); *Lactococcus lactis* (NCIMB 30117) and *Lactobacillus plantarum* (DSM16568) on the chemical composition, fermentation end-products, DM (dry matter) recovery, aerobic stability, as well as the mould development in lucerne big bale silages were studied under field conditions. The first cut of the primary growth of lucerne stand was wilted to 342 g DM kg^{-1}, than baled and stretch-wrapped for silage. The inoculant was applied at 1.5×10^5 cfu g^{-1} of crop at the time of ensiling in big bales. Big bales were stored outside for a minimum of 90 days, afterwards opened and sampled for the analyses. The results of 5 replicates (5 bales) per treatment were analysed using a non-parametric Wilcoxon Krusal-Wallis test. The inoculant significantly reduced DM loss, pH and butyric acid concentration, and significantly increased lactic acid content. Combined lactic acid bacteria strains also resulted in higher lactic:acetic ratio, lower ammonia-N concentration, and inhibited yeast and mould count in lucerne silage. Inoculation reduced visible mould growth on big-bale surface and improved aerobic stability (14 *vs.* 6 days). The inoculated silage had numerically higher, but not statistically different, organic matter digestibility.

Keywords: lucerne, lactic acid bacteria, silage, fermentation, aerobic stability

Introduction

A risk of undesirable fermentation is higher when forages with high buffering capacity are ensiled, as compared with grass species (Huhtanen *et al.*, 2012). Selected strains of homofermentative lactic acid bacteria (LAB) often result in a faster decrease in pH, lower final pH values, higher lactate:acetate ratios, lower ethanol and ammonia-N, and a 1 to 2% improvement in DM recovery (Weinberg and Muck, 1996). Thus, the use of traditional homolactic bacterial inoculants as starters for alfalfa silage has been a recommended practice to ensure rapid fermentation during the early stages of ensiling and to minimize the loss of nutrients and DM (McAllister *et al.*, 1998).

The present experiment was designed to investigate the effect of a multi-species (*Entrococcus faecium* (DSM 22502/NCIMB 11181) + *Lactococcus lactis* (NCIMB 30117) + *Lactobacillus plantarum* (DSM16568)) inoculant (FLP) on composition, fermentation end-products, DM recovery, aerobic stability, as well as the mold development in lucerne big bale silage.

Materials and methods

A homogenous plot of the primary growth of lucerne, at budding stage, was mown on 8 June with a disk mower-conditioner, set to place wide windrows and wilted to a dry matter concentration of 342 g DM kg^{-1}. The following additive treatments were applied to forage in the windrows during the baling: (1) Control – no additive (UT) and (2) a multi-species *Entrococcus faecium* (DSM 22502/NCIMB 11181-30%) + *Lactococcus lactis* (NCIMB

30117-30%) + *Lactobacillus plantarum* (DSM16568-40%) inoculant (FLP). The inoculant suspension was applied at a rate 4 L suspension t^{-1} lucerne to achieve an application rate of LAB 150 000 colony forming units (cfu) per 1 g herbage. The same volume (4 L t^{-1}) of tap water was used instead of the suspension in the control treatment (UT) for spontaneous fermentation. To avoid cross contamination untreated silage was handled first. The wrapped cylindrical bales (1.2 m wide and 1.2 m diameter) were placed gently on their flat ends without stacking and were stored outside.

Five big bales from each treatment were chosen at random, weighed when prepared and again after 90 days of storage for measuring dry matter (DM) losses. Prior to removing the plastic film surrounding each bale, it was examined carefully for visible damage. On removal of the plastic film, all visible mould colonies on the bale surface were numbered and scored. Each bale was core sampled for chemical and microbial analyses and for aerobic stability test.

All silage samples were analysed for DM, water soluble carbohydrates (WSC), pH, ammonia-N, lactic acid, fatty acids and ethanol using standard methods. Yeasts and moulds were enumerated in accordance ISO 7954:1987(E) and *Clostridium perfringens* – ISO 7937:2004.

Aerobic stability was measured in the laboratory by monitoring changes of temperature in 1000-g silage samples placed in boxes in aerobic conditions, and in parallel aerobic stability was measured on a big scale by inserting 70-cm long temperature sensors into bales at two different points after plastic film removal.

Results and discussion

Mean DM content of the forage was 342.2 g kg^{-1}, crude protein and water soluble carbohydrates concentrations were 229.1 and 48.8 g kg^{-1} DM, respectively. Buffer capacity of herbage was 564 mEq kg^{-1} DM). Consequently, water soluble carbohydrates:buffer capacity ratio was 0.09 g mEq^{-1} and lucerne was characterized as difficult to ensile.

Compared with UT, FLP treatment significantly decreased the pH, ammonia-N, butyric acid, alcohol concentrations and dry matter loss. Inoculation significantly increased lactic acid concentration and the content of water soluble carbohydrates (WSC). The lactic:acetic acids ratio was proportionally 1.9 lower in the untreated than in the inoculated silage (Table 1).

Table 1. Fermentation characteristics of the of the lucerne big bale silage.

	UT	FLP	$LSD_{0.05}$
DM §, g kg^{-1}	323.4 ± 3.93	335.1 ± 3.55*	4.916
DM loss §, g kg^{-1} DM	87.6 ± 14.81	47.1 ± 9.69*	10.274
WSC, g kg^{-1} DM	6.1 ± 1.02	10.2 ± 1.78*	1.778
Ammonia-N, % of total N	5.86 ± 0.93	4.29 ± 0.53*	1.034
pH	4.95 ± 0.085	4.41 ± 0.054*	0.035
Lactic acid, g kg^{-1} DM	32.2 ± 3.87	71.7 ± 7.29*	8.368
Acetic acid, g kg^{-1} DM	19.8 ± 5.88	20.6 ± 3.87	10.123
Butyric acid, g kg^{-1} DM	3.9 ± 0.65	0.9 ± 0.25*	0.384
Propionic acid, g kg^{-1} DM	0.2 ± 0.15	0.2 ± 0.12	0.280
Alcohols, g kg^{-1} DM	6.4 ± 1.07	4.4 ± 1.13*	0.312
Clostridia spores, log cfu g^{-1} FM	1.0 ± 0.994	<1.0 ± 0.990	0
Lactic acid bacteria, log cfu g^{-1} FM	5.00 ± 0.272	5.36 ± 0.184*	0.184
Yeast, log cfu g^{-1} FM	2.07 ± 0.305	1.18 ± 0.268*	0.147
Moulds, log cfu g^{-1} FM	2.66 ± 0.186	1.87 ± 0.162*	0.140

§ Dry matter and calculated dry matter losses are corrected for volatiles

* Significant at level 0.05

A potential inoculant strain has to grow fast to compete successfully with other microbes. The higher decline in pH and shifting fermentation towards lactic acid with FLP reflected the efficiency of added inoculant as suggested by Filya *et al.* (2007). The low proteolytic activity of the *L. plantarum* strains agrees with the observations by Winters *et al.* (2001). Appreciable decrease in the numbers of yeast (2.07 log cfu g^{-1}) and mould (2.66 log cfu g^{-1}) were detected in the inoculated silage when compared with the untreated silage (1.18 log cfu g^{-1} and 1.87 log cfu g^{-1}), respectively. Big bales treated with FLP at the time of removing the plastic film had no visible surface fungal contamination, whereas UT bales were contaminated with one visible colony each. At the end of the aerobic stability test (18 days after plastic removal) FLP big bales were scored as 1.8, and that was 2.3 times lower ($P<0.05$) if compared with the UT big bales. The lower temperature in the inoculated silages relative to the control illustrated the improved aerobic stability afforded by inoculation (Figure 1 and Figure 2).

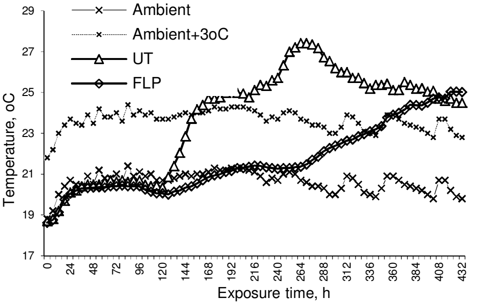

Figure 1. Aerobic stability of FLP and UT lucerne big bale silage under laboratory test.

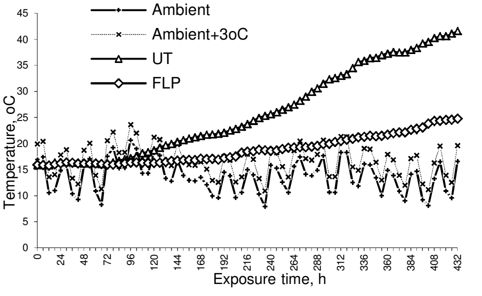

Figure 2. Aerobic stability of FLP and UT lucerne big bale silage under big scale test.

Conclusions

Baled lucerne silage treated with a multi-species (*Entrococcus faecium* + *Lactococcus lactis* + *Lactobacillus plantarum*) inoculant (FLP) was superior at the onset of fermentation, and in the decline in pH relative to the untreated baled lucerne silage. The result was higher concentration of lactic acid and lower concentration of butyric acid, alcohols and ammonia-N fraction and lower DM loss in inoculated silages compared with untreated.

FLP inhibited yeast and mould count and reduced visible mould growth on the surface of big bales. In the inoculated big bale lucerne silage, time to reach the maximum temperature was lowered; therefore, aerobic stability was improved. The improved fermentation and better aerobic stability of inoculated baled silage is a reflection of higher microbiologically inhibitory environment.

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The effects of bacterial strain blends or/and other silage inoculants on legume-grass silage fermentation

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Abstract

The effects of adding novel blends of homofermentative lactic acid bacteria (LAB) strains *Entrococcus faecium*, *Lactococcus lactis*, *Lactobacillus plantarum*; homo- and hetero-fermentative LAB strains *Lactobacillus plantarum*, *Entrococcus faecium* and *L. buchneri*; homofermentative LAB strains plus sodium benzoate; homofermentative LAB strains plus Xylanase and homofermentative LAB strains plus Xylanase and plus sodium nitrate on the fermentation and aerobic stability of legume-grass mixture (*Trifolium pratense* L. and *Lolium perenne* L.) silages were studied under laboratory conditions. The second cut [265 g kg^{-1} dry matter (DM)] of a mixture of red clover and perennial ryegrass stand was harvested with standard field equipment. After the inoculants were added, the chopped forages were ensiled in anaerobic micro-silos and compared with the control silage prepared without an inoculant. The silages were opened after 90 days of ensilage and their chemical composition and aerobic stability were measured. Overall, microbial inoculants generally had a positive effect on grass-legume silage characteristics in terms of lower pH, ammonia-N concentrations, reduced DM losses and populations of yeasts and mould compared with control silages. Homofermentative LAB in combination with *L. buchneri* or sodium benzoate shifted fermentation towards acetate and had a great effect on the aerobic stability of slightly wilted red clover-ryegrass silages.

Keywords: red clover-ryegrass mixture, inoculant, silage, fermentation, aerobic stability

Introduction

Lactic acid is the most commonly identified organic acid that helps to reduce the pH in the silage. Therefore, lactic acid-producing bacteria are the primary type of bacteria utilized in most bacterial inoculants (Wrobel and Zastawny, 2004). Another alternative is to use additives containing other antimicrobial compounds. Little information has been published on the use of sodium benzoate and sodium nitrate. The products containing sodium nitrate and sodium benzoate were used in the experiment reported in this paper, because there are no long-term studies on the effects of these combinations with bacteria strains on silage fermentation. Rammer *et al*. (1999) and Jaakkola *et al*. (2010) suggest that the combination of LAB inoculants with chemical salts has the potential to reduce spoilage of silages and to improve aerobic stability. Thus, the objective of this study was to determine the effects of inoculants containing a new combination of lactic acid-producing bacteria strains with either no other components or with enzyme or/and chemical antimicrobial compounds on fermentation end-products and aerobic stability of legume-grass silages.

Materials and methods

A moderately difficult to ensile (WSC/BC ratio- 0.226 mEq kg^{-1} DM) red clover:ryegrass (*Trifolium pratense:Lolium perenne*) (50:50) second cut sward was harvested, slightly wilted

to a DM concentration of 265 g kg^{-1} and chopped by a forage harvester under farm conditions to ≈3 cm chop length. Five samples of chopped forage were dried for 24 h at 67°C and, thereafter, analysed for chemical composition. Crude protein and soluble carbohydrate concentrations were 174.4 and 89.5 g kg^{-1} DM. Red clover:ryegrass was ensiled in 3-L glass jars at a density from 703 to 739 g L^{-1}. The experiment had 6 treatments with 5 micro-silos per treatment: T1 = uninoculated control; P1 = homo-and heterofermentative LAB – *L. plantarum* DSM 16568 (20 %), *E. faecium* NCIMB 11181 (30%), *L. buchneri* (CCM 1819) (50%) – 2.5×10^5 cfu g^{-1} of forage; P2 = homofermentative LAB – *L. plantarum* DSM 16568 (30%), *E. faecium* NCIMB 11181 (40%), *L. lactis* DSM 11037 (30%) – 3.0×10^5 cfu g^{-1} of forage, plus sodium benzoate, at 400 g t^{-1} forage; P3= homofermentative LAB – *E. faecium* NCIMB 11181 (30%), *L. lactis* NCIMB 30117 (30%), *L. plantarum* DSM 16568 (40%) – 1.5×10^5 cfu g^{-1} of forage; P4 = homofermentative LAB – *E. faecium* NCIMB 11181 (30%), *L. lactis* NCIMB 30117 (30%), *L. plantarum* DSM 16568 (40%) – 2.5×10^5 cfu g^{-1} of forage, plus Xylanase, at 0.5 HEC (hydroxy-ethylcellulose) g^{-1} forage; P5= homofermentative LAB – *E. faecium* NCIMB 11181(30 %), *L. lactis* NCIMB 30117 (30%), *L. plantarum* DSM 16568 (40%) – 2.5×10^5 cfu g^{-1} of forage, plus Xylanase, at 0.5 HEC g^{-1} forage, plus sodium nitrate, at 1.3 kg t^{-1} forage.
The LAB strains were provided by Chr. Hansen A/S. All inoculants were applied by spraying them as a water solution on the chopped crop. All inoculant solutions were analysed for LAB counts to document the correct inclusion (target +/–30%). All silage samples were analysed for DM, water soluble carbohydrates (WSC), pH, ammonia-N, lactic acid, fatty acids and ethanol using standard methods. Yeasts and molds were enumerated in accordance ISO 7954:1987(E) and *Clostridium perfringens* – ISO 7937:2004. Aerobic stability was measured in the laboratory by monitoring changes of temperature in 1000-g silage samples placed in boxes in aerobic conditions.
Silage composition data were subjected to one-way analysis of variance for a 6 (additive) factorial arrangement of treatments within a completely randomized design, using Proc GLM of SAS (SAS, 2000). Aerobic stability data for each herbage type were analysed separately by one-way analysis of variance. Significance was declared at $P< 0.05$.

Results and discussion

Inoculation resulted in higher ($P<0.05$) DM concentrations in all examined silages in comparison with the control (Table 1). The final pH and DM loss was lowest in silages inoculated with homofermentative LAB in combination with xylanase (P4) and silages inoculated with homofermentative LAB in combination with xylanase and sodium nitrate (P5). Among the five products used for inoculation there were significant differences in fermentation products. Silages inoculated with the heterofermentative *L. buchneri* plus homofermentative LAB (P1) had significantly higher pH and propionic acid concentration, significantly lower lactic acid concentration and lactic to acetic acid ratio when compared with other inoculants used in the present experiment. The reduction in the lactic acid with *L. buchneri* was reported by Oude Elferink *et al.* (2001). Among inoculated silages the largest ($P<0.05$) increase of lactic acid was observed in the silages treated with products P3, P4 and P5, and the highest ($P<0.05$) increase in acetic acid was detected in the silages treated with inoculants P1, P2 and P4. Silages inoculated with P4 had a lower ammonia-N concentration ($P<0.05$) but higher ($P<0.05$) alcohol concentration than silages treated with other inoculants. Inoculated silages had significantly lower ($P<0.05$) yeasts and mould count than uninoculated silage, whereas no differences were observed between the products used for silage inoculation.
Inoculant P1 increased silage aerobic stability dramatically, inoculant P2 increased ($P<0.05$) aerobic stability by 174 h (7 days), inoculants P3, P4, and P5 increased ($P<0.05$) aerobic

stability by 66 h (2.7 days) in comparison with untreated silages. Some inoculant lactic acid bacteria strains produce anti-microbioal compounds that inhibit mould and yeast growth or undesirable bacterial species (Gollop *et al.*, 2005). A beneficial effect of sodium benzoate on silage quality was demonstrated by Kleinschmit *et al.* (2005). Jaakkola *et al.* (2010) observed the combination of *L. plantarum* with sodium benzoate to be more efficient than the combination of *L. plantarum* and *L. buchneri*.

Table 1. Fermentation quality of the red clover-ryegrass silages and ensiling losses after 90 days of ensiling.

	T1	P1	P2	P3	P4	P5	$LSD_{0.05}$
DM§, g kg^{-1}	238^{c}	249^{b}	$251^{b,a}$	249^{b}	254^{a}	$252^{b,a}$	3.172
DM loss§, %	12.3^{a}	6.7^{b}	$5.7^{c,b}$	$6.4^{c,b}$	5.0^{c}	$5.5^{c,b}$	1.517
Ammonia-N, % of total N	9.2^{a}	5.1^{b}	4.4^{c}	$4.6^{c,b}$	3.7^{d}	$4.2^{c,d}$	0.616
pH	5.55^{a}	4.71^{b}	4.35^{c}	4.32^{c}	4.23^{d}	4.26^{d}	0.042
Lactic acid, g kg^{-1} DM	13.9^{d}	37.7^{c}	57.1^{b}	$62.6^{b,a}$	$63.7^{b,a}$	71.0^{a}	9.913
Acetic acid, g kg^{-1} DM	18.2^{b}	32.1^{a}	32.6^{a}	23.7^{b}	31.0^{a}	20.4^{b}	6.792
Butyric acid, g kg^{-1} DM	37.5^{a}	1.8^{b}	0.3^{b}	0.9^{b}	0.4^{b}	0.2^{b}	2.334
Propionic acid, g kg^{-1} DM	0.7^{b}	1.9^{a}	$0.3^{c,b}$	$0.4^{c,b}$	$0.3^{c,b}$	0.2^{c}	0.449
Alcohols, g kg^{-1} DM	14.7^{a}	$5.7^{c,b}$	$3.8^{c,d}$	$5.0^{c,b,d}$	6.1^{b}	3.1^{d}	1.951
Clostridia spores, log cfu g^{-1} FM	0.99	0.99	0.99	0.99	0.99	0.99	0.004
Yeast, log cfu g^{-1} FM	3.18^{a}	1.31^{b}	1.36^{b}	1.51^{b}	1.62^{b}	1.54^{b}	0.614
Moulds, log cfu g^{-1} FM	3.00^{a}	1.30^{b}	1.40^{b}	1.44^{b}	1.58^{b}	1.42^{b}	0.391
Aerobic stability, h	192^{d}	$>450^{a}$	366^{b}	258^{c}	$258^{c,b}$	258^{c}	4.221

$t_{0.05} = 2.086$; Error df=20

§Dry matter and calculated dry matter losses are corrected for volatiles

a, b, c, d, e – Means with different superscript letters in a line indicate significant differences of $P<0.05$

Conclusions

All inoculants had a positive effect on red clover-ryegrass silage characteristics in terms of lower pH and DM loss and shifting fermentation towards lactate with homofermentative LAB and homofermentative LAB in combination with xylanase, and toward acetate with *L. buchneri* or sodium benzoate. Inoculation with homofermentative LAB in combination with *L. buchneri* or sodium benzoate had a great effect on aerobic stability of slightly wilted red clover-ryegrass silages. Inoculation with homofermentative LAB in combination with xylanase resulted in lower protein degradation as evidenced by lower ammonia concentrations and lower DM loss.

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Preservation of moist hay with a preservative

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Abstract

In Switzerland, some farms are using chemical preservatives in order to preserve moist hay. Detailed knowledge of the hay dry matter (DM) content as well as the correct dosage of the preservative is important for a successful conservation procedure. In a trial, the efficacy of a preservative containing buffered propionic acid was investigated in hays of two different DM contents (69.2 and 74.2% DM) and three different dosages of preservative per DM-content. The hay with the lower DM-content received applications of 8, 9 and 10 L t^{-1} and the higher DM-content hay received 4, 5 and 6 L t^{-1}. Variants without additives were tested as negative controls. Temperature was continuously controlled during a 30-day period, and the DM contents and different parameters were analysed before and after this period. For hays of both initial DM levels, the untreated hay heated strongly and after 30 days it was totally mouldy. For the 69% DM hay, the heating up and the deterioration of the quality could be totally prevented with the addition of the preservative, and this did not depend on the dose rate. By contrast, in the hay with 74.2% DM, the dosage of 4 L could not prevent spoilage. In conclusion, it can be stated that the correct dosage is important for the success.

Keywords: moist hay, preservatives, dosage, spoilage

Introduction

At harvest, field-dried hay is not always dry enough for storage without problems. Mould growth and/or excessive heating are the consequences. With the use of preservatives, damage to the hay can be prevented. The right dosage is critical point for success. To determine the effect of preservative and dose rate on hays of two initially different dry matter contents, a chemical preservative was therefore tested in comparison with untreated hays, as negative controls without preservative, at a laboratory scale.

Materials and methods

Hay of a fourth cut was moistened to two different dry matter contents (69.2 and 74.2% DM). The investigated product, containing buffered propionic acid, was applied at three different dosages per DM-content: 8, 9, and 10 L t^{-1} were applied to the hay with the lower DM-content, and 4, 5 and 6 L t^{-1} to the hay with the higher DM-content. As controls, variants without additives were tested. Each variant was repeated three times. The tests were carried out in a laboratory-scale container (Meisser, 2001). The treated hay was filled into PVC containers with a capacity of 2906 cm^3 (diameter 10 cm) and a compaction of 200 kg fresh weight m^{-3}. Each container was fitted with a temperature probe. During the storage period of 30 days the temperatures were measured and recorded every 30 minutes. In the hay at the beginning, and after 30 days of storage, the DM contents, as well as various chemical parameters were determined. The crude nutrients were determined by NIRS. Statistical analysis was performed using analysis of variance and Bonferroni test (program SYSTAT 12).

Results and discussion

At both dry matter levels, the variants without preservative heated up rapidly and strongly. In the hay with 69% DM all three tested doses prevented the heating. In the hay with 74% DM only the 6-L treatment did not heat up.

After the storage period, the DM-contents in the 69% DM hay were significantly different between the treated and the untreated variants, and different to the DM% at the beginning (Table 1). Deterioration of the forage caused production of water. In the hay with the higher (74%) DM-content, differences were also found between the untreated and treated variants (Table 2). With the preservative, there was a reduction in DM content of the moist hay during the storage period, for both the 69% DM and 74% DM hays. Concerning the nutrient contents, significant differences were also found between the treated variants and the negative controls (Tables 1 and 2). The concentration of acid detergent insoluble N (ADIN) of total N, which is an important parameter for heat damage, was significantly higher in the negative control compared to the treated variants. Weiss *et al.* (1992) found that the digestibility of crude protein decreased with increasing concentration of acid detergent insoluble N of total N.

Table 1. Chemical parameters in the moist hay with 69% DM after the 30-day storage period.

		Without preservative	Preservative 8 L	9 L	10 L	SE	Significance
DM content	%	57.1^{a}	73.1^{b}	73.3^{b}	73.5^{b}	1.04	***
Ash	g kg^{-1} DM	198^{a}	106^{c}	108^{bc}	113^{b}	1.1	***
Crude protein	g kg^{-1} DM	284^{a}	185^{b}	186^{b}	187^{b}	1.3	***
Crude fibre	g kg^{-1} DM	240	237	237	238	1.5	ns
Sugar	g kg^{-1} DM	0^{a}	93^{b}	98^{b}	97^{b}	1.3	***
ADF	g kg^{-1} DM	287^{a}	274^{b}	272^{b}	275^{b}	2.2	**
NDF	g kg^{-1} DM	500^{a}	482^{b}	474^{b}	487^{b}	3.7	**
ADIN	% of total N	30.5^{a}	4.2^{b}	4.2^{b}	4.3^{b}	0.69	***
Lactic acid	g kg^{-1} DM	0.0^{a}	7.1^{b}	7.5^{bc}	7.7^{c}	0.10	***
Acetic acid	g kg^{-1} DM	0.0^{a}	0.8^{b}	0.9^{b}	0.9^{b}	0.02	***
Propionic acid	g kg^{-1} DM	0.0^{a}	5.7^{b}	6.3^{b}	7.0^{c}	0.13	***
DM-losses	%	36.3^{a}	1.1^{b}	0.9^{b}	-0.6^{b}	1.25	***

SE: standard error; *: P<0.05; **: P<0.01; ***: P<0.001; ns: non significant

Table 2. Chemical parameters in the moist hay with 74% DM after the 30-day storage period.

		Without preservative	Preservative 4 l	5 l	6 l	SE	Significance
DM content	%	74.4^{a}	79.5^{b}	79.8^{b}	80.6^{b}	0.61	***
Ash	g kg^{-1} DM	126^{a}	108^{b}	111^{ab}	109^{b}	3.4	*
Crude protein	g kg^{-1} DM	224^{a}	191^{b}	187^{b}	188^{b}	1.8	***
Crude fiber	g kg^{-1} DM	281^{a}	257^{b}	251^{b}	242^{b}	3.9	***
Sugar	g kg^{-1} DM	11^{a}	66^{b}	80^{b}	89^{b}	7.4	***
ADF	g kg^{-1} DM	334^{a}	299^{b}	279^{bc}	265^{c}	5.4	***
NDF	g kg^{-1} DM	557^{a}	505^{b}	495^{b}	480^{b}	7.4	***
ADIN	% of total N	9.9^{a}	4.9^{b}	4.1^{b}	3.9^{b}	0.29	***
Lactic acid	g kg^{-1} DM	0.0^{a}	4.5^{b}	7.2^{bc}	7.5^{c}	0.60	***
Acetic acid	g kg^{-1} DM	0.0^{a}	0.1^{a}	0.5^{ab}	0.7^{b}	0.13	*
Propionic acid	g kg^{-1} DM	0.0^{a}	1.1^{ab}	2.6^{bc}	3.5^{c}	0.39	**
DM-losses	%	13.2^{a}	2.7^{b}	-1.9^{b}	-1.1^{b}	1.1	***

SE: standard error; *: P<0.05; **: P<0.01; ***: P<0.001; ns: non significant

There were significant effects on the sugar content. In the untreated variants the sugar was degraded strongly (in the 74% DM hay) and completely (in the 69% DM hay). In addition, the fermentation acids were determined. For both DM-levels, in the negative controls without preservative neither lactic, nor acetic or propionic acids were found (Tables 1 and 2). With increasing doses of the preservative, more propionic acid was found.

The DM content and the addition of the preservative had a strong impact on the DM losses, although the dosage of the preservative did not significantly affect the DM losses (Tables 1 and 2).

After the 30-day storage period, the untreated moist hay of both DM levels was totally mouldy and had a strong odour of ammonia. In the moist hay with the initial 69% DM, and for all three dose rates of 8, 9 and 10 L t^{-1}, no or only small amounts of moulds were detected. In the moist hay with the initial 74% DM and the doses of 4 L t^{-1}, some parts with moulds were detected, although in the two variants with 5 and 6 L t^{-1} of additive, only small mouldy parts were found. The additional analyses of moulds confirmed the visual evaluation. In the moist hay with 69% DM all three doses showed values under 100,000 CFU g^{-1}. For the moist hay with 74% DM, this was only the case in the variant with 6 L (Figure 1).

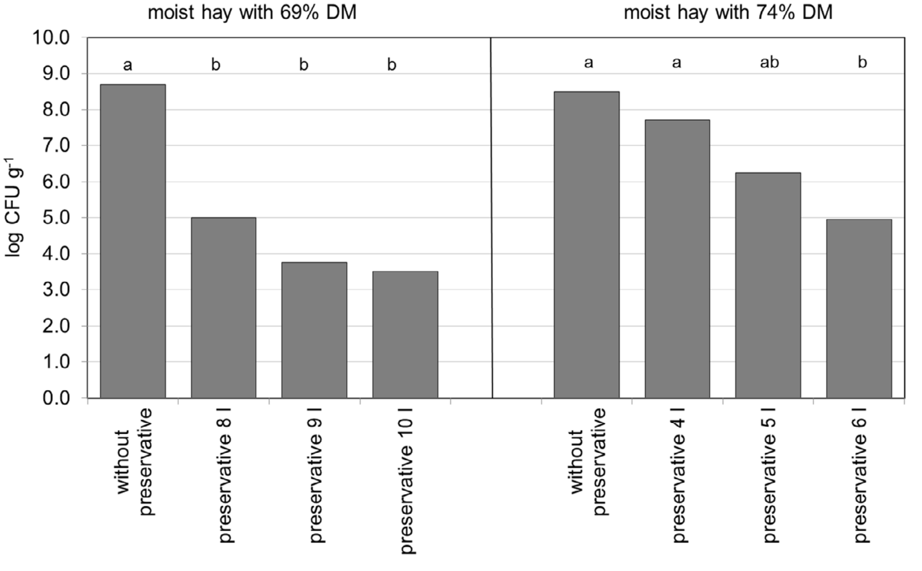

Figure 1. Mould contents in the moist hay with two DM-contents and untreated and treated variants (CFU: Colony Forming Units).

Conclusions

1. Untreated moist hay is not stable during the storage period. It heats up and gets mouldy.
2. The investigated preservative for moist hay could prevent the heating and forage spoilage, either partially or completely. The correct dosage is important for success.

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Ensilage of a meadow sward with a large proportion of red clover

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Abstract

Studies were carried out in 2009 on a permanent meadow, part of which was undersown with tetraploid red clover (*Trifolium pratense* L.). The aim of the study was to assess the suitability for ensilage of a meadow sward with a large (30%) proportion of red clover. Experimental plots were cut three times per year and pre-wilted herbage was ensiled in big bales. Herbage from the undersown part was ensiled with and without addition of inoculate containing lactic acid bacteria. The quality and nutritive value of silages were compared with silages obtained from the non-undersown part of the meadow. Beneficial changes in the chemical composition of the herbage as a result of undersowing were most evident at the third cut. Quality deterioration of silage from the sward with a large proportion of red clover was observed. The addition of bacterial inoculants improved some quality parameters but only in first-cut silages. The nutritive value of silages from the undersown part of the meadow was higher.

Keywords: bacterial inoculate, big bale silage, fermentation quality, nutritive value, red clover

Introduction

Increasing the proportion of legumes in a sward by undersowing leads to increased yield and protein content in the forage. Large proportion of legumes in the sward, amounting to over 30%, usually increases the content of protein in the sward thus reducing the suitability of the sward for ensilage (Barszczewski *et al.,* 2011). This is mainly due to lower concentration of water-soluble carbohydrates, higher buffering capacity and lower dry matter content at harvesting (McDonald *et al.,* 1991). Pre-wilting of mown herbage from swards with large proportion of legumes and the use of biological additives with LAB (Shurkhno *et al*., 2005; Krawutschke *et al.,* 2010) improve fermentation and make it possible to obtain good quality silage with increased content of crude protein. The aim of the study was to assess the suitability for ensilage of a sward with a large (30%) proportion of red clover.

Materials and methods

Studies were carried out in 2009 in a plot experiment that had been established in 2006 on a permanent meadow (0.6 ha) situated on mineral soil at the Experimental Farm in Falenty. The dominant grass species in the sward were: *Poa pratensis* L., *Alopecurus pratensis* L., *Bromus erectus* P.B., *Festuca pratensis* Huds. and *Dactylis glomerata* L. Part of the experimental meadow was undersown with tetraploid red clover (*Trifolium pratense* L.) cv. Bona at 8 kg ha^{-1} by direct sowing into the sward in spring 2006. The entire meadow surface was fertilised with solid manure (20% DM) applied in autumn 2008, after 6 months of storage, at a rate of 22 t ha^{-1}. The meadow was cut three times per year: first cut at full heading of dominant grass species; and second and third cuts at nine-week intervals thereafter. Mown grass from all three cuts was pre-wilted on the meadow surface, then collected and ensiled in big bales (about 400 kg/bale). Herbage from the undersown part was ensiled as either untreated (treatment RC) or with bacterial inoculate (treatment RC + LAB) containing *Lactobacillus plantarum, L. brevis, L. buchneri* and two enzymes (three big bales per treatment). Herbage from the non-undersown part of the meadow was ensiled without any additives (Control). In

November, silage sampling was performed with a 5-cm diameter stainless steel corer. Five horizontal cores of 40 cm depth were extracted from each bale. Quality of obtained silages was compared with silages from the non-undersown part of the meadow. Silage samples were analysed for dry matter (DM) (oven dried at 105°C) and pH of fresh mass (potentiometric method), lactic acid (LA), volatile fatty acids (VFA) and ammonia. The contents of crude protein, WSC, crude ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined in fresh herbage and silages. Analyses were made by near infrared spectroscopy (NIRS) using a NIRFlex N-500 spectrophotometer using calibrations (INGOT^R) prepared for dry forages and wet fermented forages. Nutritive value of silage was expressed as an index of relative forage quality (RFQ) (Undersander and Moore, 2002). In addition, total number of *Enterobacteriaceae*, yeasts and moulds (cultures on PetrifilmTM 3M plates) in fresh silage samples (FM) were determined. Data concerning the chemical and biological composition of silage were analysed using analysis of variance in ANOVA 1. Differences between treatments within the subsequent cuts were tested using the Tukey's HDS test (α=0.05).

Results and discussion

Meadow undersowing resulted in an increased amount of red clover in the sward, which in 2009 was 30%, as compared with only 4% on the non-undersown (control) part of the meadow. The beneficial changes in the chemical composition of the herbage resulting from the introduction of red clover to the sward were the most evident in herbage in the 3rd cut. An increase of crude protein and OMD, and decrease of crude ash, NDF and ADF were observed. A significant increase of water-soluble sugar content was also observed: this can be explained by the presence of *Lolium multiflorum* L., which spontaneously appeared in the meadow sward. Thus the carbohydrate-to-protein ratio was at a level that guaranteed a proper course of the fermentation process. There were similar tendencies in the herbage in the 2nd cut. In the herbage in the 1st cut, the beneficial effect of undersowing on chemical composition of the herbage and its usefulness for ensilage were less evident (Table 1).

Table 1. The chemical composition of the meadow sward.

	1st cut		2nd cut		3rd cut	
	Control	RC	Control	RC	Control	RC
Crude protein (g kg^{-1} DM)	137.2	137.9	114.4^a	126.8^b	107.6^a	132.1^b
Crude ash (g kg^{-1} DM)	85.8	86.3	94.4	91.8	114.5^b	93.4^a
WSC (g kg^{-1} DM)	127.0^b	101.9^a	8.08	9.62	100.6^a	141.3^b
NDF (g kg^{-1} DM)	463.0^a	512.1^b	525.4	505.9	518.4^b	463.0^a
ADF (g kg^{-1} DM)	317.6^a	343.0^b	370.3	360.7	349.6^b	311.1^a
OMD	57.3^a	50.7^b	45.2	45.6	52.5^a	57.9^b
WSC/crude protein ratio	0.94	0.75	0.71	0.77	0.94	1.08

OMD – Organic matter digestibility; Values with different letters within the same cut are significantly different (P<0.05)

First- and third-cut silages from the meadow area undersown with red clover had significantly lower DM content than silages from the non-undersown (control) area. Therefore, herbage with increased content of legumes needs more time to reach optimal DM content before ensilage. Quality deterioration of silage from the sward with a large proportion of red clover (treatment RC), shown by high ammonia concentration and increased content of VFA, was observed in silages in the 1st and 3rd cuts. The addition of bacterial inoculate containing LAB was the most effective in silage at the first cutting date. Compared with silages made without bacterial inoculate (treatment RC), they had lower pH level and higher lactic acid content. In the remaining two cuts the differences between treatments RC and RC + LAB were not statistically significant. Increased proportion of red clover in the sward improved the nutritive value of most feeds with exception those in the first cut. Compared with the control, the silages from the sward with increased content of red clover (RC and RC+LAB) had lower NDF and ADF content, and showed higher values of TDN, DMI and RFQ (Table 2).

Table 2. Fermentation quality, nutritive value and microflora of silage.

	1st cut			2nd cut			3rd cut		
	Control	RC	RC + LAB	Control	RC	RC + LAB	Control	RC	RC + LAB
DM (g kg^{-1})	550.0^{b}	399.2^{a}	427.5^{a}	429.2	398.0	421.3	550.0^{b}	420.6^{a}	389.0^{a}
pH	4.31^{ab}	4.52^{b}	4.14^{a}	4.93	4.58	4.47	5.29^{b}	4.41^{a}	4.46^{a}
NH_3-N (g kg^{-1} total N)	105.0	111.9	98.4	117.5^{ab}	124.4^{b}	100^{a}	71.8^{a}	129.3^{b}	110.1^{b}
LA (g kg^{-1} DM)	36.7^{ab}	33.1^{a}	46.4^{b}	13.7^{a}	33.6^{b}	31.7^{b}	22.8	31.8	44.1
VFA (g kg^{-1} DM)	24.3^{a}	49.4^{b}	44.7^{b}	46.8	48.2	46.3	24.2^{a}	38.4^{b}	36.3^{b}
Sum of FP (g kg^{-1} DM)	60.9^{a}	82.4^{b}	91.06^{b}	60.45^{a}	81.84^{b}	78.02^{ab}	46.92^{a}	70.19^{ab}	80.36^{b}
Proportion of LA in the sum of FP (%)	60.1 ^{b}	40.0^{a}	50.8^{b}	20.7^{a}	40.2^{b}	40.3^{b}	45.9	43.0	52.3
CP (g kg^{-1} DM)	149.9	152.9	155.2	148.7	145.7	147.9	144.7	138.8	139.0
Crude ash (g kg^{-1} DM)	74.7	73.0	80.6	82.9	79.3	82.0	82.4^{b}	68.5^{a}	74.1^{ab}
Crude fat (g kg^{-1} DM)	39.7	37.7	39.7	35.1	34.7	35.8	34.8^{b}	31.5^{a}	32.6^{ab}
WSC (g kg^{-1} DM)	76.5^{b}	82.2^{b}	65.3^{a}	85.1	79.2	77.7	100.1^{b}	89.7^{ab}	78.0^{a}
NDF (g kg^{-1} DM)	464.9^{a}	496.2^{b}	462.0^{a}	526.0	497.3	504.2	522.7^{b}	475.5^{a}	464.8^{a}
ADF (g kg^{-1} DM)	310.9^{ab}	318.2^{b}	305.6^{a}	334.2^{b}	318.6^{a}	328.0^{ab}	332.4^{b}	306.9^{a}	304.5^{a}
TDN (% DM)	64.6^{b}	63.3^{a}	64.0^{ab}	61.0^{a}	62.4^{b}	62.0^{ab}	61.2^{a}	63.9^{b}	63.9^{b}
DMI (% body weight)	3.02^{b}	2.91^{a}	2.97^{ab}	2.78^{a}	2.89^{b}	2.85^{ab}	2.80^{a}	2.99^{b}	3.00^{b}
RFQ	158^{b}	150^{a}	155^{ab}	138^{a}	146^{b}	143^{ab}	139^{a}	155^{b}	156^{b}
Enterobacteria (log_{10} cfu g^{-1} FM)	1.00	2.72	2.69	3.08	3.30	1.40	3.55a	5.85b	5.89b
Yeasts (log_{10} cfu g^{-1} FM)	1.72^{b}	1.09^{a}	1.41^{ab}	2.31	2.28	2.39	2.27	2.24	2.60
Moulds (log_{10} cfu g^{-1} FM)	1.80^{a}	3.30^{ab}	5.56^{b}	3.13	4.39	3.00	3.71	3.26	2.72

DM – dry matter; LA – lactic acid; VFA – volatile fatty acids (acetic + butyric + propionic acid); CP – crude protein; FP – fermentation products; WSC – water soluble carbohydrates; NDF – neutral detergent fiber; ADF – acid detergent fiber; TDN – total digestible nutrients; DMI – dry matter intake; RFQ – Relative Forage Quality = DMI * TDN/1.23; Values with different letters within the same cut are significantly different (P<0.05)

Conclusions

The beneficial changes in the herbage chemical composition, resulting from the increased contribution of red clover to 30% as a result of undersowing, were most evident in herbage in the third cut. Quality deterioration of silage from the sward with a large proportion of red clover was observed. Despite worse indices of chemical assessment, silage made from the sward with a high content of red clover had higher nutritive value than silages from the non-undersown part of meadow. The addition of bacterial inoculants improved some quality parameters but only in silages in the first cut.

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Yield, nitrogen and mineral content of chicory compared with perennial ryegrass, red clover and white clover over two harvest years

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Abstract

To investigate differences in the dry matter (DM) yield, nitrogen and mineral composition of forage species, pure swards of chicory (*Cichorium intybus*), perennial ryegrass (*Lolium perenne*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) were established in June 2009, with four replicate plots (12×7.5 m) of each species. Ryegrass and chicory treatments received inorganic nitrogen at 200 kg N ha^{-1} yr^{-1}. During the first and second harvest years, each plot was harvested and sub-sampled to assess DM yield and composition. Chicory contained higher concentrations of N and major minerals (except Na) when compared with ryegrass in the second harvest year. Differences in composition between chicory and either red or white clover were more variable, for example, chicory had higher P and K than red clover but similar concentrations to white clover.

Keywords: chicory, *Cichorium intybus*, mineral composition

Introduction

Comparisons of data from the 1930s and recent times show that modern agricultural products have reduced levels of micro-nutrients. For example, magnesium (Mg), which is essential for human health, has declined in meat and milk products by 10 and 25%, respectively (Thomas, 2007). Some alternative forages have been found to contain higher concentrations of essential nutrients than conventionally grown ryegrass (Fisher and Baker, 1996). Their use may help to develop more sustainable approaches to the management of nutrients within soil-plant-animal interactions, reducing the use of mineral fertilizers or feed additives to provide these nutrients for livestock. In particular, chicory (*Cichorium intybus*) possesses a deep taproot with the capacity to 'mine' deep soil resources that are inaccessible to most crop plants. Research has shown that chicory grown within mixed swards had a higher mineral content than some other forages (Belesky *et al*., 2001; Harrington *et al*, 2006). This experiment investigated the DM yield, nitrogen and mineral composition of a pure sward of chicory, compared with pure swards of other forage species, over two harvest years.

Materials and methods

Chicory (*Cichorium intybus*) (cv. Puna II), perennial ryegrass (PRG) (*Lolium perenne*) (cv. Premium), red clover (*Trifolium pratense*) (cv. Merviot) and white clover (*Trifolium repens*) (cv. Aberdai) were established as monocultures on 29 June 2009. Replicate plots (12×7.5 m) were established in a randomized block design with four blocks. Ryegrass and chicory plots received inorganic nitrogen at 200 kg N ha^{-1} yr^{-1}. During the first and second harvest years (2010 and 2011), the central 12×1.5 m strip of each plot was cut using a Haldrup plot harvester, to a height of 8 cm, on 5 occasions each year. A 100 g sub-sample of the bulked material from each cut was analysed to determine DM, N concentration (using a Leco FP 428 N analyser) and mineral composition. In 2010, plots were harvested at 4-week intervals, on 20 May, 16 June, 12 July, 10 August and 21 September. In 2011, plots were harvested on 5 May, 6 June, 27 June, 1 July, 27 July and 14 September. Calcium (Ca) data were transformed to 1/x to normalize data prior to analysis. Data were analysed by ANOVA over time using Genstat® 11.1. Multiple comparisons were based on Bonferroni adjusted LSDs.

Results and discussion

There were differences between forage species for DM yield, N content and all major minerals (Table 1). Red clover had a higher DM yield than all species except chicory. Chicory had a higher DM yield than PRG, which was higher than white clover. N concentration of chicory over two harvest years was higher than for PRG, but lower than red or white clover. There is disparity in the literature regarding N concentrations of chicory compared with ryegrass: Harrington *et al*. (2006) showed chicory had higher N than PRG, as found here, but other studies show N in chicory to be lower than in PRG (Rumball, 1986). Whilst N concentrations in chicory increase with incremental applications of inorganic N (Belesky *et al*., 1995), in this study both non-legume forages received the same N application suggesting a more efficient use of N by chicory. Chicory had a higher Ca concentration than PRG, but a lower concentration than red or white clover, except in the second harvest year for white clover. Chicory had higher phosphorus (P) and potassium (K) concentrations than either PRG or red clover but similar concentrations to those in white clover. Mg concentrations in chicory were higher than all other forages in the second harvest year, but in the first year Mg was higher in chicory than PRG and white clover, but similar to red clover. Chicory had a higher sodium (Na) concentration compared to red clover, in both harvest years, but a lower Na concentration than PRG in the first harvest year. In contrast to the findings of van Eekeren *et al.* (2006), the concentration of sulphur (S) in chicory was higher than in PRG in the second harvest year only. Differences between studies may be due to forages here being grown in pure swards whereas other studies are based on mixed swards. Concentrations of Ca, P, Mg, S and K in chicory were higher in the second year than in the first harvest year. Similarly, there were higher P, K and S levels in PRG and white clover in the second harvest year than the first year. Na concentrations of PRG decreased between the first and second harvest years.

Conclusions

In this study, chicory contained higher concentrations of N and most major minerals (except Na) compared with perennial ryegrass in the second harvest year, whereas differences in N and mineral composition between chicory and either red or white clover were more variable.

Acknowledgements

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Table 1. Mean DM yield, N concentration and mineral composition of chicory, perennial ryegrass, red and white clover over two harvest years.

	Treatment						s.e.m.		Prob.		
	Year	Chicory	Perennial Ryegrass	Red Clover	White Clover	*Mean*	Rows	Columns	Trt	Year	Trt. × Year
Yield	2010	7429	6984	9486	4122	*7005*	567.9	501.4	<0.001	0.107	0.230
(kg DM ha^{-1})	2011	7786	7446	9237	6023	*7623*	19df	12df			
	Mean	*7607*^{bc}	*7215*^{b}	*9362*^{c}	*5072*^{a}						
Nitrogen	2010	25.4	23.2	40.3	43.6	*33.1*	0.92	0.97	<0.001	0.546	0.050
(g kg^{-1} DM)	2011	28.3	23.3	37.3	45.4	*33.6*	21df	12df			
	Mean	*26.8*^{b}	*23.2*^{a}	*38.8*^{c}	*44.5*^{d}						
Calcium$^{\#}$	2010	11.1^{bA}	7.2^{a}	18.7^{d}	16.4^{c}	*13.4*	0.42	0.33	<0.001	<0.001	<0.001
(g kg^{-1} DM)	2011	15.6^{bB}	8.0^{a}	18.1^{c}	15.8^{b}	*14.4*	16df	12df			
	Mean	*13.4*	*7.6*	*18.4*	*16.1*						
Phosphorus	2010	3.1^{cA}	2.2^{aA}	2.6^{b}	3.2^{cA}	*2.8*	0.08	0.08	<0.001	<0.001	0.030
(g kg^{-1} DM)	2011	3.7^{bB}	2.9^{aB}	2.8^{a}	3.7^{bB}	*3.2*	21df	12df			
	Mean	*3.4*	*2.6*	*2.7*	*3.5*						
Magnesium	2010	3.6^{bA}	2.1^{a}	4.1^{b}	2.7^{a}	*3.1*	0.12	0.09	<0.001	<0.001	<0.001
(g kg^{-1} DM)	2011	5.1^{cB}	2.5^{a}	4.2^{b}	2.8^{a}	*3.6*	15df	12df			
	Mean	*4.4*	*2.3*	*4.2*	*2.7*						
Potassium	2010	12.8	6.8	8.5	9.9	*9.5*^{A}	1.16	1.15	0.002	<0.001	0.911
(g kg^{-1} DM)	2011	20.1	13.1	14.2	15.9	*15.8*^{B}	20df	12df			
	Mean	*16.5*^{b}	*9.9*^{a}	*11.4*^{a}	*12.9*^{ab}						
Sodium	2010	3.48^{b}	4.74^{cB}	2.64^{a}	3.31^{ab}	*3.54*	0.148	0.164	<0.001	0.002	0.015
(g kg^{-1} DM)	2011	3.37^{b}	3.59^{bA}	2.04^{a}	3.32^{b}	*3.08*	21df	12df			
	Mean	*3.43*	*4.16*	*2.34*	*3.32*						
Sulphur	2010	1.66^{abA}	1.38^{aA}	1.54^{ab}	1.73^{bA}	*1.58*	0.061	0.055	<0.001	<0.001	0.043
(g kg^{-1} DM)	2011	2.19^{cB}	1.80^{abB}	1.72^{a}	2.07^{bcB}	*1.94*	19df	12df			
	Mean	*1.92*	*1.59*	*1.63*	*1.90*						

$^{\#}$ back transformed data

Within rows, treatment values with differing lower case superscript differ significantly ($P<0.05$)

Within column, year values with differing upper case superscript differ significantly ($P<0.05$)

Trace element content of chicory compared with perennial ryegrass, red clover and white clover over two harvest years

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Abstract

To investigate differences in the trace element concentrations of chicory (*Cichorium intybus*), perennial ryegrass (PRG) (*Lolium perenne*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) each forage was established as a pure sward. Four replicated plots (12×7.5 m) of each forage species were established in a randomized block design in June 2009. Ryegrass and chicory received inorganic N at 200 kg N ha^{-1} yr^{-1}. During the first and second harvest years, each plot was harvested and sub-sampled to assess the trace element composition of the forages over two harvest years. Overall, trace element concentrations in chicory, red clover and white clover were higher than in PRG, with the exception of Se and Mo. Chicory contained higher concentrations of Cu, Zn, Co than did white clover, but levels of I were similar, and white clover contained more Fe than other forages. Red clover and chicory had similar Mn, B, Co and Fe, but chicory had higher Zn, I and (in year 2 only) Cu.

Keywords: chicory, *Cichorium intybus*, trace element composition

Introduction

Currently, many livestock farms rely unsustainably on external inputs to maintain sufficient levels of essential micro-nutrients in livestock diets. Comparisons of data from the 1930s with data from recent times have shown that modern agricultural products have reduced levels of micro-nutrients. For example, copper has declined in meat and milk products by 60% and 90%, respectively (Thomas, 2007). Some species of alternative forages have been found to contain higher concentrations of essential nutrients than conventionally grown ryegrass (Fisher and Baker, 1996). Use of these species may help to develop more sustainable approaches to the management of essential macro- and micro-nutrient within soil-plant-animal interactions, reducing the need for mineral and trace element fertilizers or feed additives for livestock. In particular, chicory (*Cichorium intybus*) possesses a deep taproot with the potential to 'mine' deep soil resources inaccessible to most crop plants. This experiment investigated the trace element concentrations in herbage of chicory in comparison with other forages when sown as pure swards and measured over two harvest years.

Materials and methods

Chicory (*Cichorium intybus*) (cv. Puna II), perennial ryegrass (PRG) (*Lolium perenne*) (cv.Premium), red clover (*Trifolium pratense*) (cv. Merviot) and white clover (*Trifolium repens*) (cv. Aberdai) were established on 29 June 2009. Plots (12×7.5 m) were established in a randomized block design with four replicates. The ryegrass and chicory treatments both received inorganic nitrogen at a rate of 200 kg N ha^{-1} yr^{-1}. During the first and second harvest years (2010 and 2011), a 12×1.5 m strip was cut from each plot using a Haldrup plot harvester, to a height of 8 cm, on 5 occasions each year. In 2010, plots were harvested on the 20 May, 16 June, 12 July, 10 August and 21 September. In 2011 plots were harvested on the 5 May, 6 June, 27 June, 1 July, 27 July and 14 September. A 300 g subsample was oven dried to estimate dry matter (DM) yield, and a further 100 g sub-sample of the bulked material from each plot was freeze dried and submitted for chemical analysis [manganese (Mn), copper (Cu), zinc (Zn), boron (B), molybdenum (Mo), cobalt (Co), iodine (I), selenium (Se) iron (Fe)]. Zn data were transformed to 1/sqrt(x), and B and Se to $\log_{10}(x)$ to normalize data prior to analysis. Data were analysed by ANOVA as a split plot over time using Genstat® 11.1. Multiple comparisons were based on Bonferroni adjusted LSDs.

Results and discussion

Results of trace element concentrations are presented in Table 1. The Mn concentration of chicory was higher than white clover. In the first harvest year, Cu concentrations in chicory were higher than in PRG and white clover, but not red clover; in the second harvest year it was higher in chicory than in all other forages. In both years, chicory had a higher Zn concentration than all other forages. These findings are in agreement with van Eekeren *et al.* (2006) who found that chicory contained higher levels of Cu and Zn when grown in a mixed sward. Zn concentration was higher in red clover than in either PRG or white clover. B concentrations were similar amongst chicory, red clover and white clover, with PRG being lower than all other forages. There were no differences in Mo or Se concentrations among the forages. Chicory forage contained similar Co concentrations to red clover, but contained more Co than PRG or white clover. There was no difference in Co concentrations between red and white clover. The I concentration of chicory was similar to that of white clover and was higher than in both PRG and red clover. There were no Fe forage × year interactions but iron (Fe) concentrations of white clover were higher than other forages (data not in table: chicory = 83.6^{a} PRG = 68.7^{a} RC = 72.2^{a} WC = 100.2^{b}). In all forages there were higher concentrations of Fe, Mn and Co in the second year than in the first harvest year, whereas there were lower concentrations of both I and Se in the second harvest year. Chicory had higher Cu and lower Mo concentrations in the second harvest year than in the first year.

Conclusions

Overall, the trace element concentrations in forage of chicory, red clover and white clover were higher than in PRG, with the exception of Se and Mo which were present in similar concentrations in all forages. Concentrations of Cu, Zn, Co were higher in chicory than in white clover, but iodine levels were similar and white clover contained more iron than all other forages. Red clover and chicory had similar Mn, B, Co and Fe concentrations, but concentrations of Zn, I and Cu (the latter in the second harvest year only) were higher in chicory than in red clover.

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Table 1. Trace element composition (mg kg^{-1} DM) of chicory, perennial ryegrass, red clover and white clover over two harvest years.

	Treatment						s.e.m.		Prob		
	Year	Chicory	Perennial Ryegrass	Red Clover	White Clover	*Mean*	Rows	Columns	Trt	Year	Trt. ×Year
Manganese	2010	94	96	76	60	*82*	7.1	5.0	0.002	0.012	0.082
	2011	106	122	74	65	*92*	15df	12df			
	Mean	*100^{bc}*	*109^{c}*	*75^{ab}*	*63^{a}*						
Copper	2010	11.9^{cA}	5.3^{a}	12.5^{c}	9.0^{b}	*9.7*	0.50	0.36	<0.001	0.003	<0.001
	2011	15.1^{cB}	6.7^{a}	11.4^{b}	9.4^{b}	*10.6*	15df	12df			
	Mean	*13.5*	*6.0*	*12.0*	*9.2*						
Zinc[#]	2010	115^{c}	37^{a}	66^{bB}	34^{a}	*53*	0.004	0.002	<0.001	<0.001	0.016
	2011	112^{c}	33^{a}	49^{bA}	30^{a}	*45*	13df	12df			
	Mean	*114*	*35*	*56*	*32*						
Boron[#]	2010	14.9^{b}	4.0^{a}	17.9^{bB}	18.2^{b}	*11.9*	0.03	0.01	<0.001	0.053	<0.001
	2011	16.6^{b}	4.3^{a}	14.1^{bA}	16.4^{b}	*11.3*	11df	12df			
	Mean	*15.7*	*4.2*	*15.9*	*17.3*						
Molybdenum	2010	0.82^{B}	0.58	0.63	0.80	*0.71*	0.104	0.054	0.160	<0.001	0.041
	2011	0.42^{A}	0.32	0.42	0.76	*0.48*	12df	12df			
	Mean	*0.62*	*0.45*	*0.53*	*0.78*						
Cobalt	2010	0.082	0.040	0.071	0.046	*0.06*	0.0054	0.0037	<0.001	<0.001	0.059
	2011	0.112	0.05	0.08	0.06	*0.08*	14df	12df			
	Mean	*0.097^{c}*	*0.045^{a}*	*0.075^{bc}*	*0.053^{ab}*						
Iodine	2010	0.390	0.342	0.338	0.379	*0.362*	0.0287	0.0352	0.001	<0.001	0.362
	2011	0.305	0.128	0.191	0.254	*0.219*	18df	12df			
	Mean	*0.347^{c}*	*0.235^{a}*	*0.264^{ab}*	*0.316^{bc}*						
Selenium[#]	2010	0.043	0.028	0.034	0.041	*0.036^{B}*	0.094	0.093	0.288	0.004	0.266
	2011	0.030	0.021	0.023	0.014	*0.021^{A}*	20df	12df			
	Mean	*0.360*	*0.024*	*0.028*	*0.024*						

[#], back transformed data; Within rows, treatment values with differing lower case superscript differ significantly (P<0.05)
Within column, year values with differing upper case superscript differ significantly (P<0.05)

The effects of sowing chicory in different ratios with ryegrass, either with or without red clover, on forage yield and chemical composition

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Abstract

The effects of chicory (*Cichorium intybus*) (cv. Puna II) in sowing mixtures with perennial ryegrass (*Lolium perenne*) (cv. Premium) (PRG) or PRG with red clover (*Trifolium pratense*) (cv. Merviot) on the chemical composition of harvested forage was investigated. Field plots (10×2.5 m) were sown with chicory and ryegrass in differing proportions, and either with or without red clover, in a replicated randomized design with four blocks. Treatments consisted of chicory sown with PRG alone or a ryegrass-red clover mix, resulting in 10 combinations with chicory included as 0.9, 0.7, 0.5, 0.3 and 0.1. Forages were sown at rates corresponding to a proportion of their usual monoculture rate, not the total seed rate. A Haldrup plot harvester was used to determine total yield and sub-samples were taken for chemical analysis. Overall, when chicory was sown in combination with ryegrass only, there was a positive effect of increasing chicory in the sward on the DM and crude protein yield. However, when sowing chicory in combinations with PRG and RC, the highest DM, CP and WSC yields were found with increasing and decreasing proportions of red clover and chicory, respectively.

Keywords: chicory, *Cichorium intybus*, protein, mixed swards, sowing rates

Introduction

Including chicory in grass seed mixtures is becoming more popular, as it offers high yields of very palatable and nutritious fodder for grazing livestock. Research has previously found that, typically, the crude protein (CP) and water-soluble carbohydrates (WSC) concentrations of chicory are higher than perennial ryegrass (PRG) (*Lolium perenne)* but crude protein concentrations are lower than red clover (RC) (*Trifolium pratense*) (Li and Kemp, 2005). However, little is known about the effects of different sowing combinations of chicory when sown with these forage species on forage yield and chemical composition. This experiment determined the effects of sowing chicory in combination with PRG only or PRG with red clover on the yield and chemical composition of the harvested forage.

Materials and methods

Field plots (10×2.5 m) were sown with chicory (cv. Puna II) and perennial ryegrass (cv. Premium) in differing proportions, and either with or without red clover (cv. Merviot), in a replicated randomized block design with four replicate blocks. Forages were sown on 25 June 2009 at rates corresponding to a proportion of their usual monoculture rate, not the total seed rate (i.e. chicory at 6 kg ha^{-1}, PRG at 33 kg ha^{-1} and PRG-red clover mix at 22 + 11 kg ha^{-1}). The treatments consisted of chicory sown with PRG alone or a ryegrass-red clover mix, resulting in 10 combinations with chicory included as 0.9, 0.7, 0.5, 0.3 and 0.1. Each plot was sown using an Oyjord experimental plot drill before the area was harrowed using an Einbock harrow and rolled. The PRG-chicory plots received 50 kg N ha^{-1} during establishment. During the establishment year, plots were cut using a Haldrup plot harvester on 14 September and 19 October. During the first harvest year (2010), plots were mechanically harvested at 4-week intervals, on the 21 May, 17 June, 12 July, 10 August and 17 September. The PRG-chicory plots received inorganic N on 4 occasions: 77 kg, 61 kg, 30 kg and 28 kg of N on 23 March, 26 May, 22 June and 22 July 2010, respectively. During the second harvest year (2011), plots were mechanically harvested at approximately 4-week intervals, on the 4 May, 6 June, 27 June, 27 July, 2 Sept and 7 November 2011. The PRG-chicory plots received inorganic N on 4 occasions: 80 kg, 31 kg, 31 kg, 32 kg on 18 March, 11 May, 8 June and 11 July 2010. Total yield was determined by weighing the material cut from an area of 10×1.5 m within each plot. A sub-sample of the bulked material from each plot was submitted for chemical analysis to determine dry matter (DM), CP (total N × 6.25) and water-soluble carbohydrate (WSC) concentrations. A further 100 g sub-sample was taken, thoroughly mixed, and then separated into chicory, PRG, red clover, white clover, broad leaf weeds and grass weeds. Data were analysed using Genstat® 11.1 by polynomial contrast analysis to determine the effects of sowing ratios and by regression analysis to compare the effects of the percentage of chicory as recorded in sward.

Results and discussion

In the first harvest year there was a linear increase in DM content and yield of harvested forage as the proportion of chicory sown decreased, but there was no effect of including red clover (Table 1a). There was an increase in the yield of PRG as the ratio of ryegrass sown increased in plots either treated with inorganic N or sown with red clover and receiving no inorganic N. Chicory yield was lower when chicory was sown in combination with PRG and red clover (and not treated with artificial N) compared with being sown with PRG (and treated with artificial N). There was an interaction between the inclusion of red clover and the sowing ratio on the total forage protein yield. When chicory was sown with ryegrass, there was a positive linear increase in forage protein yield as the amount of chicory sown was increased. In contrast, when chicory was sown with ryegrass and red clover, the highest forage protein yield was when chicory was sown at 10% of its usual monoculture rate, which was mostly due to the effects of red clover. Sowing ratio changed the WSC yield, with a linear and quadratic effect on WSC of increasing the proportion of chicory sown in combination with ryegrass. Although including red clover in chicory-ryegrass swards reduced the overall WSC yield, the most linear response of differing sowing ratios on WSC yields was found in plots where red clover was present.

In the second harvest year, the inclusion of red clover had a significant effect on all forage yield and chemical composition parameters, whereas sowing ratios, in contrast, did not alter the DM yield or WSC yield (Table 1b). Similar to the first harvest year, the yield of chicory was lower when chicory was sown in combination with perennial ryegrass with red clover (and not treated with artificial N) and there was a linear and quadratic increase in chicory yield as the sowing ratio of chicory increased. There was also an interaction between the inclusion of red clover and sowing ratio on total chicory and total forage protein yield.

In the first and second harvest year, when chicory was sown with ryegrass and red clover (and not treated with inorganic N), the highest forage protein yield was when chicory was sown at 10% of its usual monoculture sowing rate. In both years, when chicory-ryegrass was sown with red clover, the percentage of chicory present was found to increase quadratically with an increased sowing ratio of chicory. In contrast, when chicory was sown with ryegrass, the percentage of chicory in the sward did not always correspond to increasing sowing ratios, with the percentage of chicory present being similar when sown at 30% and at 90% of its usual monoculture rate. This is despite plant population data (not shown) showing differences in plant numbers. Therefore, the effects of the actual percentage of chicory present on the chemical composition of the harvested forage were compared by regression analysis. Results found that increasing the actual percentage of chicory in the sward had an effect on the crude protein but not the WSC yield of the harvested forage.

Table 1. Effects of sowing chicory in differing ratios with ryegrass and either with or without red clover (RC) on the nutritional value of the harvested forages.

a) First harvest year

	RC	Chicory sowing ratio (R)					sed	Prob.		
		0.1	0.3	0.5	0.7	0.9		RC	R	RC×R
DM content g kg^{-1}	−	187.9	165.5	163.0	151.7	147.1	7.85	ns	***	ns
	+	173.5	170.7	163.8	161.5	160.5				
DM kg ha^{-1}	−	7001	8363	7579	8336	7611	668.6	ns	*	**
	+	9438	8568	8459	7546	6006				
PRG kg DM ha^{-1}	−	5623	5667	5201	4228	3086	572.7	***	***	ns
	+	2084	1132	1468	1122	950				
Chicory kg DM ha^{-1}	−	1220	2542	2172	3881	3705	519.6	***	***	ns
	+	864	1348	2100	2294	2287				
Chicory %	−	17.1	30.0	29.6	46.5	48.2	5.64	***	***	ns
	+	8.9	15.4	24.3	30.4	37.8				
Protein kg ha^{-1}	−	1011	1149	1122	1153	1223	119.3	***	**	***
	+	1887	1646	1615	1286	994				
WSC kg ha^{-1}	−	1610	1926	1681	1889	1613	136.5	***	*	ns
	+	1442	1344	1340	1268	1009				

b) Second harvest year

	RC	Chicory sowing ratio (R)					sed	Prob.		
		0.1	0.3	0.5	0.7	0.9		RC	R	RC×R
DM content g kg^{-1}	−	202.2	173.3	183.7	176.9	174.2	6.99	***	**	ns
	+	168.6	166.7	164.5	161.3	160.1				
DM kg ha^{-1}	−	6877	7308	7769	7639	7559	373.9	***	ns	ns
	+	11588	11420	11898	11592	10616				
PRG kg DM ha^{-1}	−	6113	4882	5246	5192	5161	347.4	***	*	ns
	+	1867	1517	1416	2131	1373				
Chicory kg DM ha^{-1}	−	352	1512	1578	1436	1303	182.8	***	***	**
	+	224	500	590	614	801				
Chicory %	−	5.1	20.7	20.7	18.7	17.3	2.39	***	***	**
	+	2.0	4.4	5.0	5.2	7.5				
Protein kg ha^{-1}	−	1034	1121	1151	1120	1147	81.2	***	*	**
	+	2600	2591	2581	2509	2234				
WSC kg ha^{-1}	−	1639	1640	1743	1701	1681	75.1	*	ns	ns
	+	1569	1588	1654	1581	1542				

ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.001; RC: red clover effect; R: sowing ratio effect

Conclusions

Overall, when chicory was sown in combination with ryegrass only, there was a positive effect of increasing chicory in the sward on the DM and crude protein yield during the first and second harvest year. However, when sowing chicory in combinations with PRG and RC, the highest DM, CP and WSC yields were found with increasing and decreasing proportions of red clover and chicory, respectively.

Acknowledgements

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Seasonal fluctuation in concentrations of α-tocopherol and β-carotene in forage herb and legume species and a grass-clover mixture

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Abstract

Most studies in forages have been carried out with perennial ryegrass and legume species such as white clover. Recently, α-tocopherol and β-carotene in a number of dicotyledonous forage species were compared to a grass-clover mixture. To develop management guidelines, insight into seasonal patterns across the various harvests and years is important. Therefore, four herb species, three legumes and a perennial ryegrass-white clover mixture were investigated in a cutting trial with four harvests (May-Oct) during 2009 and 2010. Concentrations of α-tocopherol and β-carotene were highest in October and lowest in July. Biodiverse pastures could have added benefits in farming practice as various herbs outperformed the grass-clover mixture regarding α-tocopherol concentration, particularly in autumn.

Keywords: herbs, forbs, forage legumes, vitamins, α-tocopherol, β-carotene, seasonal pattern

Introduction

Grasslands provide an important part of the feed used by domestic and wild ruminants and other animals such as horses. Fresh herbage is an important natural source of vitamins in ruminant diets. Concentrations of vitamins in plants depend on factors such as regrowth stage, nitrogen, temperature, day length, and leaf proportion in the harvested herbage (Booth, 1964; Livingston *et al*., 1968). Most studies on vitamin concentrations in forages have been carried out with agronomically important grass species as perennial ryegrass and legume species such as white clover, but seldom with other grassland forage species. As yield and quality data of broad-leaf herb species grown in a sward are scarce, vitamins in a number of herb and legume species were compared to a grass-clover mixture to get insight into species differences (Elgersma *et al*., 2012). This study reports seasonal patterns in contents of α-tocopherol and β-carotene across harvests and years.

Materials and methods

The experiment was established in spring 2008 in Foulum, Aarhus University, in the central part of Jutland, Denmark (56°03', N 9°03'E). Pure stands were established with each of four herb species: salad burnet (*Sanguisorba minor)*, caraway (*Carum carvi)*, chicory (*Cichorium intybus)*, and ribwort plantain (*Plantago lanceolata*), and three legumes: yellow sweet clover (*Melilotus officinalis*), lucerne (*Medicago sativa)* and birdsfoot trefoil (*Lotus corniculatus*). Also a mixture with 85% perennial ryegrass (*Lolium perenne*) and 15% white clover (*Trifolium repens*) was sown. Net plot size was 1.5×9 m. Swards were cut with a forage harvester on 29 May, 9 July, 21 August and 23 October 2009 and 31 May, 13 July, 19 August and 21 October 2010. After cutting, samples of the harvested herbage were taken to determine yield and quality parameters. The concentrations of α-tocopherol and ß-carotene were quantified after high performance liquid chromatography (Jensen et al., 1998). The experimental design was a randomized complete block with two replications. There were eight swards (the four herb and three legume species plus the mixture) and four harvests per year. Analysis of variance procedures were applied using the MIXED procedures of SAS. Yield and vitamin concentrations were evaluated with a model that included fixed main effects of species, harvest date and their interaction. Random effect was assigned to replications, years and their interactions. All tests of significance were made at the 0.05 level of probability.

Results and discussion

All parameters showed significant differences among the species as reported earlier. Yields ranged from 3.9 to 15.4 t DM $ha^{-1}yr^{-1}$ and were lower (*P*<0.001) in yellow sweet clover, salad burnet and caraway than in lucerne and the grass-clover mixture. Concentrations of α-tocopherol were lowest (*P*<0.01) in lucerne and yellow sweet clover, and highest in salad burnet and plantain (*ca.* 22 *versus* 80 mg kg^{-1} DM, respectively); the latter two species outperformed the grass-clover mixture (*P*<0.01). Concentrations of β-carotene ranged between *ca.* 28 and 59 mg kg^{-1} DM and were lower (*P*<0.05) in salad burnet, lucerne and yellow sweet clover than in caraway, birdsfoot trefoil and ribwort plantain. (Elgersma *et al.*, 2012). Interactions between species and cut were not significant for α-tocopherol.

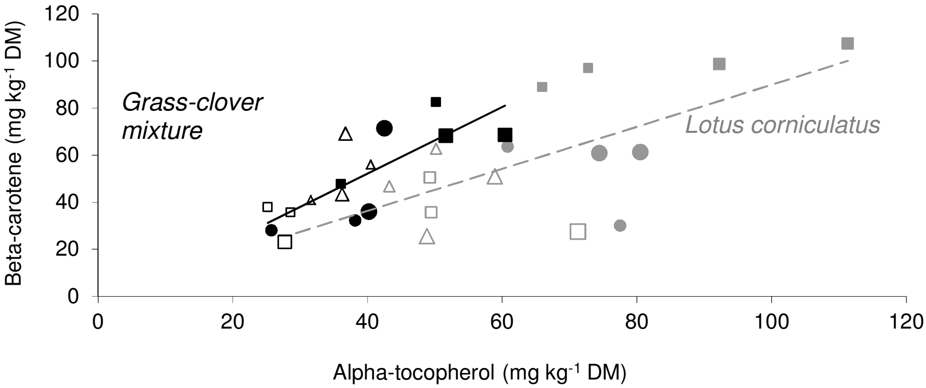

Figure 1. Concentrations of α-tocopherol and of β-carotene during 4 harvests (● late May, □ early July, Δ mid-late August, ■ mid-late October) in 2009 (small symbols) and 2010 (large symbols). Black depicts grass/clover, grey birdsfoot trefoil. Trendlines: R^2 = 0.60 and 0.39, respectively.

Large differences between harvests (*P*<0.001) were found for all parameters: yields were lowest in the 4^{th} harvest (not shown), whereas concentrations of α-tocopherol and β-carotene were generally highest in the 4^{th} harvest (illustrated for birdsfoot trefoil and the grass-clover mixture in Figure 1).

Concentrations of α-tocopherol and of β-carotene showed a positive correlation within most species except in salad burnet where no relation was found; trendlines differed (*P*<0.05) among species (e.g., Figure 1). The weather may provide an explanation for the fact that vitamin concentrations in the first harvest were much higher in 2010 than in 2009. First of all, the winter of 2009/2010 was severe and spring growth started late. The average temperature in April 2010 was only 6.5°C whereas in April 2009 it was 9.4°C. Also, in May the air temperatures in 2010 were lower than in 2009: the 20 days preceding the date of the first cut, average values were 9.6°C and 10.6°C, respectively. Therefore, the effective primary growth period differed. This implies that the forage was probably less mature on 31 May 2010 than on 29 May 2009; however, unfortunately no phenological data were recorded. The 4th harvest showed high vitamin concentrations in both years, but α-tocopherol levels again were higher in 2010 than in 2009. In 2010 there was a cold period after the 3rd cut so again the weather may have caused a delay in regrowth, resulting in physiologically younger forage in the 4th cut in 2010.

In pastures, species are mixed but here pure stands of four herbs and three legumes were studied. Interspecific interaction can affect canopy structure and leaf/stem ratio of species. Brown (1953) found four times more α-tocopherol in grass leaves than in stems, and three times more α-tocopherol in clover leaves than in petioles. Also higher contents of β-carotene have been found in plants containing a higher proportion of leaves (Livingston *et al*., 1968). Stems in birdsfoot trefoil were small, thin and green. Leaf proportions of species were highest in the 4th harvest, which coincided with generally high concentrations of vitamins.

The high α-tocopherol concentrations of the herbs and birdsfoot trefoil, as found in this study, might offer perspectives for naturally improved contents in milk, particularly in autumn.

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Tannin content and structure of selected plant extracts

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Abstract

In the context of the increasing importance of nitrogen-use-efficiency (NUE) in dairy feeding, we investigated the potential use of concentrated tannin extracts as feed additives to increase NUE for grassland-based diets. The first step is to adequately describe the material in order to correlate tannin structures with the effects on NUE. Analytical characterization of tannins in extracts is limited by matrix effects. Therefore, different extraction methods (hot water, water:methanol, acetone:water:formic acid) were tested for analysis with LC-MS to investigate tannin content and structure of some potential feed additives. Industrially produced tannin extracts from *Schinopsis lorentzii* (quebracho), *Acacia mearnsii* (mimosa), *Caesalpinia spinosa* (tara) and *Uncaria gambir* (gambier) were tested. Results indicated that extraction with hot water was the most suitable method for isolating tannins from the extract ($P<0.005$) and to minimize matrix effects. Additionally, acetone produced an interference peak, consummating several tannin peaks. This led to a measured total tannin content for quebracho extract of 139.1 g kg^{-1} with the acetone method, instead of 164.3 g kg^{-1} with hot water. Therefore, the hot water method is suggested to isolate tannins from industrially produced extracts. Observations about different tannin structures in the extracts are discussed.

Keywords: tannin, LC-MS, quebracho, mimosa, tara, gambier

Introduction

Tannins are supposed to form complexes with proteins at neutral pH, protecting them from ruminal degradation, and therefore more feed protein is expected to pass the rumen undegraded (Barry and McNabb, 1999). These complexes are assumed to dissociate again at the low pH value of the abomasum (Jones and Mangan, 1977), making the protein available for the ruminant, thus increasing nitrogen-use efficiency (NUE). Many legumes and other field herbs contain tannins, though at comparatively low tannin contents. Therefore, the objective of the project was to investigate the possible use of high concentrated tannin extracts as feed additives. It is important to characterize the material in order to determine the correct application amount. Therefore, the study evaluates different LC-MS methods in order to standardize tannin measurement and minimize interferences caused by matrix effects.

Materials and methods

Extracts of *Schinopsis lorentzii* (quebracho), *Acacia mearnsii* (mimosa), *Caesalpinia spinosa* (tara), and *Uncaria gambir* (gambier) were examined. For reversed-phase-liquid-chromatography different preparation methods were tested: purification using hot water, water:methanol (1:1) and acetone:water:formic acid (70:29.5:0.5). Measurements were done by liquid chromatography (1200 series with diode array detector; Agilent Technologies), using 0.1% formic acid and methanol:formic acid (99.9:0.1) as eluents. An Accucore RP-MS (150×3.0 mm 2.6 µm Solid Core LC Column) with precolumn was used. An ion-trap mass

spectrometer (Esquire3000, Bruker) was used, operating with an electrospray ionization source in the negative ionization mode and scanning from m /z 100 to 1800. The total analysis time of a sample was 42 min. All preparations and analyses were performed twice.
The UV/VIS peaks produced at 280 nm were integrated if they showed a typical tannin spectrum. Tannin content was quantified on the basis of calibration curves of tannin standards with similar structure to the tannins in the respective extract. Statistical evaluation was performed by simultaneous comparisons of means using multiple contrast tests.

Results and discussion

Table 1 shows the tannin content as quantified after the different preparation methods for all four extracts. Hot water extraction provided higher values than extraction with methanol or acetone ($P<0.005$). For mimosa and gambier, no differences were observed between extraction with methanol and acetone, while for quebracho and tara there were lower values for acetone ($P<0.0001$). Comparability to other methods may be limited, for example the photometrical measurement of butanol/HCl method led to very different numbers (see Schweigmann *et al*., 2012).

Table 1. Tannin content of the extracts in g kg^{-1} by LC-MS analysis with different preparation methods, quantified based on Procyanidin C1 (C1) or epigallocatechin gallate (EG) as calibration standard (s.e. = 1.3).

	Hot water	Water : Methanol	Acetone:Water:Formic acid
Quebracho (C1)	164.3 ^{aB}	152.4 ^{bB}	139.1 ^{cC}
Mimosa (C1)	108.2 ^{aC}	101.9 ^{bC}	98.2 ^{bD}
Tara (EG)	647.5 ^{aA}	633.6 ^{bA}	572.9 ^{cA}
Gambier (C1)	169.3 ^{aB}	154.3 ^{bB}	152.9 ^{bB}

^{a,b} different small letters indicate significant differences between methods within one extract ($P<0.005$)
^{A,B} different capital letters indicate significant differences between extracts within one method ($P<0.005$)

Methanol or acetone in the preparation process resulted in noticeable increase of interferences in the tannin peak spectra, rendering tannin identification more difficult. Utilization of acetone caused a disturbing peak at 2.5 min, consuming several others and therefore contributing to an underestimation of the tannin content.
Venter *et al*. (2012a) also investigated industrially produced quebracho extract and identified *ent*-fisetinidol-4*β*-ol (negative m/z value 289) as main monomer as well as its oligomers (up to heptamer). With the scan mode set to a maximum of 1800, the hexamer was the largest oligomer detectable. The mono- and all oligomers were found in the present quebracho extract, but only with low intensity. Another molecule with m/z 915 appeared throughout the sample travel time at quite high intensities (Figure 1a). Fragmentation of this particular mass revealed its fragments to be quebracho tannin oligomers; therefore, it has to be some kind of modification product. This thesis was substantiated by another study of Venter *et al*. (2012b), describing a sulfited quebracho trimer, supposedly making the product cold-water soluble. Apparently, the present quebracho extract was treated with sodium hydrogen sulfite extensively, raising the question of its harmlessness regarding animal feeding. On the other hand, cold-water solubility might be essential for adequate application. The tannin structure of tara differed from the other extracts. The mass spectrum displayed several tannins with mass differences of continuously 152. Mane *et al*. (2007) found the tara tannin to consist of quinic acid with one or more galloyl moieties. Figure 1c shows the tara mass spectrum, including single quinic acid (m/z 191). Only few studies were found to help interpret the spectra of mimosa and gambier (Figure 1b, 1d). Drewes *et al*. (1966) indicated the mimosa monomer to be leucorobinetinidin. For gambier tannins, Nonaka and Nishioka (1980) found the main component to be gambiriin.

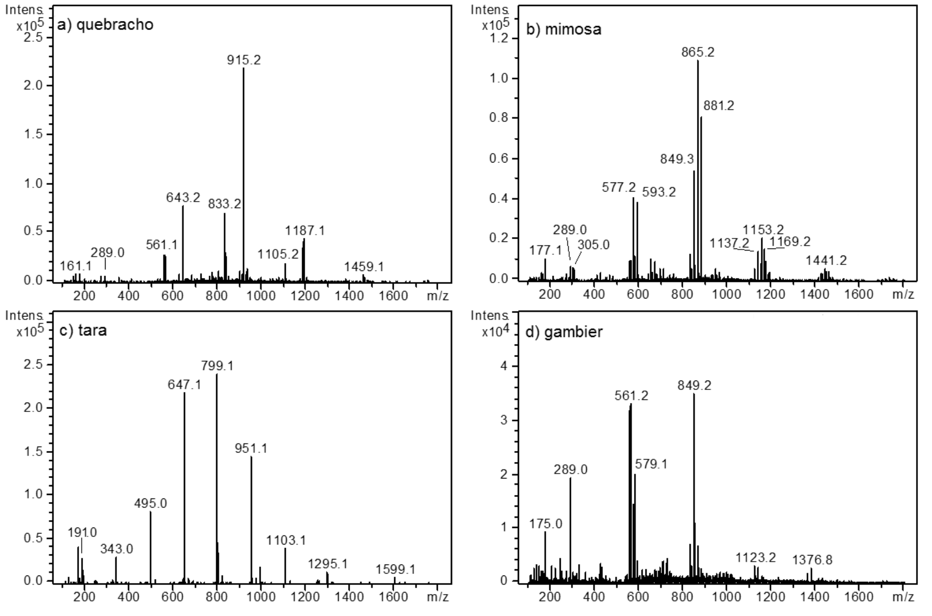

Figure 1. Mass spectra of quebracho, mimosa, tara, and gambier extract (hot water method).

Conclusions

Preparation of tannin extracts by hot water seems to be the most suitable method to isolate tannins and minimize matrix effects. Standardization will be difficult, as one single standard for quantification would provide overall comparability, but heterogeneity in tannin molecule size and structure prevents usage of the same standard for different tannins.

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Effect of stocking rate and lactation stage on nitrogen balance of dairy cows on pasture under Galician grazing conditions (NW Spain)

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Abstract

The effect of stocking rate (SR), low *vs.* high (L, 4.0 *vs.* H, 5.2 cows ha^{-1}), and cows' lactation stage (LS), early *vs.* middle (E, 31 *vs.* M, 140 days in milk) was studied on N-balance of cows (n = 72). ΣN inputs (grass, silage and concentrate) and ΣN outputs (milk and body weight gain) were evaluated in two periods, with medium (P1) *vs.* low (P2) level of supplementation at pasture. ΣN inputs and ΣN outputs were higher (P<0.001) in cows at E (189±14 and 121±9 g N cow^{-1} d^{-1}) than at M lactation stage (163±2 and 105±5 g N cow^{-1} d^{-1}). Supplement intake was higher (P<0.05) in H than in L stocking rate groups (10.4±0.2 *vs.* 9.2±0.9 kg DM cow^{-1} d^{-1}). ΣN inputs, ΣN outputs and N excretion were no different for SR treatments. ΣN inputs and ΣN outputs (g N cow^{-1} d^{-1}) were higher (P<0.001) in P1 (237±8 and 140±10), with higher supplementation, than in P2 (114±19 and 86±8). N excretion was also higher (P<0.001) in P1 than in P2 (479±67 *vs.* 68±24 g N cow d^{-1}), but no differences were found between cows at both LS and SR. N surplus was the highest in cows at E lactation stage and H stocking rate.

Key words: dairy cattle, grazing pressure, days in milk, N excretion, supplementation

Introduction

Galicia is located in the humid part of Spain with suitable soil types and climatic conditions for developing dairy grazing systems. Nevertheless, only 2 mt out of 6.0 mt of Spain's milk quota are produced in this region by 41% of our cattle and 56% of our farms using a supplemented (silage + concentrate) grazing system. Treacy *et al.* (2008) reported that lower stocked clover-based farms generate lower N surpluses (and lower N losses) compared with higher stocked farms. Taking into account the animal feed part from the N-total farm level balance, studies are being carried out in Galicia to determine the N-conversion rate from inputs (grass, grass/maize silage and concentrate) to outputs (milk and body weight gain) in order to decrease the N-surplus by improving efficiency of N utilization at animal level. Considering that the main N input at cow level is via feed, the possibility is evaluated of improving grazing management by applying appropriate stocking rates (SR) while decreasing supplementation according to cows' lactation stage (LS). The objective is to investigate the effect of SR and cows' LS on animal N-balance in two periods of supplementation at pasture. This work will contribute to validation of the N-balance as a tool for Galician grazing dairy systems and it will help to focus our new research projects on environmental aspects related to milk production under local conditions.

Materials and methods

A trial was carried out at the Agrarian Research Centre of Mabegondo (NW Spain) to study the balance of nitrogen under a grazing system, considering N inputs from feed (grass, grass/maize silage and concentrate) and N outputs (milk and body weight gain) from cows, to see if N-offer is in accordance with animal nutritional N-requirements. Holstein-Friesian cows (n = 72), at two lactation stages (LS), early (E, 31 days in milk) *vs.* middle (M, 140 days in milk), were managed at two stocking rates (SR), low (L, 4.0 cows ha^{-1}) *vs.* high (H, 5.2 cows ha^{-1}), in spring-summer. Four groups (EL, EH, ML and MH) were grazing rotationally on mixed perennial ryegrass and white clover pastures in two periods: (P1) with medium level of silage (3.5 kg DM cow^{-1} d^{-1}) and concentrate (3.5 kg DM cow^{-1} d^{-1}) supplementation in addition to pasture (20.5 kg DM cow^{-1} d^{1}) *vs.* (P2) without silage and low level of concentrate supplementation (0-2 kg DM cow^{-1} d^{-1}) in addition to pasture (17.5 kg DM cow^{-1} d^{-1}). Five random samples (0.33×0.33 m) were taken per paddock before and after grazing, cutting to 4 cm above ground level, for chemical composition determination using NIRS System 6500. Statistical analysis was performed by SPSS15.0. Significant differences were declared at *P*<0.05.

Results and discussion

On average, milk yield was higher (*P*<0.05) in cows at E lactation stage (25.7 kg cow^{-1} d^{-1}) than in cows at M lactation stage (19.6 kg cow^{-1} d^{-1}). Nevertheless, on average, milk protein content (31.1 *vs.* 29.0 g kg^{-1}), milk fat content (38.1 *vs.* 36.0 g kg^{-1}), body weight (BW) (568 *vs.* 587 kg) and body condition score (BCS) (2.8 *vs.* 3.0) were higher (*P*<0.05) in cows at M lactation stage than in cows at E lactation stage, respectively. No significant differences (*P*<0.05) were found between SR treatments for average milk yield, milk protein content, milk fat content, BW and BCS (Table 1).

Table 1. Animal performance and milk quality responses in four groups of cows at two stages of lactation and two stocking rates.

Herds[1]	Cows (number)	Lactation (days)	Stocking rate (cows ha^{-1})	Milk yield (kg cow^{-1} d^{-1})	Protein (g kg^{-1})	Fat (g kg^{-1})	BW[2] (kg)	BCS[3] (1-5)
EL	22	33[a]	4.3[a]	24.9[a]	29.2[a]	35.6[a]	573[ab]	2.8[a]
EH	22	28[a]	5.8[b]	26.5[a]	28.7[a]	36.3[ab]	564[a]	2.7[a]
ML	14	139[b]	3.6[a]	20.4[b]	30.6[b]	39.3[b]	600[b]	2.9[b]
MH	14	140[b]	4.6[b]	18.9[b]	31.6[b]	36.8[ab]	574[ab]	3.0[b]

[1]Herds: Stage of Lactation by Stocking Rate [2]BW (Body Weight); [3]BCS (Body Condition Score)
[a-b]Values in the same column not sharing a common superscript are significantly different (*P*<0.05)

Average grass intake was higher (*P*<0.05) in cows at M (21.4 kg DM cow^{-1} d^{-1}) than at E (16.9 kg DM cow^{-1} d^{-1}) lactation stage and lower (*P*<0.05) concentrate supplementation was observed in cows at M (3.0 kg DM cow^{-1} d^{-1}) than at E (1.5 kg DM cow^{-1} d^{-1}) lactation stage. No differences were found in silage supplementation between cows at both lactation stages. Lower (*P*<0.05) grass intake was reached by cows at H (18.3 kg DM cow^{-1} d^{-1}) than at L (20.0 kg DM cow^{-1} d^{-1}) stocking rate and no differences were found between both SR treatments for supplementation (Table 2). Lower (*P*<0.05) DM (16.8 *vs.* 18%), ADF (264 *vs.* 277 g kg^{-1} DM) and NDF (479 *vs.* 496 g kg^{-1} DM) content were reached by cows managed at H than at L stocking rate.

Table 2. Total feed intake of four dairy cow groups and sward quality characteristics.

	Total intake								Sward characteristics[2]					
	Grass		Grass silage		Maize silage		Concentrate							
	P1	P2	P1	P2	P1	P2	P1	P2	DM	CP	ADF	NDF	WSC	IVOMD
Herds[1]	(kg DM cow^{-1} day^{-1})								%	(g kg^{-1} DM)				
EL	18.8[a]	14.2[a]	1.5	0	1.7	0	4.1[a]	1.8[a]	17.3[a]	131[a]	275[a]	487[a]	185[a]	749[a]
EH	16.4[a]	18.2[a]	1.5	0	1.7	0	4.1[a]	1.8[a]	16.9[b]	149[ab]	261[b]	475[b]	193[a]	759[ab]
ML	25.9[b]	21.0[b]	1.8	0	2.0	0	2.6[b]	0[b]	18.5[a]	146[ab]	278[a]	505[a]	174[b]	757[ab]
MH	21.0[b]	17.6[b]	1.8	0	2.0	0	3.3[b]	0[b]	16.7[b]	157[b]	266[b]	483[ab]	177[b]	790[b]

[1]Herds: Stage of Lactation by Stocking Rate [2]Sward characteristics: DM, Dry Matter (%); CP, Crude Protein; ADF, Acid and NDF, Neutral Detergent Fibre; WSC, Water Soluble Carbohydrates; IVOMD, *In vitro* Digestibility of Organic Matter
[a-b]Values in same column not sharing a common superscript are significantly different (*P*<0.05)

N inputs from silage (g N cow^{-1} d^{-1}) were higher (*P*<0.001) in P1 (30 and 20) than in P2 (0 and 0) for grass and maize, respectively (Table 3). N inputs from concentrate (g N cow^{-1} d^{-1})

were higher (*P*<0.001) at E than at M lactation stage (87 *vs.* 44), higher (*P*<0.05) at H than at L stocking rate (69 *vs.* 62) and also higher (*P*<0.001) in P1 than in P2 (103 *vs.* 28). N outputs from milk (g N cow^{-1} d^{-1}) were higher (*P*<0.001) at E than at M lactation stage (116 *vs.* 99) and also higher (*P*<0.001) in P1 than in P2 (135 *vs.* 86). ∑N inputs and ∑N outputs (g N cow^{-1} d^{-1}) were higher (*P*<0.001) at E (189 and 121) than at M lactation stage (163 and 105), respectively. Higher ∑N inputs (*P*<0.001) and ∑N outputs (*P*<0.05) (g N cow^{-1} d^{-1}) were reached by cows managed at H (182 and 115) than at L stocking rate (170 and 111), respectively. ∑N inputs and ∑N outputs (g N cow^{-1} d^{-1}) were also higher (*P*<0.001) in P1 (237 and 140) than in P2 (114 and 86), respectively. There were no significant differences between LS and SR treatments for ∑N inputs - ∑N outputs and N excretion. Period showed a significant effect on ∑N inputs - ∑N outputs (g N cow^{-1} d^{-1}), with higher (*P*<0.001) values in P1 (97) than in P2 (28), and also on N excretion (g N cow^{-1} d^{-1}), with higher (*P*<0.001) values in P1 (460) than in P2 (53). Our results highlighted that supplementation with silage and concentrate in P1 strongly increased N-excretion in cows managed at both LS and SR.

Table 3. ∑N inputs (grass, silage and concentrate as g N cow^{-1} day^{-1}), ∑N outputs (milk and body weight gain as g N cow^{-1} day^{-1}) and N excretion (g N cow^{-1} day^{-1}) in four groups of cows at two lactation stages (E, early *vs.* M, middle) and two stocking rates (L, low *vs.* H, high) considering two periods (P1 *vs.* P2) of supplementation at pasture.

Herds[1]	EL		EH		ML		MH		Significance[3]						
Period[2]	P1	P2	P1	P2	P1	P2	P1	P2	LS	SR	P	LS×SR	LS×P	SR×P	LS×SR×P
Grass	68	67	83	95	105	95	83	87	ns	ns	ns	ns	ns	ns	ns
Grass silage	26	0	30	0	31	0	31	0	ns	ns	***	ns	ns	ns	ns
Maize silage	17	0	20	0	20	0	21	0	ns	ns	***	ns	ns	ns	ns
Concentrate	121	51	121	56	74	3	97	3	***	*	***	ns	*	ns	*
∑N inputs	232	118	254	151	230	98	232	90	***	***	***	***	ns	**	*
Milk output	143	90	150	101	124	78	122	73	***	ns	***	**	ns	ns	ns
Body weight gain	−6	−3	10	−1	10	7	8	−2	ns	ns	ns	ns	ns	ns	ns
∑N outputs	137	87	160	100	134	85	130	71	***	*	***	**	**	***	**
∑N inp.-∑N outp.	95	30	94	50	96	12	102	18	ns	ns	***	ns	ns	ns	ns
N excretion	442	56	356	119	466	−6	576	41	ns	ns	***	ns	ns	ns	ns

[1]Herds: Stage of Lactation by Stocking Rate [2]P, Period of the Grazing Season (P1, medium level *vs.* P2, low level of supplementation at pasture); [3]Significance:*** (*P*<0.001); ** (*P*<0.01); * (*P*<0.05); ns, not significant

The N-overall calculation to the animal presented a surplus of 63, 73, 54 and 60 g N cow^{-1} d^{-1} for each of the EL, EH, ML and MH groups, respectively. The average N-conversion rate (from inputs to outputs) was of 63% for the four cited groups, without any significant differences between cows managed at both LS and SR treatments.

Conclusions

The results show the importance of evaluation of lactation stage and stocking rate on ∑N inputs and ∑N outputs to minimize N-losses, and to validate N-balance as a tool for assessing dairy systems. Supplementation highly increased N-excretion in grazing cows.

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Feeding value of late autumn cut timothy-meadow fescue silage under Nordic conditions

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Abstract

The highest grass silage digestibility over the whole growing season under Nordic conditions is achieved using a three-cut strategy. The late-autumn cut grass has a low content of fibre and high organic matter digestibility. Thus the feeding value of the third-cut silage should be high in terms of supporting milk production. The aim of this study was to compare the feed intake and milk production of the third-cut grass silage with the first and second cuts. Experiment 1 included three timothy-meadow fescue silages supplemented with 9, 11 or 13 kg of concentrate d^{-1}. The harvest dates were 12 June, 26 July and 4 September 2011. Experiment 2 included only the second and third cuts with the same harvesting dates as in Experiment 1. The concentrate supplementation was 4, 6.5, 8 or 11.5 kg d^{-1}. The energy content of the third-cut silage was the highest, compared to earlier cuts, which was also confirmed by *in vivo* digestibility. However, when fed to cows, the dry matter intake of the third-cut silage was similar to, or lower than, the other cuts. Due to the reduced energy intake and/or lower energy utilization in milk production, the feeding value was similar or lower in the third-cut silage compared to that of the other two cuts.

Keywords: grass silage, harvest timing, milk production

Introduction

The timing of cuts during the growing season affects markedly the digestibility of ensiled grass. Early cut grass produces highly digestible primary growth for silage, but the digestibility of the second cut decreases (Kuoppala *et al.,* 2006; Hyrkäs *et al.,* 2012). Over the whole growing season under Nordic conditions, the highest average digestibility is achieved using three cuts. The third-cut grass typically has a low content of fibre and high organic matter digestibility (Hyrkäs *et al.*, 2012). Thus the feeding value of the autumn cut should be high. However, there are only few milk production studies that have compared three cuts with each other.

The aim of this study was to compare the milk production potential of the third-cut grass silage with the first- and second-cut silages.

Materials and methods

All silages within the experiment were cut consecutively from the same timothy-meadow fescue field and stored in round bales with an additive in Experiment 1, and in separate bunker silos in Experiment 2. The soil in the field of Experiment 1 was organic soil and in Experiment 2 it was mixture of mineral and organic soils. The silages were prepared on 12 June (A), 26 July (B) and 4 September 2011 (C). The pelleted concentrate in both experiments was a mixture of barley, oats, beet pulp and rapeseed meal.

In Experiment 1, silages from three cuts were used. The study included 36 dairy cows with an average milk yield of 33.6 ± 6.6 kg at the beginning of the experiment. Silages were supplemented with 9, 11 or 13 kg d^{-1} of concentrate. The diet digestibility was measured using acid insoluble ash as an internal marker in 9 cows.

In Experiment 2, only the second- and third-cut silages were used. The initial milk yield of the 40 experimental cows was 34.7 ± 7.4 kg. The amount of the concentrate was 4, 6.5, 8 or 11.5 kg d^{-1}.

Both experiments were conducted using change-over designs (Davis and Hall, 1969) with 4 (Experiment 1) and 3 (Experiment 2) periods. The digestible organic matter (D-value, g kg^{-1} DM) was analysed using pepsin-cellulase solubility and calculated using different equations for primary growth and regrowth silages.

Results and discussion

In Experiment 1 the energy content of C was the highest, which was also confirmed by *in vivo* digestibility measurements (Table 1). Different fields were used in Experiment 1 (organic soil) and 2 (mixture of organic and sandy soil), which explains the slight differences in silage quality between the experiments. The high digestibility of C is most probably a consequence of the low average temperature during the autumn (Thorvaldsson *et al.,* 2007) and the low fibre content of the grass (Hyrkäs *et al*., 2012).

In a milk production study, Huhtanen *et al.* (2001) compared timothy-meadow fescue and tall fescue silages including both the second and third cut. Both DM intake and milk yield were higher in the third cut compared to the second cut, and the effect was similar for both plant species. However, Huhtanen *et al*. (2001) concluded that the energy utilization in milk production was lower with the third cut compared to the second. In contrast to the findings presented by Huhtanen *et al*. (2001), the silage intake was the lowest with C, resulting in both the lowest metabolizable energy (ME) intake and milk yield when C was fed. There were no differences in feed efficiencies in milk production between harvests, but if liveweight changes are taken into account, the energy utilization was lowest with C.

In Experiment 2, the silage D-value was clearly higher in C compared to B. This resulted in higher ME intake and energy-corrected milk (ECM) production with C, despite the same silage intake for both cuts. However, the ECM response to increased silage D-value was very low, only 0.1 kg ECM 10 g^{-1} kg^{-1} DM, whereas for primary growth silages Kuoppala *et al.* (2008) reported 0.61 kg ECM and Rinne (2000) 0.27 kg ECM per a 10 g kg^{-1} DM change in D-value.

Table 1. Effects of silage harvest on feed intake and milk production of dairy cows. Harvests A, B and C are first, second and third cut from the same field within an experiment.

	Experiment 1					Experiment 2			
	Harvest			SEM	*P*-value	Harvest		SEM	*P*-value
	A	B	C			B	C		
Dry matter (DM) intake, kg									
Silage	13.4 a	13.2 a	12.4 b	0.29	<0.01	12.9	13.0	0.39	0.72
Concentrate	9.6	9.6	9.6	0.02	0.86	6.6	6.6	0.01	0.43
Total	23.0 a	22.8 a	22 .0 b	0.29	<0.01	19.5	19.6	0.39	0.73
ME intake, MJ [1]	261 a	256 b	254 b	3.2	<0.01	208	218	4.1	<0.01
ME intake *in vivo*, MJ [2]	236	239	232	5.8	0.12				
Organic matter digestibility	0.73	0.74	0.76	0.01	<0.01				
k_l [3]	0.60	0.61	0.61	0.010	0.28	0.70	0.65	0.026	0.12
Live weight change, kg d^{-1}	0.64	0.19	-0.02	0.097	<0.01	0.18	0.23	0.098	0.69
Milk, kg d^{-1}	33.9 ab	34.5 a	33.3 b	0.89	<0.01	30.3	30.6	0.87	0.43
ECM [4], kg d^{-1}	33.8 ab	34.4 a	33.3 b	0.76	0.03	29.3	30.1	0.76	0.03

[1] Metabolizable energy (ME) intake based on feed tables and in vitro organic matter digestibility for silage
[2] ME intake based on in vivo apparent diet OM digestibility
[3] ME efficiency in milk production
[4] Energy corrected milk

The ECM responses to increased concentrate supplementation (0.7 and 1.1 kg^{-1} DM, for Experiments 1 and 2, respectively) were linear and independent of the cut within both experiments. Kuoppala *et al*. (2008) reported a low milk response (0.34 kg ECM kg^{-1} DM) to supplementation with highly digestible first-cut silage, whereas the response was 1.01 kg for low digestibility first-cut silage. In their study this digestibility × concentrate response interaction was not obvious with regrowth silages, as also seen in the current study.
The reason for the unexpectedly low milk production of silage C is unclear. All the silages had only a moderate fermentation quality but C was comparable to the other silages. The intake capacity of a cow is high when low fibre silage is offered so the answer has to be found on chemostatic intake regulation or silage palatability. It would be possible that moderate fermentation quality combined with low fibre content of silage limits intake.

Conclusion

The organic matter digestibility of the third-cut silage was higher than the second-cut silage. The feeding value of the third-cut silage, in terms of its effect on milk production, was similar to or lower than the silage from the other two cuts, and not as high as could have been expected based on its D-value.

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Festulolium – an interesting forage grass for high-latitude regions?

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Abstract

Longer growing seasons in high-latitude regions will require forage species with good regrowth and sufficient nutritive value. Yield and forage quality were investigated in commercial cultivars of ×*Festulolium* (Hykor, Felopa) and timothy (*Phleum pratense* L.) (Grindstad) at two Norwegian locations. In addition, forage of timothy (Vega) and ×*Festulolium* (Felina, Felopa) was harvested at a NDF content of 50% and the silage was fed to growing Norwegian Red bulls. Concentrate was limited to 1 kg per animal and day. No significant differences in carcass weight or carcass classification were found between the three silage groups, demonstrating the importance of the NDF content in the Nordic feed evaluation system (NorFor) and good quality forage. In the main cut, 50% NDF content was reached between heading and anthesis in the loloid entries, and before early heading and with a slow rate of increase in the festucoid type (Hykor). For timothy, this level was reached at the vegetative stage with a high rate of increase as a consequence of maturity stage. Forage nutritive quality and the regrowth capacity of ×*Festulolium* make it an interesting species also for high-latitude regions.

Key words: ×*Festulolium*, timothy, feeding, NDF, iNDF, DDMY

Introduction

Longer growing seasons are predicted for high-latitude regions (Hanssen-Bauer, 2008) and ×*Festulolium* may be an interesting forage grass species due to its good regrowth capacity and high nutritive value. Fescues (*Festuca* spp.) and ryegrasses (*Lolium* spp.) can be hybridized fairly easily, which makes it possible to combine the superior forage quality of ryegrass species with the high persistency and stress tolerance of fescues in interspecific *Lolium* × *Festuca* hybrids (×*Festulolium*) (Humphreys *et al*., 2003). In the Nordic countries a feed evaluation system (NorFor) for ruminants has been developed in which roughage as the main source of energy has increased importance, and digestibility of neutral detergent fibre (NDF) is an important factor affecting digestibility of organic matter.

The aim of this investigation was to examine the impact of silage from cultivars of ×*Festulolium* and timothy harvested at a NDF content of 500 g kg^{-1} DM in a feeding experiment with bulls and to study the nutritive values of forage at different maturity stages in similar cultivars in field trials.

Materials and methods

Field trials were established at two Norwegian locations in 2006: Bodø (67°18’N, 14°29’E; 30 m a.s.l.) and Fjaler (61°18’N, 5°4’E; 10 m a.s.l.). Commercial cultivars of ×*Festulolium* (Hykor, Felopa) were compared with timothy (*Phleum pratense* L.) (Grindstad) in the first cut at four maturity stages: vegetative stage; early heading (inflorescence emerging), heading (50% of the inflorescence emerged); and anthesis. The fibre characteristics neutral detergent fibre (NDF), the indigestible part of NDF (iNDF), and digestibility of dry matter (DDM) were studied in relation to the maturity stages recorded at plot level in 2007 and 2008. In these years individual cuts were taken according to the maturity stages. A feeding experiment was carried out in Bodø using silage of cultivars of ×*Festulolium* (Felina, Felopa) and timothy (Vega), which were all harvested individually at a NDF content of 500 g kg^{-1} DM in 2008 in the first cut of a first-year ley. The silage was fed to growing Norwegian Red bulls and concentrate supply was limited to 1 kg per animal and day. The bulls were slaughtered at a live weight of 600 kg.

Results and discussion

Despite limited intake of concentrates, the animals had very good daily weight gain in all groups (Table 1), indicating that the silage was generally of good quality.

Table 1. Daily weight gain, intake and NDF digestibility in the different feeding groups, presented as mean ± standard deviation.

Species / cultivar	Timothy	×*Festulolium*		
	Vega	Felopa	Felina	*P*
Daily weight gain (g)	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	<0.01
Forage intake (kg DM/100 kg live weight)	2.0 ± 0.06	1.7 ± 0.9	2.2 ± 0.7	<0.01
NDF digestibility %	73.6 ± 0.18	71.9 ± 0.45	70.2 ± 2.03	<0.01

The carcasses were classified according to the EUROP system. The classification status of the carcasses was not affected by the type of silage, with an average fat class of 3 and a saleable meat yield class of O/E+. These are good classifications for Norwegian Red bulls, and demonstrate the importance of the NDF content in evaluation of feed for cattle. On average, the animals in the timothy group were slaughtered two weeks before the animals in the cv. Felopa group and one week before the animals in the cv. Felina group.
In the plant maturity study, a NDF content of 50% was reached between heading and anthesis in cv. Felopa, and between vegetative stage and early heading in cv. Hykor and cv. Grindstad. However, there was a very slow rate of increase in ×*Festulolium* cv. Hykor and a fast rate of increase in timothy (Figure 1). The changes in NDF and iNDF at different maturity stages were quite similar for the entries investigated.

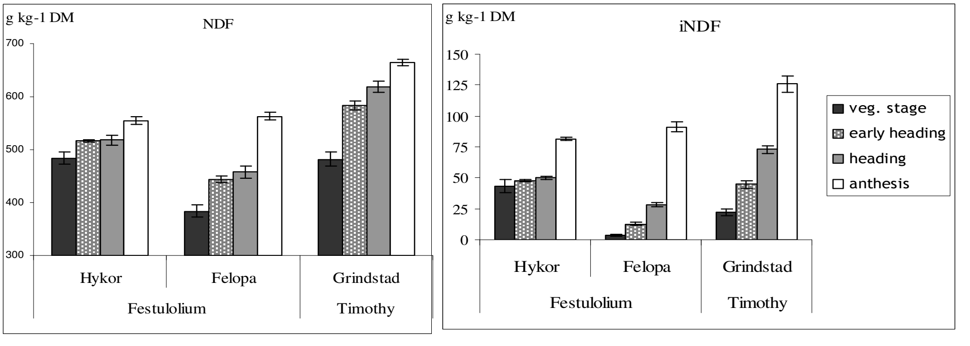

Figure 1. Fibre characteristics (NDF and iNDF) in the first cut of four maturity stages averaged over two locations (Fureneset, Vågønes) in 2007, presented as mean ± standard deviation. N=6.

The first cut corresponded to the main cut in high-latitude regions and forage DMY and quality considerably affect the winter feed used for cattle husbandry. When harvested at heading, digestible dry matter yield (DDMY) averaged over three cuts at both locations and years was 7.6, 6.3 and 5.7 t ha^{-1} for cv. Hykor, cv. Grindstad and cv. Felopa, respectively, of

which the first cut constituted 46%, 57% and 37% of total DDMY for the three entries. The lower DDMY in the loloid type Felopa (*F. pratensis* × *L. multiflorum*) was mainly caused by lower winter survival. A loloid ×*Festulolium* candivar (FuRs0463, *F. pratensis* × *L. perenne*) from the Norwegian plant breeding company Graminor AS (Ltd) has been proven to have similar DDMY to cv. Hykor (Østrem *et al*., manuscript), and due to their tolerance to winter stress, loloid type ×*Festulolium* cultivars for Nordic conditions should be generated from hybrids of *F. pratensis* × *L. perenne* (Østrem and Larsen, 2010).
Climate differences between the two experimental sites led to different cultivars being used in the feeding experiment and the field trials. ×*Festulolium* cv. Hykor and cv. Felina are both of the festucoid type, originating from tall fescue (*F. arundinacea* Schreb.) and Italian ryegrass (*L. multiflorum* Lam.), and cv. Felina was chosen for Bodø due to its slightly better winter survival than cv. Hykor at that site (Molteberg and Enger, 2005). The cultivars are otherwise very similar, although cv. Hykor is the marketed cultivar. Timothy cv. Vega was grown for the feeding trial due to its more northern adaptation compared with cv. Grindstad, which is the main timothy cultivar in Norwegian agriculture.

Conclusions

The carcass classification and daily weight gain data indicate that silage produced from ×*Festulolium* cultivars harvested at 50% NDF constitutes an adequate roughage. The results also demonstrate that the concentrate quantity can be greatly reduced when good quality silage is used. Timothy is expected to remain an important forage grass species due to its high persistence. However, if winter conditions allow the use of ×*Festulolium* cultivars, their DDMY and forage quality surpass those of timothy. Harvesting timothy at 50% NDF content is not cost-effective in terms of taking advantage of its yield potential, and it might also affect the persistency. For the ×*Festulolium* entries, this NDF content is reached at late maturity, when the yield potential has already been exploited.

Acknowledgements

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Effect of extended grazing season of suckler cows on yield, quality and intake of sward

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Abstract

The aim of this investigation was to evaluate the effect of suckler cow late-autumn and early winter grazing on sward diversity, yield, quality, herbage intake and rate of its utilization. Experiments were carried out in 2010-2011 on semi-natural lowland pastures on mineral soil, pre-utilized by grazing at the beginning of August. Afterwards the sward regrew until the autumn/winter period for rotational stocking with suckler cows. Five cows (Angus and Angus × Limousin hybrids) were used to graze the sward on three paddocks starting in the last week of October, then in the last week of November and in December. Forage from the pasture was the only feed the cows received. It can be concluded that, in western Poland, extension of the grazing season for suckler cows is possible. The effect of grazing in late autumn and early winter in terms of the decrease of sward yield, sward intake and herbage quality should be taken into consideration. No impact of the extended grazing by suckler cows was observed on the pasture botanical composition. The sward intake and utilization rates were highly influenced by weather conditions in the experimental years.

Keywords: extending grazing, herbage quality, pasture, suckler cows, sward intake

Introduction

Extending the grazing season and reducing the need for stored feed has become highly desirable. It can reduce the cost of cattle rearing and less labour is required for grazing than under stored feed systems. In Central Europe, extension of the grazing period for suckler cows or beef cattle herds is possible until the end of the year (Opitz v. Boberfeld *et al.*, 2006). Manipulation of the grazing rotation length and sward management can be used to increase biodiversity as well as to minimize environmental impact (Farruggia *et al.*, 2008).
The aim of this study was to evaluate the influence of late autumn and early winter grazing of suckler cows on sward diversity, yield, quality, intake and rate of its utilization.

Materials and methods

The experiment was established in August 2010 on semi-natural lowland pastures at the Brody Experimental Station of PULS (52°26'N, 16°18'E; 92.0 m a.s.l.; long-term average annual rainfall 601 mm; average air temperature 8.3°C) located on mineral soil, fertilized at 50 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O, applied in spring. The fenced experimental area (3600 m^2) was pre-utilized by grazing (15 suckler cows during one week) at the beginning of August. After that, the sward was allowed to regrow until autumn/winter period. In 2010 and 2011, three paddocks each 1200 m^2 were prepared for rotational stocking of suckler cows. The 5 experimental cows were Angus and Angus × Limousin hybrids, 460 to 520 kg body weight, and they were grazing in each paddock in the last weeks of October, November and December. In 2010, from the beginning of Dec. until the first days of January 2011, severe winter conditions occurred with about 20 cm snow cover. Therefore the last grazing was carried out in the third week of Jan. that winter. The monthly average air temperature of Oct., Nov., Dec. and Jan. 2010 was 6.2, 4.4, -5.6 and 0.5°C, and rainfall was 7.5, 133.8, 74.1 and 31.1 mm, respectively. Weather conditions for extending the grazing season in 2011 were favourable. The monthly average air temperature of Oct., Nov. and Dec. was 9.5, 3.2 and 3.4°C, and rainfall 18.2, 0.6 and 45.7 mm, respectively. Forage from the pasture was the only feed the cows received. During the day, the animals were on the pasture, and during the night they were in the barn where fresh water and barley straw was offered. Before grazing of each paddock, in ten randomly selected plots (1×2 m), sward botanical composition (samples were separated and the fractions were weighed) and sward yield (measured on the total area of 20 m^2) were determined. The herbage samples from the plots were dried in a forced-draught oven at 60°C and their chemical composition determined using commonly accepted methods. After grazing of each paddock, the residual mass yield, sward utilization rate, and sward intake per cow were evaluated. Tests of the main effects were performed by F-test. Means were separated by LSD and were declared different at $P<0.05$. Generally, years were considered separately in the statistical model due to the great importance of weather conditions during late autumn and early winter.

Results and discussion

Botanical composition was dominated by *Lolium perenne* (from 44.7% to 53.7%). *Trifolium repens* occupied 4.7-10.3%. *Festuca rubra* and *Poa pratensis* comprised 6.7-23.7% and 4.0-12.3%, respectively. The total number of species in the pasture sward ranged from 24 to 29, depending on the year and period of grazing. No significant impact of the extended grazing by suckler cows was observed on the pasture botanical composition in the next years. Golińska and Goliński (2005) reported that the pre-utilization in August, applied in this trial, was found to be the best method of stabilization of the botanical composition of winter pasture. The dry matter yield of pasture before grazing showed significant variations between grazing periods in both years. The highest sward yield at the beginning of the grazing was recorded in Nov. 2011, which can be attributed to very good weather conditions for the vegetation (Table 1).

Table 1. Pasture sward yield in the extended grazing of suckler cows (t ha^{-1} DM).

	2010				2011			
	Oct.	Nov.	Jan.	$LSD_{0.05}$	Oct.	Nov.	Dec.	$LSD_{0.05}$
Before grazing	2.010	1.926	1.750	0.115	2.806	3.479	2.750	0.438
Residual mass	0.377	0.401	0.386	ns	0.736	1.201	0.884	0.186

Utilization rate of the pasture, which in rotational management is largely determined by the target residual DM left at the end of the grazing period relative to the mass at the beginning of the grazing period, was significantly greater in 2010 (79.5%) than in 2011 (69.0%). The highest utilization rate was in Oct. 2010 (81.2%) and the lowest in Nov. 2011 (65.5%).

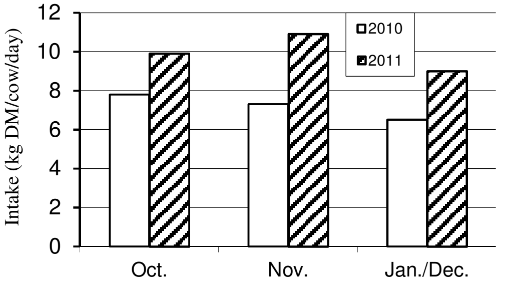

Figure 1. Sward intake in the extended grazing season of suckler cows.

Herbage intake was influenced by weather conditions in the experimental years. Good availability of the pasture sward in Nov. 2011 connected with high yield and favourable weather conditions were the reasons for the highest herbage intake in this grazing period (Figure 1). In Jan. 2011, the intake was 17.4% lower than in Nov. 2011. In 2010, intake decreased during the extended grazing season from 7.8 to 6.5 kg DM cow^{-1} d^{-1}.

Climatic conditions of Central Europe fluctuate much more than the Atlantic climate. This is reflected also in annual fluctuations of the quality of the pasture sward. In general, the quality of sward before grazing was better in 2011 than in 2010. A delayed utilization of herbage mass is generally connected with a decrease in forage quality due to the advanced stage of maturity (Opitz v. Boberfeld *et al.*, 2006). We observed the same relationships in both years of the study. In the third grazing period, compared to grazing in Oct., the concentrations of crude protein, sugars, calcium, potassium and sodium in the sward were significantly lower, while the ash content was higher (Table 2). The concentrations of ADL and magnesium did not differ significantly in the specific grazing periods in both years.

Table 1. Chemical composition of sward in the extended grazing season of suckler cows (g kg^{-1} DM).

	2010				2011			
	Oct.	Nov.	Jan.	$LSD_{0.05}$	Oct.	Nov.	Dec.	$LSD_{0.05}$
Crude protein	196.1	183.4	145.5	15.50	210.2	211.3	173.5	8.13
ADL	224.1	240.6	229.6	ns	295.1	254.2	251.4	ns
Hemicelluloses	199.3	193.8	214.9	8.84	201.6	204.7	207.1	ns
Sugars	107.3	72.3	43.9	21.95	59.6	75.6	66.7	ns
Ash	87.8	97.5	128.2	28.07	80.7	86.7	117.2	31.99
Calcium	10.8	13.4	8.2	2.19	12.9	12.1	10.6	ns
Magnesium	1.3	1.0	0.9	ns	1.5	1.7	1.3	ns
Phosphorus	3.6	3.7	4.2	0.38	3.8	3.7	3.5	ns
Potassium	24.8	21.0	20.7	3.13	24.5	17.1	17.1	4.68
Sodium	2.6	1.9	0.8	0.83	2.4	4.6	1.2	0.83

Skládanka (2010) reported that, under climatic conditions of the Czech Republic, the grazing period can be extended to the beginning of December. The same situation occurred in western Poland where, depending on weather conditions in individual years, extending the grazing season until the end of the year is possible. In some cases, the lasting snow layer limited the utilization of the autumn growths as winter pasture for suckler cows.

Conclusions

In the western part of Poland, the extension of grazing season for suckler cows is possible. In December and January, a decrease in sward yield and sward intake was determined. In these periods, in comparison with late October, concentrations of crude protein, calcium, potassium and sodium in the sward were significantly lower, while the ash content was higher.

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Using mobile milking robots for special quality dairy products based on site-specific grazing

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Abstract

An option for combining grazing and automatic milking is Infield Automatic Milking, with more or less mobile infield automatic milking systems (IAMS), which have been practiced since 2008 in several European countries. This emerging technology can be used to harvest milk from specific areas, giving specialized dairies the possibility to market concept milk, cheese or butter. Terroir dairy can be a new way to increase marginal product value and thereby farm income. Three different options are described: i) the IAMS placed on a stationary platform in the field, ii) movable together with facilities necessary for the functionality from field to field, and iii) as a self-propelled moving unit that changes positions in the field every day, requiring transportation of the milk to the field border. All systems could guarantee dairy products that are based on maximum grazing for the period that the pasture can provide sufficient feeding. Batch size depends on the dairies minimum process volume for the specific products. Local conditions will be decisive for the number of days needed for pooling before production.

Keywords: automatic milking, mobile, infield, sward, milk quality, grazing systems

Introduction

'Site specific' can be used in the wine production industry to describe a product which has its own qualities associated with soil, altitude, angle of sun incline and other factors that can influence the quality of the grape and the corresponding wine. Parallel to this, in dairy production based on grazing, it is often claimed that milk, cheese or butter are different in consistency, taste, smell and colour, depending on the pasture (Monnet *et al*., 2000).
Dairy industries are already seeking new market share by introducing special product types; like, for example, raw-milk cheese, a cheese made of milk not homogenized and not pasteurized and no older than one day. It is claimed that the gentle handling and avoidance of heating gives a better taste. In addition, the organic milk sector is growing, and here the summer grazing is the key benchmarking factor. The dairy industry has lately been especially focused on increasing the milk quality, e.g. lower free fatty acids (FFA) and higher conjugated linoleic acid (CLA). FFA and CLA concentrations in milk are positively influenced by grass diets, especially grazed grass (Oudshoorn *et al*., 2012).
When a dairy industry wants to collect milk, based on pasture, the pasture ratio of the total feed diet has to be dominating. This can often be a problem for farmers lacking sufficient grazing fields adjacent to the barn, where they have to gather the herd for milking once or twice daily. In some countries (Denmark, the Netherlands) the expanding sizes of the herds have forced many farmers to increase the barn-feeding ration to ensure the milk yield, which in practice sometimes even means the outside time for the cows is mostly dedicated as exercise for avoiding hoof and leg problems. In other countries only remote grazing areas exist (mountains, valleys, marshes). Increasing experience and resilience of automatic milking technology has inspired initiatives in several countries to experiment with infield automatic

milking systems (IAMS). In Denmark (DK), the Netherlands (NL), and Belgium (B), different mobile automatic milking systems have been designed and tested (Figure 1).

Figure 1. Mobile Automatic Milking Systems in the field in Denmark, The Netherlands and Belgium.

The experiments were conducted in 2008-10 in Denmark, in 2008-11 in the Netherlands, and they started in 2010 and continue in Belgium. All three prototypes have in common that the automatic milking system is made mobile and therefore can be moved in the field, where the grazing is taking place.

Design and functionality

Denmark: The IAMS was designed as a semi-mobile unit which could be moved to another site. Due to the gates, fences, vacuum slurry tank, diesel generator, water hose connection, and preparation of the waiting area with wood chips, the process of moving took about 4-5 hours. Afterwards the urine and manure-soaked wood chips were removed and stored as manure, to be spread in the spring on arable land. Soil samples underneath the waiting area revealed no increased nitrate, phosphate or potassium concentrations. The IAMS, consisting of two milking units, was tested with a herd of 70 Red Danish milking cows (RDM). The total amount of concentrates in the robot amounted to between 2 and 3 kg; the amount was automatically regulated according to the milk yield average of the last 5 days. Milk yields were around 22 kg per cow in the spring, decreasing to 19 kg in autumn. Major problems were increasing somatic cell counts in the milk, mud around the smart gates and access paths, and declining milk yields in the autumn due to worsening grass quality (Oudshoorn, 2008).
The Netherlands: The IAMS was designed as a fully mobile unit, self-propelled with its own power and traction source, driving on caterpillar tracks. Later in the design process a shuttle was constructed to fetch the milk and drive supplies of diesel and fresh water to the site every 2 days. Due to the daily location shift (or sometimes more frequently), no notable soil pollution or disturbance was induced. The IAMS was tested on an experimental location (Zegveld) with 55-60 Holstein Friesian (HF) and 'Blaarkoppen' cows. Using permanent grazing with free cow traffic, forced strip grazing, and free cow traffic with strip grazing, 20.3, 19.4 and 24.9 kg of milk per cow was obtained, respectively. No extra roughage was supplied, with an average of 5.9 kg concentrates in the robot (De Haan *et al*., 2010).
Belgium: The IAMS in Belgium was designed as a satellite milking station, where the waiting area around the IAMS was hardened with perforated concrete, and drained into a flexible slurry tank. Electricity and water were connected from source, and a small concentrate silo was placed aside the unit. The unit was tested with a herd of 45 HF cows and in the robot an average of 2.15 kg of concentrate was provided. In dry periods some supplementary maize was fed in the pasture. Water was provided in the fields. The cows yielded an average of 19.6 kg milk (Dufrasne *et al*., 2012).

Results of grazing

The systems as described all had the main objective to guarantee a high ratio of pasture grass in the diet. In Denmark, both permanent grazing and high intensive rotational grazing combined with strip grazing was tested. Free cow traffic, with as little manual fetching as possible, was the goal. The rotational grazing system increased the milking frequency to slightly more than two, but also induced some stress in the herd, which can increase somatic cell count. Cows were guided along fenced paths to the IAMS, only allowing them to go back to the pasture after milking. In the Netherlands, permanent grazing was practiced, avoiding manual labour. However, the milking frequency and milk yield seemed to decrease with increasing freedom, in some cases to below two daily the first year. Introducing controlled strip grazing by moving the fence up to three times a day, with free cow traffic, increased milking frequency and milk yield. In Belgium, rotational grazing on 11 paddocks was used, and here the cows were fetched twice a day but, in between, free visits were possible.

Conclusion

Stand-alone units in separate grazing areas automatically milking the cows are possible with milk yields per cow up to 24 kg a day, and will be able to collect milk primarily based on the pasture they are situated in. However, the unit will have to be serviced with concentrates (3-5 kg per cow per day) in order to secure sufficient milking events, water for flushing the system, and electricity for driving the milking system and the cooling of the milk. Depending on the system design, 45-80 cows can be milked, collecting 900-1600 kg of milk daily. The milk yield and composition will be very much dependent on the quality of the pasture, as the concentrates only contribute a small part of the diet. The system could potentially be used by dairies that want to produce special products, associated with exactly the specific pasture composition or location. Benchmarking of the product as 'site specific' and tagging the product with information on location and habitat could justify a higher price, as compensation for the extra costs of infield automatic mobile milking systems.

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Sustainable milk production using low concentrate input

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Abstract

The current experiment belongs to a series running in Europe as a part of the SOLID Project (Agreement no. 266367, http://www.solidairy.eu/) financed by EU FP-7. The general aim is to compare different cow genotypes to identify the degree of adaptation of different breeds to organic and low input systems. In Finland, the breeds were Holstein and Finnish Red. Cows were subjected to two different dietary energy levels: 11.5 MJ ME kg^{-1} DM^{-1} and 11.9 MJ ME kg^{-1} DM^{-1}. The experiment lasts over the whole lactation, and the preliminary results presented here cover the first 100 days in milk. The Finnish Red group had lower milk production (29.2 vs. 31.5 kg d^{-1}) and fewer claw disorders compared to the Holstein group during the first 100 days in milk. Primiparous cows could not fully utilize the high dietary energy content at the beginning of lactation; their milk production was 23.5 kg and 24.0 kg d^{-1} for the low and high energy-level diets, respectively.

Keywords: feeding intensity, health, claw disorder, forage, breed, cow genotype

Introduction

The global increase in food demand combined with climate change will probably reduce the possibilities for production of grain for cattle feed in most parts of the world. Annual precipitation and mean ambient temperature has been predicted to increase in Northern Europe, and these changes in climate conditions would lead to improved possibilities to produce grass. These effects would likely increase the demand to produce milk from forage. This means either the use of highly digestible silage or a decrease in milk yield. Reduced feeding intensity and, consequently, lower milk yield is acceptable only if the production costs are also lowered at the same time. Improved longevity, linked with less intensive milk production, would offer a possibility to decrease the production costs.
The current experiment belongs to a series running in Europe as a part of the SOLID Project (Agreement no. 266367, http://www.solidairy.eu/) financed by EU FP-7. The general aim is to compare different cow genotypes to identify the degree of adaptation of different breeds to organic and low input systems. The breeds included are: Finnish Red and Holstein in Finland, Jersey × Holstein × Swedish Red crossbred and Holstein in Northern Ireland, and lifetime-performance selected Holstein and Swiss Brown in Austria. Every herd includes 'adapted' (the former) breed for low input production and 'conventional' (the latter) breed. Results from Finland only are reported here.

Materials and methods

In Finland the experiment includes 15 Finnish Red (FR) and 32 Holstein (Hol) cows. Cows were fed according to feed recommendations during the dry period and they entered the experiment 21 d prior to calving. The calving dates varied between 6 March and 9 September in 2012. After calving, the cows were subjected to two different dietary energy levels: 11.5 MJ metabolizable energy (ME) kg^{-1} DM^{-1} (Low) and 11.9 MJ ME kg^{-1} DM^{-1} (High). Feeding included first-cut grass silage supplemented with variable amounts of grain and rapeseed

meal. The experiment lasts over the whole lactation and the preliminary results presented here cover the first 100 days of the lactation.
There were fifty cows at the start of the experiment. Three cows were culled at the beginning of the lactation for reasons not related to the experimental treatments. Three primiparous cows in the High group had difficulties in intake from the beginning of the lactation and two of them had lameness symptoms. Despite these problems the cows were included in the analysis.

Results and discussion

The concentrate proportions were 28% and 47% (on a dry matter basis) for the Low and High, respectively. The cows in the High group consumed 1.3 kg DM more feed compared to the Low group (16.2 *vs.* 17.5 kg DM). The difference corresponded to 19.8 MJ ME d^{-1}. The intake was low because of the early stage of the lactation, and the primiparous cows especially had a low intake (13.7 kg DM for primiparous and 20.1 kg DM for multiparous cows). There was no significant difference in intake between the breeds.
Multiparous cows produced 13.2 kg d^{-1} more milk compared with primiparous cows (P<0.001). The difference decreases as lactation proceeds because the persistency in milk production is higher with primiparous cows compared to multiparous. The difference in milk yield between Low and High with multiparous cows was 4.3 kg, whereas the difference was only 0.45 kg with primiparous cows, and showing only a negligible response for them. The Hol cows produced 2.7 kg more milk day^{-1} compared to FR cows (P=0.04).
An average milk response to additional feed energy with multiparous cows was 0.18 kg milk MJ^{-1} ME^{-1}; this is relatively high compared to an average value of 0.11 kg ECM MJ^{-1} ME^{-1} that was reported by Huhtanen and Nousiainen (2012). The response to additional energy decreases as the energy balance becomes more positive, and the high response observed in the current study is most probably due to the early stage of the lactation. Kokkonen *et al*. (2000) reported low responses to additional energy with primiparous cows but the value of only 0.03 kg milk MJ^{-1} ME^{-1} for primiparous cows that was obtained here is exceptional. The primiparous cows in High had the same increase in live weight as that of the primiparous Low cows during the first part of lactation, so changes in body condition did not explain the poor energy utilization.
The claw health was good for the FR group (Table 1). There were no lameness records in Low FR group, and only two records in High FR group. The group size was so small that the overall claw health did not differ between the breeds, according to the results of a chi square test, but in heel erosions the breeds differed significantly (P=0.05). More ketosis could have been expected in the Low group, but the High group had a numerically higher incidence compared to the Low group.

Table 1. The proportion (%) of Finnish Red (FR) and Holstein (Hol) cows in groups fed either Low (11.5 MJ^{-1} kg DM) or High (11.9 MJ kg^{-1} DM) energy content in the diet that were treated for various health problems.

	Low		High	
	FR	Hol	FR	Hol
Claw infections	0	35	13	12
Heel erosion	0	35	0	6
Other claw disorders	0	13	13	24
Ketose	13	29	0	18
Mastitis	13	24	13	24

Conclusions

The preliminary results suggest that the Finnish Red group had lower milk production and fewer claw disorders compared to the Holstein group. Primiparous cows did not respond to the high dietary energy content at the beginning of lactation, whereas multiparous cows had relatively high response to high energy content in the diet. Thus the concentrate proportion for primiparous cows should be low when high digestibility silage is used. This may require the need to group cows according to their parity.

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Response of five introduced forage grass cultivars to salinity stress under irrigation

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Abstract

Some introduced grass cultivars have shown promising adaptability when evaluated under the growing conditions of central Saudi Arabia. Salinity is a limiting factor for forage productivity in this region. The objective of this study was to assess the productivity and measure the relevant characteristics of several introduced grass species using irrigation water with different salinity levels (control, 4000 and 8000 ppm NaCl). Plants were seeded in pots with three replications for each salinity level. The following grasses were used: perennial ryegrass (*Lolium perenne* cvs. Aries and Quartet), endophyte-free tall fescue (*Festuca arundinacea* cv. Fawn), orchardgrass (*Dactylis glomerata* cv. Tekapo), and Rhodes grass (*Chloris gayana* cv. Katambora). Herbage dry weights decreased significantly as the salinity level of the irrigation water increased. Rhodes grass produced the highest level of dry weight under the control treatment. However, its dry weight was 30 and 50% lower at 4000 ppm salinity at the two sample cuttings, respectively. At a salinity of 4000 ppm, the Aries-perennial ryegrass had the highest dry weight at both sample cuttings. Results indicated that salinity treatments had no major impact on forage quality.

Keywords: cool season grasses, salinity stress, forage quality, central region of Saudi Arabia

Introduction

Soil salinity is one of the most significant abiotic stressors, and it is widespread in both irrigated and the non-irrigated regions of the world (Ashraf *et al.,* 2008). The Qassim region is one of the major areas of irrigated agriculture in central Saudi Arabia. The increasingly large areas being used for agriculture in this region have led to salinity problems in both the soil and the water. The present level of salinity in the water in this area ranges from 2000 to 5000 ppm, which creates an obstacle to growing forage crops that are sensitive to the salinity of irrigation water. The responses of some field crops to varying salinity stresses in arid and semi-arid regions have been the subject of investigations (e.g. Pesarraki, 1999). In the most recent studies conducted in Saudi Arabia, a few introduced cool-season grass species showed promising levels of adaptability when evaluated under the conditions found in the central region (Al-Ghumaiz and Motawei, 2011). However, there has been no research on how these species perform under salinity stress. The aim of this study was to assess the responses of these species to different levels of salinity in their irrigation water, in Saudi Arabia.

Materials and methods

The experiment was conducted in 2012 in a greenhouse at the Qassim University Agricultural Research and Experimental Station. The average temperature of the greenhouse during the day was 28.5°C. Five grass cultivars belonging to four species were screened for their salt tolerance levels: perennial ryegrass (*Lolium perenne* cvs. Aries and Quartet), endophyte-free tall fescue (*Festuca arundinacea* cv. Fawn), orchardgrass (*Dactylis glomerata* cv. Tekapo), and Rhodes grass (*Chloris gayana* cv. Katambora). Two salinity levels of irrigated water

(4000 and 8000 ppm NaCl) and a control were applied. The cultivars were seeded in plastic pots on 20 December 2011, in a randomized complete block design with three replications of each salinity treatment. After the trial was established, the plants were clipped and the salinity treatments were applied at the concentrations indicated above. The first and second sample cuttings were obtained on 7 May and 12 June, 2012, respectively. For each pot, the plants were manually clipped and the plant material was then dried at 70°C for 72 hr to obtain the dry weights (g pot^{-1}). The dried samples were ground to provide samples for chemical analyses of the following forage quality parameters: crude protein (CP%), crude fibre (CF%), sugar content (%), ether extract (EE) (%), and ash (%). The chemical analyses were completed at the Forage Analysis Laboratory at the Department of Animal Production and Breeding at Qassim University, according to the procedures used by the AOAC (1990). A Foss TECATOR apparatus (Model:2300 Kjeltec) was used to measure CP and a Model:Fibertec2010 was used for CF analysis. The results were analysed by ANOVA (MSTATC, 1990) and treatment means of four cultivars were compared using the LSD test. Because the dry weight yield of Rhodes grass (cv. Katambora) was so superior the data of this cultivar were analysed separately. Coefficients of variation (CV) were listed to measure the precision of the experiment.

Results and discussion

Results presented in Table 1 indicated significant differences (P<0.05) in dry weight among the four cultivars (PGR/Aries, TF/Fawn OG/Tekapo, and PRG/Quartet) as a result of the salinity stress. There was a significant decrease in dry weight as the salinity level of the irrigation water increased. There were significant salinity × cultivar interactions. OG/Tekapo exhibited the lowest dry weight and had the lowest persistence to salinity stress. PRG/Aries exhibited moderate tolerance to salinity stress when compared with all of the other cultivars tested. However, when salinity was under 4000 ppm, PRG/Aries produced the highest dry weight at both samplings compared with its dry weight at 8000 ppm. At both salinity levels, TF/Fawn produced a similar dry weight at both sample cuttings.

Table 1. Dry weight (g pot^{-1}) of PRG/Aries, TF/Fawn OG/Tekapo, and PRG/Quartet cultivars at different salinities of irrigation water.

Species /Cultivar	1st cut (g pot^{-1})				2nd cut (g pot^{-1})			
	Control	4000 ppm	8000 ppm	Mean	Control	4000 ppm	8000 ppm	Mean
PRG/Aries	10.5	10.7	5.0	8.8a	5.1	6.1	4.4	5.2bc
TF/Fwan	12.4	7.6	5.5	8.5a	9.3	4.8	4.9	6.3b
OG/ Tekapo	9.8	6.8	3.4	6.7b	7.7	3.4	2.9	4.7c
PRG/Quartet	14.3	8.4	4.4	9.0a	10.5	8.2	5.0	7.9a
Mean	11.7a	8.4b	4.6c		8.1a	5.6b	4.3b	
LSD for interaction		2.8				2.2		
CV %		19.9				21.6		

Means within same column and row followed by similar letters are not significantly different at P= 0.05 for cultivar and salinity levels, respectively; PRG= Perennial ryegrass, TF=Tall fescue, OG= Orchardgrass

The results showed RG/ Katambora had the highest dry weight under the control treatment (Table 2) and although it produced the highest total dry weight among the cultivars examined, its dry weight was 30 and 50% lower at 4000 ppm at the two sample cuttings, respectively. The results of the forage quality component analysis indicated that there were significant treatment × cultivar interactions between ash and OM (Table 3). In addition, significant differences between the levels of salinity in OM, ash and EE were found.

Table 2. Dry weight (g pot^{-1}) of Rhodes grass cv. Katambora at different salinities of irrigation water.

Species /Cultivar	1^{st} cut (g pot^{-1})				2^{nd} cut (g pot^{-1})			
	Control	4000 ppm	8000 ppm	Mean	Control	4000 ppm	8000 ppm	Mean
RG/ Katambora	35.1a	24.5b	22.2b	27.3	55.2a	26.8b	23.5b	35.1
CV %		13.7				12.3		

Means within same row followed by similar letters are not significantly different at *P*= 0.05

Table 3. Effects of salinity treatments and cultivars with their interactions on forage quality.

	%ash	%OM	%CP	%CF	%EE	%CAR
			Salinity level (A)			
Control	11.6c	85.2a	21.5	22.5	3.3a	37.9
4000ppm	14.5b	82.0b	20.9	21.2	2.5b	37.4
8000ppm	16.3a	80.2c	21.9	21.1	2.1b	35.0
Sig.	**	**	ns	ns	**	ns
			Cultivars (B)			
PRG/Aries	15.4ab	81.3c	22.1b	19.7c	2.8a	36.7b
TF/Fwan	11.1d	85.6a	24.5a	20.9c	2.7a	37.5ab
RG/ Katambora	13.3c	83.2b	17.0c	25.0a	2.1b	39.1a
OG/ Tekapo	14.1bc	82.5bc	20.8b	22.8b	3.0a	35.8bc
PRG/Quartet	16.8a	79.6d	22.8ab	19.5c	2.7a	34.7c
Sig.	**	**	**	**	*	**
A×B	*	*	ns	ns	ns	ns
CV %	11.4	2.0	9.6	6.6	22.2	4.9

Means within same column and row followed by same letters are ns at $P>0.05$ for cultivar and salinity levels, respectively; * and ** are significant at $P<0.05$ and $P<0.01$ levels of probability, respectively; OM =Organic Matter; CP = Crude Protein; CF = Crude Fibre, EE = Ether Extract; CAR = Carbohydrates (%), PRG = Perennial ryegrass, TF = Tall fescue, RG = Rhodes grass, OG = Orchardgrass

Conclusions

The five introduced grass cultivars showed variations in their adaptation to different salinity levels in their irrigation water. PRG cv. Aries exhibited potential salt tolerance that might allow it to compete with Rhodes grass with regards to productivity under salt stress. Orchardgrass and tall fescue had no potential tolerance of salt stress. In future research, additional sampling events should be considered to evaluate cultivar stability under salt stress.

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Root depth and biomass of tall fescue vs. perennial ryegrass

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Abstract

Tall fescue (*Festuca arundinacea* Schreb.; Fa) is gaining interest due to its good drought resistance compared to other grass species. Although this attribute is commonly explained by the deeper roots of tall fescue, quantitative data on rooting depth are scarce and the effects of soil type and season are hardly known. We measured the root biomass of diploid perennial ryegrass (*Lolium perenne* L.; Lp) and Fa in a series of yield trials differing in soil type, location and management. Soil core samples were taken up to 70 or 90 cm depth in spring and in autumn. Higher root biomass was found for Fa compared to Lp in the deeper soil layers. Both species had higher root biomass in autumn than in spring.

Keywords: root biomass, tall fescue, perennial ryegrass

Introduction

Tall fescue (*Festuca arundinacea* Schreb.; Fa) is gaining interest due to its good drought resistance compared to other grass species (Wilman *et al*., 1998; Reheul *et al*., 2012). The drought resistance of Fa compared to ryegrasses is explained by the deeper rooting of the former especially in deeper soil layers (Wilman *et al.*, 1998; Eekeren *et al.,* 2010; Deru *et al.,* 2012). Durand and Ghesquière (2002) found that tall fescue extracted water down to 180 cm whereas Italian ryegrass (*Lolium multiflorum* Lam.; Lm) was limited to 80 cm, based on data from neutron probe measurements. Quantitative data on rooting depth in the field for Fa in comparison with other species are scarce; hence it is not clear whether the deeper rooting of Fa compared to other species occurs in all soil types and under different management conditions. We compared the rooting depth of Fa and diploid perennial ryegrass (*Lolium perenne* L.; Lp) in different field trials that included both species. We hypothesised that below 30 cm, root biomass would be consistently higher for Fa than for Lp and that the root biomass would differ between soil types and seasons.

Materials and methods

Four herbage yield trials, comprising both Fa and diploid Lp, were sampled for root biomass. Trials were located on different soil types and conducted under different management regimes (Table 1). All trials were complete randomized block designs with three replicates. In the Belgian trials, located at Melle, Merelbeke and Poperinge, the Fa and Lp varieties were 'Castagne' and 'Plenty' respectively. In the Dutch trial located in Helvoirt, the Fa and Lp varieties were 'Barolex' and 'Bargala'. Using a root auger (Eijkelkamp, Giesbeek, the Netherlands), soil cores with a diameter of 8 cm were extracted at six depths (0-15, 15-30, 30-45, 45-60,60-75, 75-90 cm) in the Belgian trials and at seven depths (0-10, 10-20, 20-30, 30-40,40-50, 50-60, 60-70 cm) in the Dutch trial. Two samples for each depth were taken per plot. Soil samples were stored at -18°C prior to washing. Soil was washed from unfrozen samples with tap water on a sieve (0.4mm) and the roots were dried for 24 h at 75°C. The effect of the grass species on the root biomass at the different depths was tested using one-way Anova. In Melle and Helvoirt, we tested the effect of the season (spring vs. autumn) on the root biomass at different depths. Statistics were performed in R, using the aov() function (R Development Core Team, 2011).

Table 4 Trial identification, soil type and management regime.

Trial	*Location*	*Sowing date*	*Soil type*	*Sampling date*	*Management regime*
1 A	Melle (B)	April 2009	Sandy loam	27/4/2011	Mowing (1st cut) followed by grazing
B				16/10/2011	350 kg N yr^{-1}
C				1/10/2012	
2 A	Helvoirt (NL)	September 2007	Sand	13/5/2011	Cutting (4 cuts yr^{-1})
B				5/8/2011	300 kg N yr^{-1}
3	Merelbeke (B)	April 2009	Sandy loam	15/10/2012	Cutting (5 cuts yr^{-1}) 300 kg N yr^{-1}
4	Poperinge (B)	April 2009	Loam	7/11/2012	Cutting (5 cuts yr^{-1}) 300 kg N yr^{-1}

Results

In the upper soil level, no significant differences in root biomass were found between Fa and Lp (Table 2). Below 15 cm, significant differences between Fa and Lp occurred at different locations for different depths (Table 2). At Helvoirt, on the sandy soil, no significant differences were found in root biomass between Fa and Lp (Table 3).

In Melle and in Helvoirt, a significant seasonal effect on the root biomasses was found at some depths. In Melle, Fa had a higher root biomass at 75-90 cm depth in autumn than in spring (P=0.02), but for Lp there was no significant seasonal effect. In Helvoirt, higher root biomasses were found in mid summer than in spring for Fa in the layers 10-20 cm (P=0.008), 20-30 cm (P=0.009) and 40-50 cm (P=0.01) but for Lp only in the 40-50 cm layer (P=0.09).

Table 5. Root biomass (g dry matter m^{-2}) for *Festuca arundinacea* (Fa) and diploid *Lolium perenne* (Lp) measured in sandy loam and loam soils. Soil profile 0-90 cm. Significance of differences between species indicated as *** P<0.001; ** P<0.01; * P<0.05; ns = not significant.

Trial		*0-15 cm*	*15-30 cm*	*30-45 cm*	*45-60 cm*	*60-75 cm*	*75-90 cm*	*0-90 cm*
1 A	Fa	1082.3	167.0	47.0	26.9	17.6	10.1	1350.9
	Lp	847.5	183.3	32.2	14.6	11.0	6.2	1094.8
		ns	ns	ns	ns	ns	ns	ns
1 B	Fa	763.3	108.8	48.6	36.5	37.0	37.4	1031.6
	Lp	811.7	162.6	39.9	31.6	23.7	13.1	1082.6
		ns	ns	ns	ns	ns	*	ns
1 C	Fa	730.9	142.8	59.5	39.3	37.4	26.1	1036.1
	Lp	894.1	118.2	35.1	28.3	13.8	13.8	1103.3
		ns	ns	ns	ns	*	ns	ns
3	Fa	841.0	181.0	113.1	55.0	42.4	38.3	1270.8
	Lp	509.3	106.5	53.6	13.1	1.5	1.0	685.0
		ns	*	*	*	*	ns	*
4	Fa	742.3	71.7	53.1	58.8	45.8	28.5	1000.1
	Lp	692.0	62.4	24.4	12.5	5.2	2.1	798.7
		ns	ns	ns	*	**	***	ns

Table 6. Root biomass (g dry matter m^{-2}) for *Festuca arundinacea* (Fa) and diploid *Lolium perenne* (Lp) measured on a sandy soil in Helvoirt at six different depths. Significance of differences between species indicated as *** P<0.001; ** P<0.01; * P<0.05; ns = not significant.

Trial		*0-10 cm*	*10-20 cm*	*20-30 cm*	*30-40 cm*	*40-50 cm*	*50-60 cm*	*60-70 cm*	*0-70 cm*
2 A	Fa	669.1	83.6	42.6	44.7	15.8	11.8	10.0	877.6
	Lp	1039.2	91.1	76.4	63.4	14.6	8.9	5.1	1298.7
		ns	ns	ns	ns	ns	ns	ns	ns
2 B	Fa	821.2	195.6	120.5	101.8	42.2	55.1	45.6	1382.0
	Lp	1263.5	165.6	135.6	55.6	40.2	18.7	7.9	1687.1
		ns	ns	ns	ns	ns	ns	ns	ns

Discussion

The results found for root biomass were in line with the results of Hejduk and Hrabe (2003) who found a root biomass of 976 g m^{-2} in a sandy loam soil in the Czech Republic in a soil layer of 0-200 mm averaged over a period of five years on grassland with different grazing managements and fertilizations. Eekeren *et al.* (2010) found root biomasses of 1034 g m^{-2}, 217 g m^{-2} and 135 g m^{-2} for the 0-10 cm, 10-20 cm and 20-30 cm layers averaged over five grass treatments under a cutting regime on a sandy loam soil in the Netherlands. Significant differences in root biomass between Fa and Lp occurred mostly in the soil layers below 30 cm and the difference between the Fa and Lp increased with depth. In contrast to Eekeren *et al.* (2010) and Deru *et al.* (2012) we found no significantly higher root biomass for Fa on the sandy soil in Helvoirt, neither in spring nor in mid summer of 2011. The methodology might explain these differences: the samples from Helvoirt contained a lot of organic material (peat) that was hard to separate from the roots, especially from the fine roots of Lp.
In the sandy loam and loam soils, root biomass of Fa below 45 cm tended to be substantially higher than that of Lp, indicating better access to water in the deeper soil layers. The differences were most accentuated (and nearly always significant) in the samples taken in the autumn in the plots under an intensive cutting regime. Root biomass in these plots below 45 cm was approximately 10% and 2% of total root biomass for Lp and Fa respectively.
While total root biomass in the autumn was comparable for Fa and Lp in the predominantly grazed trial, Fa had at least 20% to 50% more root biomass than Lp in the trials with an intensive cutting regime, indicating that the potential benefit of Fa may be more important under a cutting regime.
As the leaves of Fa have a longer lifespan than leaves of Lp, it is recommended to analyse the difference in lifespan of roots in order to get a better view on root dynamics of Fa.

Conclusions

A consistently higher root biomass for Fa compared to Lp was found below 40-45 cm, but over the whole soil profile the root biomass of Fa was not necessarily higher than that of Lp.

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Effects of pre-acclimation temperature on cold tolerance and photoinhibition in forage species

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Abstract

The expected temperature rise in late summer - early autumn can change the conditions for acclimation and affect the winter survival of perennial crops. This is especially relevant in arctic regions where light is limited. Here we studied the effect of pre-acclimation temperature on cold tolerance of timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.), including entries (cultivars/breeding populations) adapted to the northern and southern parts of Norway. A phytotron study was performed in order to test 1) if increasing pre-acclimation temperature decreases cold acclimation ability, delays growth cessation and reduces photosynthetic acclimation to cold, and 2) if northern populations are more affected than southern populations. The results showed that a rise in pre-acclimation temperature decreases cold acclimation and photosynthetic acclimation. However, there were quite large differences between the species. On the other hand, there were no consistent differences in responses of the northern compared with the southern adapted entries.

Keywords: acclimation, chlorophyll fluorescence, red clover, timothy, perennial ryegrass

Introduction

Cold acclimation is a quantitative trait controlled by a complex interaction between light and temperature. Rising temperatures at the end of the growing season (i.e. late summer-early autumn) may delay growth cessation and reduce cold acclimation ability, including photosynthetic acclimation to cold (Gray *et al.*, 1997). Photosynthetic activity may be inhibited when the relative amount of absorbed photochemical energy is higher than the transfer of this energy to final acceptors (ATP, NADPH), known as photoinhibition. The photosynthetic rate in the dark phase is considerably reduced at lower temperatures that trigger photoinhibition. Photosynthetic acclimation to cold-induced photoinhibition seems to be closely related to freezing tolerance (Rapacz *et al.*, 2004). In this study, we used chlorophyll fluorescence-based techniques and traditional freezing tests to study pre-acclimation to cold of promising entries of common perennial forage species in Norway.

Materials and methods

Seedlings of perennial ryegrass, timothy and red clover, adapted to northern and southern parts of Norway (Table 1), were established in a phytotron. After four weeks at 20°C and 24 h photoperiod (150 µmol m^{-2} s^{-1}) the seedlings were subjected to three different pre-acclimation temperature treatments, 9°, 12° and 15°C for 5 weeks, the first 2 weeks at 14 h photoperiod followed by 3 weeks at 12 h photoperiod, before all entries were cold acclimated at 2°C for 3 weeks. Plants were grown under artificial light conditions (150 µmol m^{-2} s^{-1}) during the whole experiment. The effect of photoinhibition was studied by means of chlorophyll fluorescence measurements (Pam-2500 Portable Chlorophyll Fluorometer; Heinz Walz GmbH, Germany) performed after high-light treatment (800µmol, 5 min., at room temp.). The measurements were taken before and after pre-acclimation and after cold acclimation. Freezing tests at the end of the experiment were performed as described by Larsen (1978) where crown segments were placed in plastic boxes covered with humid sand. A total of 6 different temperatures were used in addition to the control for which the crown segments were kept at 2°C. For each temperature there were two replicates, with 10 plants in each replicate. The LT_{50} values, the temperature at which 50% of plants are killed, were estimated based on scoring the regrowth of the plants after 3-4 weeks of growth at 20°C using probit analysis.

Results and discussion

Generally, frost tolerance increased in plants grown at lower pre-acclimation temperatures, though the differences were not significant within all entries (Table 1). There were no significant differences in LT_{50} values between the different temperature treatments in perennial ryegrass *FuRa9805* and red clover *B1D2+D3+VåRk0734*. Furthermore, there was no clear trend in how southern-adapted entries responded to different temperatures compared to the northern-adapted. The grasses expressed reduced photoinhibition (i.e. higher F_v/F_m values) when exposed to pre-acclimation temperatures (Figure 1). Moreover, plants pre-acclimated at lower temperatures expressed less photoinhibition after either pre- or cold acclimation. There were no differences between northern and southern adapted entries of timothy, but the southern adapted perennial ryegrass *Fura9805*, was particularly sensitive to cold-induced photoinhibition without pre-acclimation. Red clover was more tolerant to photoinhibition than grasses, and during the experiment no photosynthetic acclimation to cold was observed during pre-acclimation.

Table 1. Frost tolerance as LT50 values and 95% confidence interval for the entries tested. *estimated values: LT_{50} interval not reached.

Entry (cultivar/breeding population)	Species (origin)	Pre-acclimation temperature treatment (°C)	LT_{50}	95% Confidence interval	
				lower	upper
FuRa9805	Perennial ryegrass (southern adapted)	9	**-16.0**	-16.0	-15.9
		12	**-16.0**	-16.0	-15.9
		15	**-15.0**	-15.0	-15.0
Fagerlin	Perennial ryegrass (northern adapted)	9	**-17.4***	-	-16.2
		12	**-15.6**	-16.1	-15.0
		15	**-14.1**	-15.0	-13.4
MTL9701+Grindstad	Timothy (southern adapted)	9	**-14.8**	-15.8	-13.2
		12	**-14.7**	-15.4	-13.7
		15	**-11.0***	-13.5	-
MTV0508-3	Timothy (northern adapted)	9	**-19.3**	-20.4	-18.3
		12	**-14.8**	-15.5	-13.9
		15	**-14.1**	-15.0	-12.5
LøRk0393/0395	Red clover (southern adapted)	9	**-14.1**	-15.0	-13.3
		12	**-14.2**	-15.0	-13.6
		15	**-11.9**	-12.8	-11.1
B1D2+D3+VåRk0734	Red clover (northern adapted)	9	**-15.1**	-16.7	-14.1
		12	**-14.2**	-15.2	-13.5
		15	**-14.6**	-15.4	-14.0

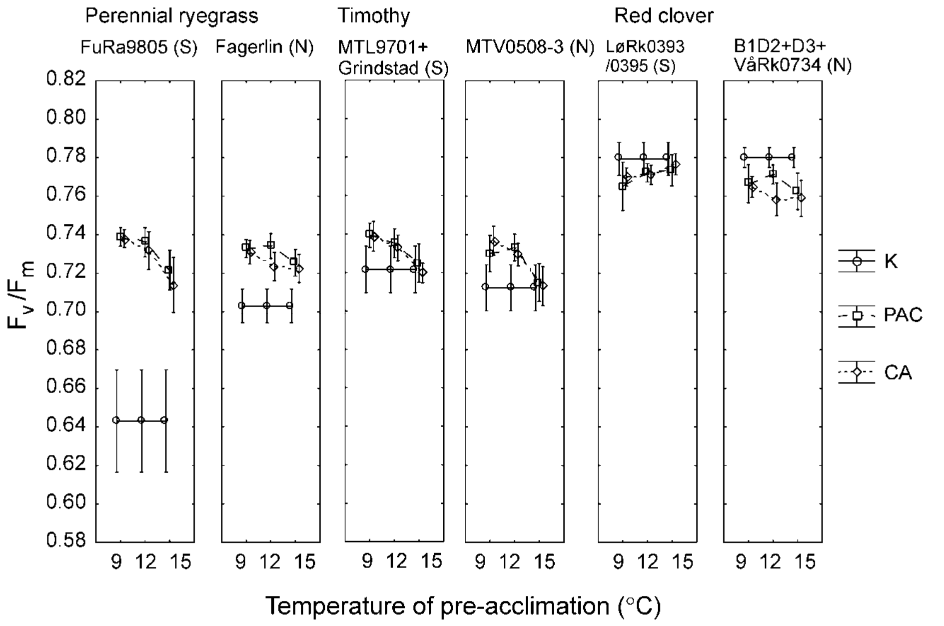

Figure 1. Values of F_v/F_m as indication of photoinhibition measured before pre-acclimation (K), after pre-acclimation (PAC) at different temperatures and after subsequent cold acclimation (CA) in the entries of perennial ryegrass, red clover and timothy. (S) and (N) denotes southern and northern adaptation of the entry, respectively. Means ± confidence intervals for P=0.05.

There were no differences between northern and southern adapted entries of timothy, but the southern adapted perennial ryegrass *Fura9805*, was particularly sensitive to cold-induced photoinhibition without pre-acclimation. Red clover was more tolerant to photoinhibition than grasses, and during the experiment no photosynthetic acclimation to cold was observed during pre-acclimation.

Conclusion

Increasing pre-acclimation temperatures reduced the frost tolerance in red clover, perennial ryegrass and timothy. The grass species expressed reduced photoinhibition when exposed to lower pre-acclimation temperatures.

Acknowledgements

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Molecular regulation of flowering in timothy (*Phleum pratense* L.)

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Abstract

Timothy (*Phleum pratense* L.) is the most widely grown perennial forage grass in Scandinavia. It is a long-day plant that flowers when the critical day length has been exceeded. Our earlier results show that vernalization accelerates flowering in timothy. Flowering is regulated by several endogenous and environmental factors, and numerous genes have important role in the complex regulation system. The genomic information available for timothy is limited, compared to that for other monocots and grass species. To study the flowering regulation of timothy at molecular level, five cDNA libraries (vernalization, gibberellic acid, photoperiod treatments) were sequenced and annotated. Several putative homologs of timothy flowering genes were identified, such as *VRN1*, *VRN3, LpMADS10, PpD1a* and *GaMYB,* and the expression was studied in timothy genotypes of different origin exposed to different vernalization or photoperiod conditions. Both phenotyping and molecular studies showed that northern genotype required 10 weeks vernalization for flowering, whereas southern genotype did not respond to vernalization.

Keywords: cDNA library, *Phleum pratense* L., timothy, vernalization, *VRN1*, *VRN3*

Introduction

Timothy is a widely grown forage grass species in temperate areas. The timothy canopy consists of three different tiller types (VEG, ELONG and GEN) and the contribution of tillers determines the quality and quantity of the yield. It has vegetative elongating tillers, which have true pseudostems, but they do not flower. In the field, flowering tillers are dominant in the primary growth and the proportion of vegetative and vegetative elongating tillers is minor (Virkajärvi *et al*., 2012). Timothy requires long days for flowering, but it has also been shown that vernalization accelerates flowering, although it does not necessary have an obligatory requirement for it (Seppänen *et al*., 2010).

A complex network of genes regulates flowering induction in monocots. In cereals there are three genes which determine the vernalization requirement (Trevaskis *et al.*, 2007); *VRN1* gene induces flowering after low temperature exposure, *VRN2* represses floral induction in order to avoid flowering before winter and prevents the induction of *VRN3* under long day conditions and *VRN3* promotes the expression of *VRN1* and finally allows flowering (Distelfeld *et al*., 2009).

The aim of this study was to i) determine the vernalization requirement for two genotypes of different origin, and ii) gain more information on flowering regulation at the molecular level in timothy.

Materials and methods

cDNA libraries from cultivar 'Iki' were prepared and sequenced after five different vernalization, photoperiod and gibberellic acid treatments. Produced contigs were compared to sequences of closest model species (*Lolium perenne*, wheat, *Brachypodium distachyon*) and this information was used to design timothy specific primers for gene expression studies.

Timothy breeding lines originating from southern and northern latitudes (later referred to as genotypes) were tested in a greenhouse for vernalization and day length responses. Clonal plants were propagated and vernalized in growth chambers for 0, 2, 10, 12 or 15 weeks and transferred to a greenhouse with 12, 16 or 20 hours day length, where the heading date, tiller composition and final leaf number were observed. RNA samples were taken to investigate *VRN1*, *VRN2* (*LpMADS10*) and *VRN3* expressions using q-RT-PCR.

Results and discussion

Vernalization time affected the canopy structure of the two tested genotypes (Figure 1). The southern genotype did not have any response to vernalization and heading time remained unchanged in all treatments. In contrast, the northern genotype had 10 weeks vernalization requirement for flowering and it only flowered after 10 and 12 weeks vernalization in 16 hours photoperiod. In the 20-hour photoperiod the northern genotype flowered without vernalization. Previous findings have defined timothy as a plant species that only requires long day lengths to flower (Heide 1994). However, vernalization has an important role for the yield formation of timothy (Seppänen *et al*., 2010). Here we indicate that genotypes from different origin can have significantly different flowering regulation and vernalization requirements.

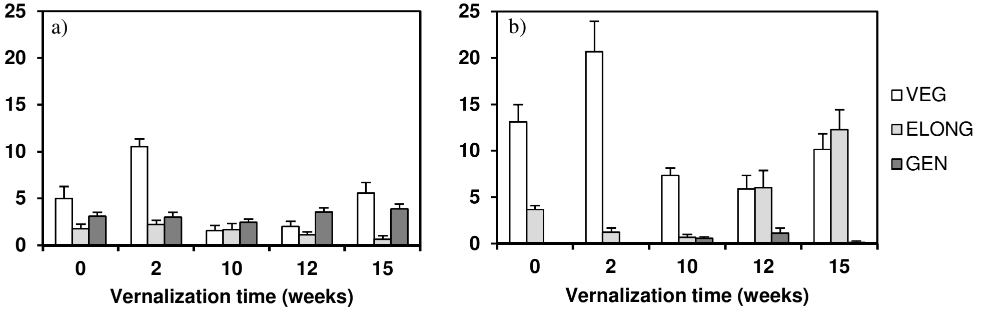

Figure 1. The number of vegetative, vegetative elongating and generative tillers in a) southern and b) northern timothy genotype after 0 to 15 weeks vernalization and 16 hours photo period. N=9 ± SE.

The 454 sequencing produced a total of 435258 reads, which were assembled using 454 Newbler assembler. A total of 70083 contigs were assembled with an average contig size of 311 bp, and 14884 large contigs with an average size of 853 bp. Annotation revealed numerous flowering genes, which included putative homologs of the widely studied *VRN1*, *VRN2* (*LpMADS10*) and *VRN3* genes.

VRN1 was expressed in the southern genotype after all vernalization treatments with highest expression after 10 weeks vernalization (Figure 2). For the northern genotype a minimum of 10 weeks vernalization was required for the expression of *VRN1,* supporting the results of phenotyping (Figure 1). *VRN2* transcript level was higher in the northern genotype especially in plants exposed to short vernalization. As the vernalization time prolonged, the expression of *VRN2* decreased. Abundant *VRN3* expression was detected in both non-vernalized and vernalized plants of the southern genotype, whereas in northern genotype the level increased as the time of vernalization increased. The regulation of the studied flowering genes seems to be similar in timothy compared to other monocots (Dubcovsky *et al*., 2009). Our earlier studies have shown, that the expression of *VRN3* is essential for the flowering stimulus and the high transcript of *VRN1* alone is insufficient to allow flowering in timothy (Jokela *et al*., unpublished).

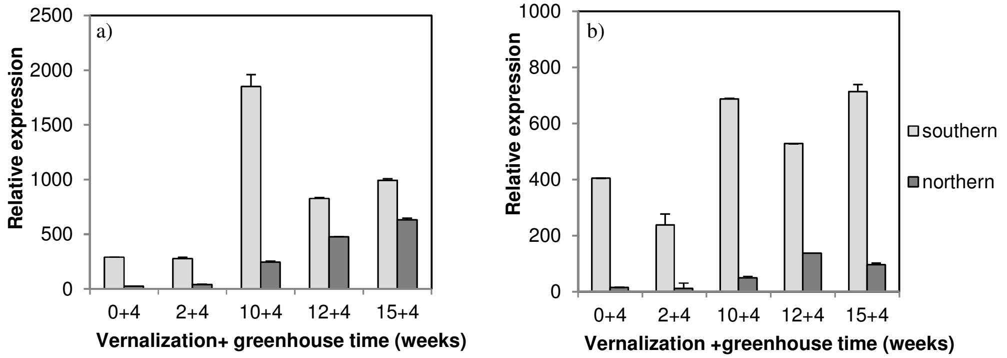

Figure 2. The relative expression of a) *VRN1* and b) *VRN3* homologs after 0 to 15 weeks vernalization and four weeks in a greenhouse in 16 hours photoperiod.

Conclusion

These results reconfirm the essential role of vernalization and photoperiod in the regulation of flowering in timothy. The expression studies of vernalization genes supported different strategies and requirements for flowering in two tested genotypes from different latitudes.

Acknowledgement

This study has been supported by Finnish Cultural Foundation.

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Yield performance of forage grasses on both shores of the Baltic Sea

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Abstract

We evaluated 27 forage grass cultivars bred in the Baltic States and Sweden for their agronomic performance with the aim of identifying those with export potential. Field plots were managed according to local practices in 2010-2011. The highest rate of fertilizer input contributed to the highest herbage dry matter yield (DMY) in Sweden, followed by Estonia, Latvia and Lithuania. Over a sum of years *Dactylis glomerata* L. (*Dg*) mainly ranked first and *Lolium perenne* L. (*Lp*) last. *Festulolium* Asch. (*Fl*) was the most productive in Latvia. Domestic cultivars of *Festuca pratensis* Huds. were superior to foreign cultivars at all testing sites. ×*Festulolium* 'Punia', *Lolium* × *boucheanum* Kunth. 'Saikava' and *Phleum pratense* L. 'Switch' ranked first across the testing sites, where they were present. In addition, *Lp* 'Elena' and 'Birger', *Dg* 'Aukštuole' and 'Priekulu 30' showed evidence of their export potential through wide adaptation, approved by realization of their DMY potenial. DMY values of *Dg* were stable during consecutive harvest years. In Sweden the grass production varied least during the two years. Species' mean DMYs, summed over the testing period, varied 2.1-2.7 times across the locations. All the examined cultivars withstood the specific natural conditions and forage production systems.

Keywords: *Lolium, Festulolium, Phleum, Dactylis, Festuca*, herbage yield

Introduction

Phleum pratense L. (*Pp*), *Festuca pratensis* Huds. (*Fp*), *Dactylis glomerata* L. (*Dg*), and *Lolium perenne* L. (*Lp*) are important components of permanent grasslands in sustainable grassland ecosystems throughout temperate climate zones, especially in northern Europe. In addition, intensive farmers seek productive species with higher forage quality; e.g. ×*Festulolium* Asch. (*Fl*) and *Lolium* ×*boucheanum* Kunth. (*Lb*) can improve livestock performance. In order to assess the value of the released grass cultivars from the perspective of the seed trade, knowledge about their agronomic performance is essential. Crossover interactions between cultivars are common in variety evaluation schemes with the implication that a cultivar that is superior in one environment may not maintain this superiority in another (Conaghan *et al*., 2008). Breeding programmes currently target the standardized national testing systems rather than the actual management systems used by European farmers. For the purposes of evaluation schemes the relative performance of cultivars is more important than the absolute performance as this is only relevant to the test site and conditions (Burns *et al*., 2011). National evaluation schemes for forage grasses evaluate cultivars based on yield, persistence, disease resistance and, to a lesser degree, quality attributes.

Our paper compares the seasonal rankings of forage grass cultivars based on their dry matter yield (DMY). Mutual testing aimed at selecting the cultivars that fit into a wider array of soil, climatic and management conditions, as well as those prevailing in their country of origin.

Materials and methods

Plants and harvest. Four plant breeding institutions (Table 1) provided 6 cultivars of *Lp*, 1 *Lb,* 1 *Fl,* 6 *Fp,* 6 *Dg, and* 7 *Pp* for the exploration of their agronomic value. The initial harvests were done at heading; the aftermaths' harvests were decided independently in each site. The sum of DMYs harvested during two years was considered a measure of production potential.
Experiment setup and soils. Standard field trials with plot sizes between 5.9 and 18.5 m^2, sown in three to four replicates, were established by the methods of randomized complete blocks or lattice design in spring and summer 2009 in four countries – Estonia (EE), Latvia (LV), Lithuania (LT), and Sweden (SE). The experiments were drilled in May (LV, SE), June (LT), and July (EE) into calcic luvisol (EE), sod-podzolic loam (LV), endocalcari-epihypogleyic cambisol (LT), and eutric cambisol (S). Table 1 summarizes the managements.
Fertilization. A single nitrogen (N) input onto the grass swards in the pasture should not exceed 70 kg ha^{-1}. Therefore the fertilizer doses applied to *Lp* in EE in 2010 remained lower than these used in the tall species. Four harvests of *Dg* per season in SE show its full yield potential. To exploit the vigour and to preclude herbage quality deterioration, extra NK-fertilizer was applied after the third harvests of *Dg* on both years.
Weather. The sums of precipitation from May to October in 2010 and 2011 were highest in SE (459 and 555 mm), followed by LV (526 and 405 mm) and LT (470 and 388 mm); lowest values were in EE (405 and 307 mm). Mean air temperatures for the same periods were between 12.4 and 13.6°C in SE in 2010 and 2011, and between 14.7 and 14.9°C in LT.
Statistical analysis. Significance of the differences (P<0.05) among the cultivars was detected by ANOVA, performed by using software Agrobase™ 20. The cultivars were ranked in each location on the basis of total DMYs, using the rank analysis tool of Microsoft Excel.

Table 1. Seeding rates, harvest regimes, and fertilization of *Lolium* species *(L.*sp.*), Festulolium (Fl), Phleum pratense (Pp), Festuca pratensis (Fp),* and *Dactylis glomerata (Dg)* at four testing locations.

Site, country	Seed rates *L.*sp,*Fl/Pp/Fp/ Dg* (g m^{-2})	No. cuts 2010/ 2011	Nutrient application (kg ha^{-1}) and yearly totals (∑)	
			2010	2011
Jõgeva (EE)	3.0/1.0/3.3/2.0	4 / 3	*L.*sp.: 4 x N60-P4-K7 *L.*sp.: ∑ 240-16-28 *Pp, Fp, Dg*: N80-P6-K10 + 3 x N70-P5-K8 ∑ 290-21-34	N80-P6-K10 + 2 x N60-P4-K7 ∑ 200-14-21
Skrīveri (LV)	2.0/1.2/2.5/1.5	3 / 2	3xN60, ∑ 180-0-0	3 x N60, ∑ 180-0-0
Dotnuva (LT)	3.1/1.0/2.2/1.8	3 / 3 *L.*sp: 3 / 2	N60+2xN45-P26-K75 ∑ 150-26-75	N60 + 2 x N45-P26-K75 ∑ 150-26-75
Svalöv (SE)	2.5/1.5/2.3/1.9	3 / 3 *Dg*: 4 / 4	N95 + N80-K60 + N60-K45 (*Dg*: + N60-K45) + P42-K150 ∑ 235-42-255 *Dg*: ∑ 295-42-300	N95 + N81-K116 + N62-K88 (*Dg*: + N62-K88) ∑ 238-0-204 *Dg*: ∑ 300-0-292

Results and discussion

The highest average DMYs per site in 2010 and 2011 were 14.28 and 14.55 Mg ha^{-1} (SE); the lowest DMYs were 6.93 (LV) and 3.45 Mg ha^{-1} (LT), respectively. This was mainly caused by divergent nutrient rates, and to a lesser extent by the weather as the coolest seasons in SE and driest in EE produced higher DMYs than in more favourable circumstances in LV and LT. In *Fp* at all sites the domestic cultivars were superior to the foreign ones. *Fl* 'Punia', *Lb* 'Saikava', and *Pp* 'Switch' ranked first at each testing site. Cultivars 'Raidi', 'Arni', 'Minto', ‘Silava’, ‘Regenta’, ‘Luxor’, ‘Jauniai’, and ‘Jõgeva 54’ demonstrated their superiority in one location and inferiority in another. Their genotypes interact more with local environments.

The DMYs declined during two consecutive years at most locations and species, but not in *Pp* in SE, nor for *Dg* in SE and EE. *Dg* was the most productive species in both years at LT and SE and in the second year at EE. In LV, timothies produced the highest herbage yields. The rank of species there (*Fl*>*Lb*>*Pp*>*Lp*>*Dg*>*Fp*) was disinct from the remaining countries (*Dg*>*Pp*>*Fp*>*Fl*>*Lb*>*Lp*). This is mainly caused by the limited nutrient application in LV, which hindered the growth of *Dg* in particular. It is competitive under abundant fertilization.

Herbage yield was very dependent on regional precipitation. The production rise in SE in the second year was apparently conditioned by 1.2°C higher temperatures, and by 96 mm more rainfall than in the first growing season. In the Baltic States, conversely, the growing periods were only by 0.1-0.6°C warmer in 2011, but considerably drier (by 82-121 mm) than in 2010. Thus the DMYs of species in the second harvest year varied considerably in relation to the first year. The ratio was most pronounced (1.20) for *Pp* in SE. In EE, LV, and LT these maximum ratios appeared in *Dg*, being 1.05, 0.93, and 0.67, respectively. *Dg* contrasted with *Lp*, in which annual herbage production typically declines with age.

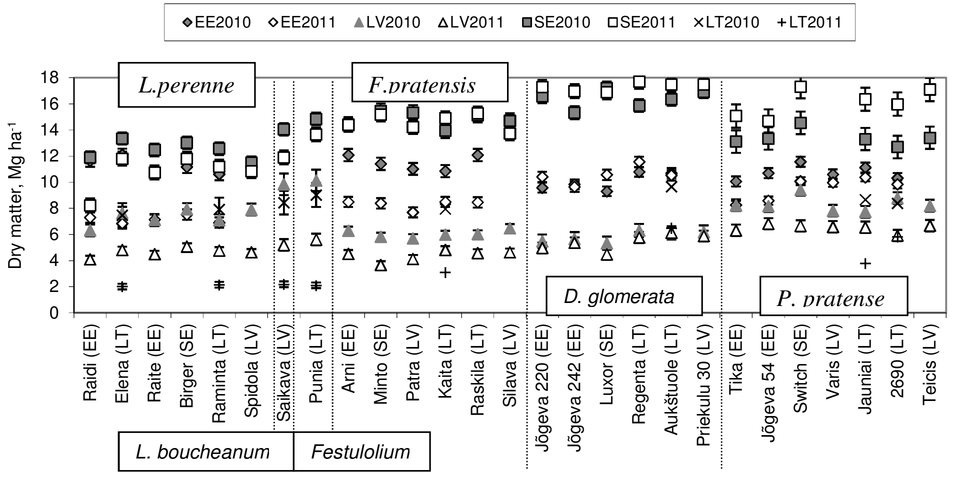

Figure 1. Annual herbage dry matter production of forage grass cultivars in Estonia (EE), Latvia (LV), Sweden (SE), and Lithuania (LT) in 2010 and 2011. Bars indicate least significant differences at $P<0.05$ for a species in a testing location.

Conclusions

Widely adapted *Fl* ‘Punia’, *Lb* ‘Saikava’ and *Pp* ‘Switch’ can be marketed to complement the set of domestic forage grasses in these four countries. *Dg* and *Pp* were most productive under specific growing conditions and utilization methods in EE, LT, and SE, *Fl* and *Lb* in LV.

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The role of vernalization in freezing tolerance and tiller composition of forage grasses

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Abstract

In Finland, leys for silage are typically productive for three to four years after which they are renewed. For high dry matter (DM) yields adequate winter hardiness of perennial forage grass species is required. In winter, cold acclimation occurs simultaneously with vernalization, i.e. induction of flowering, after which the winter hardiness is gradually lost. In forage grasses vernalization results in a rapid development of stem-forming tillers and affects yield formation significantly. Our results from field experiments show that the vernalization saturation in timothy, meadow fescue and perennial ryegrass genotypes occurred in January-February, which was later than the initiation of deacclimation. It indicates that these processes may not be as tightly linked together in forage grasses as in winter cereals. The tiller composition of 1st and 2nd harvests was strikingly different in all studied forage grass species. In timothy, genotypic variation in DW and number of flowering tillers in the 2nd harvest was observed, so that in the northern genotypes that required vernalization, fewer flowering tillers were measured.

Introduction

Low freezing temperatures and long winters are the major limiting factors restricting the number of forage grass species and cultivars for leys in Finland. In winter cereals, low temperature acclimation and improved freezing tolerance is a cumulative process until the vernalization saturation and the veg/gen transition of apices takes place (Fowler *et al*., 1996). Although recent molecular studies have shown that the regulatory pathways for cold acclimation and vernalization are independent (Bond *et al*., 2011), the close association between these processes can be utilized in the breeding of cultivars for various overwintering conditions. As the vernalization saturation of winter cereals has been achieved, the decrease in heading date (HD) and final leaf number (FLN) levels off, and there is no further response to prolonged vernalization time (Mahfoozi *et al.,* 2005). To our knowledge there have been are no previous studies on vernalization saturation in forage grasses.

In intensive forage-silage production, leys are harvested two to three times a year. The first harvest consists of vernalized tillers where both stem elongation and flowering are induced, whereas the second and third harvests consist of non-vernalized tillers. Recent studies on timothy (*Phleum pratense*) have shown that vernalization has a major impact on the canopy structure and tiller composition of harvested herbage biomass (Seppänen *et al*., 2010; Virkajärvi *et al*., 2012). In timothy, three tiller types have been characterized: vegetative (VEG), vegetative elongating (ELONG) and generative (GEN) (Seppänen *et al*., 2010; Virkajärvi *et al*., 2012), and it seems that vernalization accelerates the veg/gen transition and decreases the number of ELONG tillers (Jokela *et al.,* 2012). Transition to the generative stage and ability to develop flowering tillers are associated with transcript accumulation of two major regulatory genes in the vernalization pathway, *VRN1* (Seppänen *et al*., 2010) and *VRN3* (Jokela *et al*., 2012). The quantity of the second and third harvests also correlates well with the abundance of stem-forming tillers (Virkajärvi *et al*., 2012). Therefore, the regulation of stem elongation and flowering in forage grasses is of great interest. The aim of this study was to reveal i) when vernalization saturation takes place under field conditions; ii) how it is related to deacclimation; and iii) how vernalization affects the tiller composition of different forage grass species.

Materials and methods

Freezing tolerance (LT_{50}) and the development of vernalization (FLN – final leaf number, FTN – final tiller number) were monitored in field experiments located at the Viikki Experimental Farm, Finland during winters of 2009-2010 and 2010-2011 (eight timothy, five perennial ryegrass, meadow fescue, tall fescue and festulolium cultivars) and in 2011-2012 (six timothy, three meadow fescue and one perennial ryegrass cultivars or breeding lines).
Samples were collected from fields once a month from November to April and subjected to freezing tests (Lauda Proline RP3530). The viability was estimated by regrowth 4 to 6 weeks after the freezing treatments (Seppänen *et al.*, 2010). Days to heading (HD) was defined as the time required from planting to the emergence of the first visible heads in a studied genotype. Herbage biomass was harvested twice during the growing period and the tiller composition was analysed by calculating the number and dry weight of generative (GEN), elongating vegetative (ELONG) and vegetative (VEG) tillers.

Results and discussion

Cold acclimation processes proceeded in forage grasses till December, after which deacclimation and a gradual loss of freezing tolerance were observed. Timothy cultivars differed in their ability to produce flowering tillers in early autumn. The northernmost cultivar (cv. Tuure) required vernalization for flowering, whereas other studied genotypes had already developed flowering tillers in October (49 to 67 days after transfer to greenhouse). In March the difference between timothy genotypes disappeared and all genotypes flowered after 34 days. Meadow fescue and perennial ryegrass flowering tillers were detected starting from December. FLN and FTN decreased as the winter proceeded in all species (Figure 1), except in some tall fescue and festulolium cultivars where FTN increased towards spring. Vernalization saturation occurred after mid-winter in early February, which was much later than the observed deacclimation. FTN continued to decrease until April. The results indicate that in forage grasses the veg/gen transition and deacclimation may not be as tightly linked together as they are in winter cereals. The field observations here are supported by our earlier results where the veg/gen transition coincided with elevated expression of *VRN1* without any significant loss of freezing tolerance (Seppänen *et al.*, 2010).

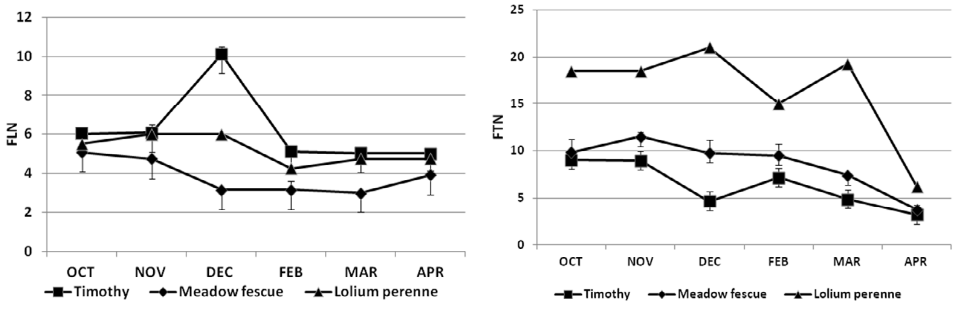

Figure 1. Development of vernalization in timothy (n = 6), meadow fescue (n = 3) and perennial ryegrass (n = 1) genotypes during winter 2011-2012. Samples were collected from field experiment once a month and transferred to greenhouse where final leaf number (FLN) and final tiller number (FTN) was monitored.

The tiller composition of the 1st and 2nd harvests was strikingly different in all species (Figure 2). GEN tillers were heavier than ELONG or VEG tillers and the DW of the 1st harvest was dominated by GEN tillers. In meadow fescue and perennial ryegrass the 2nd harvest was composed mainly of VEG tillers, whereas in timothy the number and DW of ELONG and GEN tillers was higher. The results are preliminary, representing the first year of the field experiment. It seems, however, that there exists genotypic variation in timothy in the number and DW of GEN tillers in the 2nd harvest.

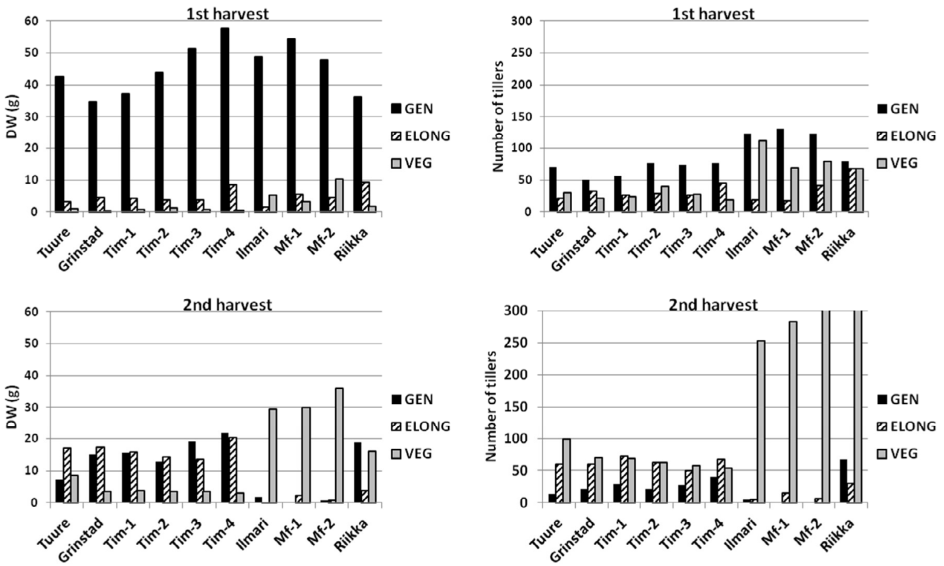

Figure 2. Tiller composition (DW, dry weight (g) and number of tillers) of 1st and 2nd harvest of timothy (Tim), meadow fescue (Mf) and perennial ryegrass genotypes. GEN = generative, ELONG = elongating vegetative and VEG = vegetative tillers. N = 4.

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Session 2

Conservation of Extensive Grasslands

Semi-natural grasslands as a source of genetic diversity

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Abstract

Semi-natural grasslands are grasslands created by long-term human management with variable species richness and productivity. Populations from such grasslands are often collectively termed ecotypes. Most of the gene bank accessions of forage grasses and legumes have been collected in such semi-natural grasslands. Genetic diversity is indispensable for breeding new cultivars. Although breeders today cross genotypes from existing cultivars to create base material for breeding, most of the current cultivars trace back to breeding material originally created from collected ecotypes. Genetic resources are usually characterized genetically by neutral molecular marker variation, and often also phenotypically by a range of descriptor traits. Forage species are outbreeding crops; thus a major part (63-98%) of genetic variation is found within populations. The geographic structuring of molecular and phenotypic variation often does not coincide, and phenotypic variation in ecotypes is often larger than in cultivars. Unique phenotypic variation is present in ecotypes and demonstrates the value of semi-natural habitats as reservoirs of genetic resources. Therefore genetic resources need to be properly characterized both using molecular markers and phenotypic traits. Association of molecular marker variation with phenotypic variation and utilization of this information in breeding improved cultivars is, however, a big challenge.

Keywords: semi-natural grasslands, genetic diversity, molecular markers, phenotypic diversity, grasses, legumes

Introduction

The 'International Forage and Grazing Lands Terminology Committee' (Allen *et al.*, 2011) define semi-natural grassland as 'a managed ecosystem dominated by indigenous or naturally occurring grasses and other herbaceous species', while naturalized grassland contain 'forage species primarily introduced from other geographical regions that have become established and have persisted under the existing conditions of environment and management over a long time'. According to Hejcman *et al.* (2012) grasslands in Europe can be divided into three major types depending on their origin: i) natural grasslands such as the steppes of Eastern Europe and alpine and arctic grasslands, conditioned by low precipitation and low temperature/short growing seasons; ii) semi-natural grasslands created by long-term human intervention and with a wide range of species richness and herbage productivity; and iii) improved grasslands which are products of modern intensive agriculture (fertilization, mowing, etc.) containing sown grass and legume species. The European Grassland Federation (EGF) has organized a separate working group aiming to 'establish a common concept on the characteristics that distinguish 'semi-natural grasslands' from other types of grasslands (EGF, 2012). Since the same species present in various grasslands are also found in wild populations, a continuum of populations from wild, unmanaged vegetation to natural and semi-natural grasslands exists. It is thus difficult to distinguish between wild, semi-natural, and naturalized populations and Boller and Greene (2010) proposed to call all such populations ecotypes. In addition landraces, i.e. populations which have adapted to a specific region or location (farm) by repeated seed harvest and reseeding by humans at the same location, are important in forage crops. Good examples are lucerne in Italy (Annicchiarico, 2013), timothy in Norway (Schjelderup *et al.*, 1994) and red clover in Switzerland (Kölliker *et al.*, 2003).

Semi-natural grasslands, like old permanent pastures and meadows which are constantly managed by cutting or grazing, can be very stable as regards botanical composition. They might also be in transition to improved grasslands if cultivation, re-sowing and fertilization are imposed on them, or revert to shrubs and forests if interventions by humans or grazing animals are completely relaxed (Bullock *et al.*, 2011). A key feature of semi-natural grasslands as opposed to improved grasslands (leys and meadows) is that they are among the most species rich habitats harbouring a large number of plant, animal and fungal species. An excellent review of the evolution of European grassland biodiversity and the ecological rules for maintaining high biodiversity was presented by Pärtel *et al.* (2005). The high biodiversity (species richness) would also indicate that semi-natural grasslands contain a high genetic diversity (within species diversity). This is not necessarily true since a high biodiversity and interspecific competition usually lead to reduced population sizes of individual species (Peter-Schmid *et al.*, 2010; Nestmann *et al.*, 2011). In this presentation we will not make a clear distinction between semi-natural and naturalized grasslands since it is often difficult to distinguish between indigenous and introduced/naturalized species. Besides, most of the available data on genetic diversity comes from studies of forage crop species that are most productive under more intensive management, representing in most cases ecotypes/naturalized grasslands. In this presentation we will focus on examples of genetic diversity in key grassland species mainly from the Mediterranean, the Alps and Northern Europe, regions that span a large range of latitudes and altitudes, with large variability in environmental conditions.

Distribution of semi-natural grasslands in Europe

Before modern agriculture introduced intensive management and fertilization, semi-natural grasslands were the dominant part of the agricultural landscape in Europe. In some regions, e.g. the Swiss Alps, semi-natural grasslands still cover 89% of the utilized agricultural area below the timberline (Peter *et al.*, 2008). Permanent grasslands cover about 33% (57 mill ha in 2007) of the European Utilized Agricultural Area (Peeters, 2012). In Italy, grasslands constitute 50% (6.1 mill ha in 2011) of the agricultural land, of which about 75% is classified as permanent grasslands (>10 yrs old). In Norway grasslands constitute about 68% of the agricultural land of which 35% is classified as short-term (temporary) leys (<10 yrs old), 12.5% as leys over 10 yrs old, and about 20% as semi-natural and pasture grassland (Daugstad, 2013). In northern and highland regions of Norway more than 90% of the agricultural land is grassland. In many parts of Western Europe, semi-natural grassland has greatly declined during the last part of the 20th century due to intensification of agriculture with subsequent conversion into arable land or forest after abandonment of grazing and mowing. Examples are the UK lowlands where 90% of semi-natural grasslands are estimated to be lost and only 2% of the grassland area comprises high diversity semi-natural grassland (Bullock *et al.*, 2011). However, the decrease has been substantially less in UK in the last 10 years. The same loss of semi-natural grasslands is reported from Sweden (Hansson and

Fogelfors, 2000). Although the general trend in Europe is loss of semi-natural grasslands and reduced biodiversity, there are quite large country and regional differences due to structural, e.g. farm size, topographical and policy characteristics. Semi-natural grasslands are for example completely lost from Denmark and The Netherlands, countries with flat cultivated land, large farms and highly intensive agriculture, while such grasslands are still present in Mediterranean, the Alps and in Northern Europe – mountainous regions with relatively small farm units. Relics of very old natural meadows which have turned into re-naturalized grasslands after cultivation was abandoned 30-40 years ago are present in Italy, e.g. grasslands with sainfoin (*Onobrychis viciifolia* Scop.) along the calcareous range of Apennines, or sulla (*Hedysarum coronarium* L.) in the clayish hills of Central Italy.

Factors shaping the genetic diversity within and among local grassland populations

Semi-natural grasslands are mostly found fragmented as patches embedded in other vegetation types (Hejcman *et al.*, 2012). This means that for a given species local populations can be viewed as metapopulations with diversity being shaped by immigration and extinction. Genetic diversity within local populations is determined by genetic drift, selection and migration. The relative magnitude of these evolutionary forces acting within local populations depends on population size, management (harvesting, grazing, fertilization, etc.), pedoclimatic factors and gene flow by pollen or seed. The extent of gene flow by seed might historically have been much more extensive than we tend to think (Hejcman *et al.*, 2012). In the early ages of the development of agriculture in Europe, people and livestock moved frequently over quite large distances spreading seeds found in fodder and on animals. In modern times the anthropogenic influence of gene flow from improved cultivars into semi-natural grasslands and by establishing naturalized grasslands is a major contribution to the observed distribution of genetic variation within and between populations in many species.

Kölliker *et al.* (1998) found that fertilization and frequent defoliation reduced molecular diversity, estimated with RAPD (Random Amplified Polymorphic DNA) markers, within six natural populations of *F. pratensis* sampled from long-term experiments which had received treatments for 11-38 years. Also molecular diversity within cultivars was lower than within natural populations. Analysis of agronomic traits was only partially congruent with the results of RAPD analysis.

Peter-Schmid *et al.* (2010) studied genetic diversity of *Festuca pratensis* Huds. and *Lolium multiflorum* Lam. ecotypes assessed with SSR markers and related this to the species composition and abundance of the collection sites. They found small differences in genetic diversity among the grassland types (*Mesobromion*, *Festuco-Agrostion*, *Lolio-Cynosuretum* and *Heracleum-Dactylis* for *F. pratensis*; *Heracleum-Dactylis*, *Trifolio-Alopecuretum* and *Lolietum multiflori* for *L. multiflorum*) for both species. Genetic diversity within *F. pratensis* ecotypes was negatively correlated with species diversity at their collection sites, while no correlation was found in *L. multiflorum.* As a result, intermediately managed grassland habitats with lower species diversity contained *F. pratensis* ecotype populations with significantly more rare alleles than extensively managed habitats. These results point to a possible conflict between conserving species diversity and genetic diversity when designing management regimes for *in situ* conservation of semi-natural grasslands.

Importance of genetic diversity from ecotypes/semi-natural grasslands

Genetic diversity is indispensable for breeding new cultivars. Most important is to choose the best genetic resources to build a new breeding programme on, and select appropriate exotic materials for introgression of new variability into existing breeding programmes (Boller and Greene, 2010). Forage crop breeders are mostly utilizing released cultivars, improved breeding materials and ecotypes in active breeding programmes. It is difficult to judge how many cultivars have been developed completely or partly from ecotypes collected in semi-natural grasslands. Boller and Greene (2010) presented figures on the origin of 93 French cultivars (cocksfoot, timothy, meadow fescue, tall fescue, perennial ryegrass and Italian ryegrass) released in the period 1954-1987 showing that 47% originates from ecotypes and 16% from a combination of ecotypes and varieties. Although breeders today cross genotypes from existing cultivars to create base material for breeding, most of the current cultivars trace back to breeding material originally created from collected ecotypes. This is reflected by the small genetic distances between cultivars and ecotypes found in molecular studies, for example in perennial ryegrass (Bolaric *et al.*, 2005; McGrath *et al.*, 2007), meadow fescue and Italian ryegrass (Fjellheim and Rognli, 2005b; Peter-Schmid *et al.*, 2008b; Fjellheim *et al.*, 2009) and timothy (Fjellheim *et al.*, 2013). In Norway nearly all forage grass and clover cultivars listed in the last decades stem from collections of ecotypes collected in the 1970's and 1980's (grasses and white clover) and landraces (red clover). In the case of timothy cultivars have been developed also from crossing of ecotypes with the widely grown landraces 'Engmo' (northern adapted) and 'Grindstad' (southern adapted). The Norwegian meadow fescue cultivar 'Norild' is an interesting example of the value of ecotypes for breeding new cultivars and how breeding is changing the genetic structure. 'Norild' was based on surviving plants after 3 yrs field testing at 70°N (Alta, Finnmark). Intense selection is reflected by 'Norild' being distinct from all other Nordic cultivars and having lowest within-population molecular diversity (Fjellheim and Rognli, 2005a). 'Norild' originates from a local North-Norwegian population which does not differ from other Norwegian local populations as regards phenotypic and molecular diversity (Fjellheim and Rognli, 2005b).

Characterization and evaluation of genetic resources

Genetic resources are in most cases characterized genetically, usually by molecular marker variation, and also phenotypically. Characterization focuses on traits that are simply inherited while evaluation focuses on traits that have quantitative inheritance (Boller and Greene, 2010). The most common markers used in forage crops are nuclear AFLPs (amplified fragment length polymorphisms) and SSRs (simple sequence repeats or microsatellites), and cpDNA (chloroplast) markers (sequence variation or SSRs). Phenological, morphological and agronomic descriptor traits (collectively termed phenotypic traits herein) are usually scored following the IBPGR (the International Board for Plant Genetic Resources, now Bioversity International) descriptor list (Tyler *et al.*, 1985) with local modifications. Phenotypic evaluation of populations of outbreeding crops is costly and challenging. There is a general agreement that proper evaluation of agronomic traits, like persistency and yield, must be based on swards rather than single plants commonly used in characterization because of interplant competition. It is practically unfeasible to evaluate agronomic traits on a large number of accessions; also the amount of seed available preclude establishment of larger sward plots without prior multiplication. Even if cost-effective protocols using unreplicated simulated swards with clonal controls have been devised (Annicchiarico, 2004), testing of genotypes and populations in several locations to estimate genotype × environment interactions (GEI)/phenotypic plasticity is preferable to single common garden tests. Estimates of heritability of simple traits that show little GEI, e.g. heading time and disease resistance, can be obtained using clones, and simulated swards/rows which give interplant competition can be established by half-sib or full-sib families. However, the number of accessions that can be evaluated in this way is very restricted and is probably only feasible for core collections.

Phenotypic and genetic/molecular diversity studies of semi-natural grassland populations/ecotypes of key species

Meadow fescue

Several investigations of molecular and phenotypic diversity of European meadow fescue ecotypes and genebank accessions have been reported (Kölliker *et al.*, 1998, 1999; Fjellheim and Rognli, 2005a, b; Fjellheim *et al.*, 2007; Peter-Schmid *et al.*, 2008a; Peter-Schmid *et al.*, 2008b, 2010). In general the studies show that there is very little differentiation between populations; 80-90% of the molecular variation exists within populations. Rather restricted genetic variation among ecotypes of meadow fescue might be caused by extensive gene flow, restricted diversity due to bottle-necks with subsequent radiation from a few refugia (Fjellheim *et al.*, 2006), gene flow from a few sown broad-based cultivars (naturalized grasslands), or most probably a combination of these factors. Kölliker *et al.* (1998) found that fertilization and frequent defoliation led to a reduction in genetic variability within natural meadow fescue populations in Switzerland, and molecular and phenotypic diversity were considerably lower within cultivars of meadow fescue compared to cultivars of *L. perenne* and *D. glomerata* (Kölliker *et al.*, 1999). AFLP analysis of local populations from Norway showed that they are structured into three groups, western, southern, and inland, probably reflecting different routes of introduction of the species into Norway (Fjellheim and Rognli, 2005a). Similar geographic structuring was also found for twelve Swiss ecotype populations using SSR markers (Peter-Schmid *et al.*, 2008b). The Norwegian inland populations are closely related to the cultivars and have most probably been established as a result of migration from sown meadows (Fjellheim and Rognli, 2005a). The analyses of Nordic and Baltic meadow fescue local populations and cultivars found little variation between local populations and cultivars, and the level of variation within cultivars was even higher than within local populations (Fjellheim and Rognli, 2005b; Fjellheim *et al.*, 2009). Natural populations from the Alps were found to be clearly separated from cultivars, indicating that the natural populations from this region are much older than the Nordic populations (Peter-Schmid *et al.*, 2008a). Larger phenotypic diversity within meadow fescue ecotypes compared to cultivars have been detected for a number of traits within Nordic (Fjellheim *et al.*, 2007) as well as Swiss populations (Peter-Schmid *et al.*, 2008b).

Perennial ryegrass

Charmet and Balfourier (1991) scored 15 agronomic traits in 70 wild perennial ryegrass populations originating from Germany, Norway, Romania and Wales and grown at two locations in France. Clustering of the populations based on the agronomic traits was consistent with the geographic origin of the populations except for the Norwegian populations. This indicates that the Norwegian wild populations are younger than other European populations and have not had enough time to develop local adaptation.

The earliest studies of genetic variation within local populations using markers were conducted in perennial ryegrass populations from a wide geographic range (Europe and the Middle East) using isozyme markers (Balfourier and Charmet, 1994; Oliveira *et al.*, 1997; Balfourier *et al.*, 1998). The general findings were that a major part of the variation was present within populations, clinal trends (north-south and altitude) were present for some isozymes, and geographic structure could be detected with lowest diversity present in populations from UK and Ireland and highest diversity among populations from the Middle-East.

In order to identify exotic germplasm for broadening the gene pool for Norwegian perennial ryegrass breeding, especially for improving winter survival, Russian and Swiss ecotypes were compared with Norwegian breeding populations and foreign cultivars in field experiments at two locations (coastal and continental) in Norway (Solberg *et al.*, 1994). Swiss Alp populations, presumably adapted to long lasting snow-cover, did not show better adaptation to the continental climates in Norway than the domestic breeding populations.

A study of molecular diversity of 22 ecotypes originating from three geographic areas in Germany showed that variation within ecotypes was much larger (71%) than variation among ecotypes (29%) and that ecotypes from North Germany were significantly different from ecotypes from South and Middle Germany (Bolaric *et al.*, 2005). Molecular diversity was very similar in ecotypes and cultivars sharing 98% of the molecular variance.

The European Cooperative Programme for Plant Genetic Resources (ECPGR) *L. perenne* core collection was characterized for AFLP molecular variation (800 plants from 80 accessions including 4 reference cultivars) by Ghesquiere *et al.* (2003). Analysis of molecular variation showed that a major part of the genetic variation could be attributed to variation within populations (93.8%) while differences between populations within geographical groups and differences between regions accounted for only 3.7 and 2.5%, respectively. Evaluation of families from pair-crosses between elite breeding materials and 30 core collection accessions, selected based on geographic distance, molecular and phenotypic characteristics, showed that none of the families performed better than the standard cultivars (Ghesquiere and Baert, 2013).

Italian ryegrass

Analyses of molecular variance of 12 ecotypes of *L. multiflorum* collected from permanent meadows in Switzerland showed that 97.1% of the variation was present within ecotypes, 2.6% between ecotypes, and as little as 0.6% between ecotypes and cultivars (Peter-Schmid *et al.*, 2008a). Contrary to the situation in a species like *F. pratensis*, ecotypes and cultivars of *L. multiflorum* seem to form one gene pool in the Alps. However, the ecotypes were shown to be superior to cultivars as regards yield, vigour and resistance to snow mould (Boller *et al.*, 2009). Resistance against crown rust was significantly poorer in ecotypes compared to cultivars. Ecotypes collected in permanent meadows with low species diversity at eastern and northern locations performed best. Genetic diversity seems not to be correlated with species diversity at their sites of origin (Peter-Schmid *et al.*, 2010).

Timothy

In *P. pratense* only 6% of molecular variation (SSR markers) was found between Nordic populations in a joint analyses of diversity in local populations and cultivars, and no variation was found between cultivars and local populations (Fjellheim *et al.*, 2013). This means that essentially the same variation exist in all populations and cultivars of timothy in the Nordic countries; a picture which is quite different from the situation described for meadow fescue. Contrary to meadow fescue where local Nordic populations show clear geographic structuring based on molecular variation (Fjellheim and Rognli, 2005b; Fjellheim *et al.*, 2009), in timothy there is a complete lack of geographic structure. This might be due to a more recent, common introduction of timothy following human agricultural activity. Timothy is hexaploid with a large effective population size; it might therefore harbour more genetic diversity within populations than diploid species. This will contribute to the very low geographic structuring observed. The Nordic populations of timothy show little differentiation in phenotypic traits and no structure could be found using multivariate analyses. However, comparisons of data for several descriptor characters between populations showed that differentiation was present between populations from northern, intermediate and southern locations in Europe for most of the characters (Fjellheim *et al.*, 2013).

White clover

Relatively few European investigations of ecotypes of white clover have been reported. Finne *et al.* (2000a) found highly significant genotypic variation for a range of traits among 11 local populations of white clover (*Trifolium repens* L.) collected from a wide range of latitudes and altitudes in Norway. Populations originating from the northernmost locations had lower biomass and seed yield, flowered early, had smaller leaves, were more prostrate and exhibited shorter internodes and stolons compared to southern populations (Finne *et al.*, 2000a). Path coefficient analysis showed that there is enough genetic variation in these white clover populations to develop new cultivars that combine upright growth habit (higher yielding) with sufficient winter-hardiness and persistency (Finne *et al.*, 2000b). Similar conclusions were drawn by Helgadóttir *et al.* (2008) based on studies of full-sib families from crosses between white clover populations of northern origin and commercial cultivars of more southern origin.
Natural populations of white clover are widespread in hill and mountain areas of the Alps and northern Apennines. Annicchiarico (2012) compared eleven small-leaved or medium-leaved natural populations from these areas with eight medium-leaved or large-leaved varieties of different origin and one natural population from Sardinia for DM yield and forage quality under mowing and grazing, seed yield and fourteen phenotypic traits. Large-leaved populations had highest competitive ability in association with cocksfoot for mowing, but this relationship was not present under grazing. Most natural populations from northern Italy were acyanogenic (incapable of producing cyanide), several exhibited high yield under grazing and high seed yield, and one medium-leaved natural population outperformed medium-leaved cultivars for both forage and seed yield traits. The natural population from Sardinia displayed low seed yield and high cyanogenic potential.
Ladino white clover (*Trifolium repens* var. *giganteum*) is a white clover characterized by very large leaves originating from irrigated permanent meadows of the Po Valley. Natural populations were extensively collected in the Po Valley in the late 1980's (Annicchiarico, 1991). Comparisons of ecotypes and landraces showed that ecotypes had lower seed yields, equal DM yield and slightly higher persistency than landraces, indicating that development of landraces at the farm level only had improved seed yield. Ecotypes demonstrated distinct canopy attributes (smaller leaves, longer internodes and denser stolons), and variation within populations (both landraces and ecotypes) was larger than among populations (Annicchiarico, 1993). Relatively high heritabilities were found for all components of DM and seed yield, but negative genetic correlations makes it difficult to combine dry DM yield, persistency and seed yield in Ladino clover (Annicchiarico and Piano, 1995; Annicchiarico *et al.*, 1999).

Red clover

Red clover natural populations from semi-natural grasslands and pastures in Italy proved very variable with very high broad-sense heritabilities for various phenotypic traits (Pagnotta *et al.*, 2011). Prostrate habit was a common feature in populations evolved in pastures, such as those from Sardinia. Populations originating from Northern Italy and from peninsular and insular Mediterranean areas showed good winter hardiness and drought tolerance, respectively (Annicchiarico and Pagnotta, 2012). Geographical distances among landraces and natural populations were correlated with distances based on phenotypic traits but not with genetic distances based on AFLP markers. The average within-population molecular variation was about 2.6 times higher than the variation between populations, and within-population variation was similar in natural populations and cultivars, but somewhat lower than in landraces. Fifty-seven accessions (of which forty were European) from the U.S. core collection and one population cultivated in Southern Brazil were characterized for 21 phenotypic traits and molecular variation using seven SSR markers by Dias *et al.* (2008).

Analysis of molecular variance revealed that 83.6% of the variation was found within populations. The populations could be separated and clustered based on both phenotypic traits (flowering time, persistency, growth habit and DM yield), and molecular marker variation. However, there was no relationship between the clusters based on phenotypic and molecular data.

Swiss Mattenklee is a particular form of persistent red clover developed by on-farm seed production over long time (Kölliker *et al.*, 2003; Kölliker and Boller, 2010). AFLP analysis (410 loci) of Mattenklee landraces, Mattenklee cultivars, Swiss wild clover populations and foreign red clover cultivars from different countries (total number of 114 populations) showed that wild clover populations are distinctly different from other germplasm investigated (Herrmann *et al.*, 2005). Gene flow between adjacent wild clover populations and Mattenklee landraces was evident from patterns of AFLP diversity.

Lucerne

Lucerne landraces were extensively grown in Italy until recently but is declining due to the ban on commercialization of landrace seed from 2003 (Annicchiarico, 2013). Landraces from Northern Italy with a long track of multiplication on the same farm have been collected and compared to cultivars. Landraces were at least as good as cultivars as regards yield and persistency, and they exhibited specific adaptation to drought reflecting their site of origin. One outstanding native lucerne gene pool that can be found in extensive areas with an annual rainfall ranging from about 350 to 600 mm is represented by the wild 'Mielga' populations in Spain (Prosperi *et al.*, 2006). Mielgas possess specific traits such as prostrate habit and rhizomes which contribute to excellent tolerance towards grazing and drought.

Distribution of genetic variation – population differentiation

A major challenge in studies of genetic variation is to interpret and evaluate the significance of the low between-population variability [2-37%, see Boller and Greene (2010)] usually seen in outbreeding species like grasses and clovers. In theory this could be due to i) extensive gene flow, usually by pollen, between populations; and/or ii) common ancestry of local populations, e.g. the same plant material (landraces) introduced and distributed to a number of sites in a region as is likely the case for timothy in Scandinavia (Fjellheim *et al.*, 2013) or gene flow from a few improved cultivars into local populations as indicated for continental local populations of meadow fescue from Norway (Fjellheim and Rognli, 2005b). These factors combined with large effective population sizes, which makes genetic drift negligible, will effectively prevent population differentiation. Contrary to this, spatial differentiation for phenotypic traits is often found, indicating local adaptation to pedoclimatic factors and management (Pagnotta *et al.*, 2011; Fjellheim *et al.*, 2013). Local adaptation is evident among European timothy populations for traits like winter survival, growth habit and traits related to seed production capacity, the latter being a sign of human-mediated selection influence (Fjellheim *et al.*, 2013).

Conclusions

A major aim both for management of accessions in gene banks and utilization of genetic resources in breeding is to associate molecular marker variation with phenotypic variation. As evident from the results summarized in this article this is not easy and there are few if any good examples. The best example is probably the association of some AFLP markers with low mean January temperature and freezing tolerance (LT_{50} values) obtained in a study of 47 perennial ryegrass European populations with origins representing clines in temperature (Skøt *et al.*, 2002).

When there is so little molecular variation between populations of outbreeding species; why do we keep such a large number of accessions in the gene banks? Should we just keep a few in a core collection? We should be cautious since lack of genetic structuring and differentiation in a limited number of neutral molecular markers does not necessarily mean that there is no interesting functional variation. Data from phenotypic screening in common garden experiments often show clear signs of local adaptation that is not captured by neutral molecular markers. We need to understand the nature of this local adaptation. Is it due to unique allelic or allele frequency variation? Are epigenetic mechanisms regulated by environmental cues important components?
The most interesting traits for breeding are quantitative and it is unlikely that a few single markers obtained from low-density marker screening will be useful across unrelated breeding materials. Large genomes among forage grass species, population structure, low and variable linkage disequilibrium (LD), and general lack of historical information about the origin of populations from semi-natural grasslands exaggerate the challenges. The rapid development of low-cost next-generation sequencing (NGS) techniques, e.g. genotyping-by-sequencing (GBS), holds great promise for genome-wide, high-resolution SNP (Single Nucleotide Polymorphism) marker screening of populations (Poland and Rife, 2012). This makes it possible to obtain extremely detailed pictures of the genetic architecture of populations and bridge the current genotype-phenotype gap.

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Livestock grazing and biodiversity in semi-natural grasslands

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Abstract

The loss of semi-natural grasslands and the fragmentation of remaining habitats is seen as a major problem for biodiversity across Europe. Many European countries use livestock grazing as a nature conservation tool, but national differences in ecological conditions and agricultural history have led to contrasting views on the opportunities and threats posed by livestock grazing. Here, we review conceptual frameworks, the underlying ecological mechanisms, and experimental evidence from different biogeographical areas across Europe in order to discuss the consequences of cattle, sheep or horse grazing on grassland biodiversity (i.e. plant species richness, vegetation and insect communities). In the process, we see how grazing livestock, if appropriately managed, can play a positive role for biodiversity conservation and more generally for the ecosystem services provided by grassland-based livestock farming systems.

Keywords: competitive exclusion, disturbance, ecosystem services, livestock, patch stability

Semi-natural grasslands across Europe

Approximately one-third of the Earth's vegetative cover is savannah, grassland and other grass-dominated ecosystems. In their now-classic paper, Milchunas *et al.* (1988) distinguished between what they called climatically-determined grasslands, like savannah and shrub steppe, and successional/agricultural grasslands maintained by agronomic or other management practices. The borderline between these 'natural' and 'semi-natural' grasslands appears more diffuse than previously thought, as grasslands that had been considered as natural in Africa (Muchiru *et al*., 2008), North-America (Foster *et al*., 2002) and Australia (Lunt *et al*., 2007) often prove to have a more anthropogenic background. However, under this classification, most European grasslands fall into the successional/agricultural category as they have been shaped and maintained by grazing and other agronomic practices for millennia (Emanuelsson, 2009). Agriculture was first introduced in south-east Europe about nine thousand years ago and by medieval times had conquered most of the Middle- and South-European landscape. European agriculture was an integrated system of cultivation and grazing systems. Farmed animals grazed on outfields, but also on cultivated fields after harvests, and grazing systems were either stationary or based on transhumance. Differences in ecological conditions and the use of different grazing animals resulted in various semi-natural vegetation types. In Scandinavia, agriculture and grazing by domestic animals have influenced the vegetation since Neolithic times about six thousand years ago (Berglund, 1991). Pre-industrial agriculture was based on husbandry and semi-natural grasslands. The permanent infields were first and foremost used for food production, while outlaying land was grazed by livestock and largely used to harvest winter fodder (hay, straw and leaf fodder). Most uncultivated grasslands below the treeline are thus semi-natural, even if landslides and fluctuating water levels also keep some 'natural' grassland areas open (Norderhaug *et al*., 1999). Iceland has a much shorter grazing history than other European countries, since it had no ungulate grazers before the Norse settlement in the late 9th century. Iceland has also suffered massive erosion and land degradation, and there is a long-held consensus that sheep grazing was the culprit, as the indigenous vegetation was ill-adapted to grazing. Recent research, however, has revealed that erosion started before the time of settlement (Geirsdottir *et al.,* 2009; see also Thórhallsdóttir *et al*., 2013 for discussion). In this context, ecosystem restoration efforts in Iceland have mainly aimed to increase biomass and soil fertility by grazing exclusion and fertilizer application. Biodiversity has only recently emerged as an issue.

Land use changes during the 20th century have resulted in grasslands being either abandoned or more intensively used (cultivated, fertilized, etc.). Today, Spain and other Mediterranean countries, together with Romania and Poland, seem to be the countries with the largest areas of semi-natural grasslands left (Emanuelsson, 2009). In Scandinavia, Norway appears to hold the most valuable areas. Semi-natural grasslands, which are exposed to little if any fertilizer input, are characterized by low P and N content and are mostly species-rich. The loss of semi-natural grasslands and the fragmentation of remaining habitats is thus seen as a key problem for biodiversity conservation (Reidsma *et al.,* 2006). Many of the species that depend on these habitats are now threatened by either management intensification or shrub encroachment. Grazing by livestock has therefore been used as a nature conservation tool, as it is considered one of the main drivers of global vegetation dynamics, which can have positive effects on plant species richness (Diaz *et al*., 2007). However, quality of the management regime is decisive in this connection, and knowledge of grazing history is highly important (Dahlström, 2006). The effect of grazing also depends on livestock species, pasture productivity and grazing intensity, and in some conditions even on livestock breed and grazing period. Here we bring a state-of-play review on the connection between grazing and biodiversity and the role of grazing in ecosystem health, including for ecosystem services.

Grazing and biodiversity: conceptual framework and core processes

A global framework connecting grazing and biodiversity first emerged in 1988 in the Milchunas–Sala–Lauenroth (MSL) model from a background in the competitive exclusion hypothesis (Grime, 1973). The model, in which grazing is considered the disturbance factor, predicts a bell-shaped relationship between diversity and disturbance. Diversity is limited by stress and competitive exclusion on the extremes, but peaks at an intermediate grazing intensity. Milchunas *et al*. (1988) also suggested that the relationship between grazing and biodiversity is a function of environment moisture and of the evolutionary grazing history of the ecosystem (Figure 1). In regions with a long grazing history, the effect of large grazing herbivores on biodiversity would be minor in semiarid areas but stronger in sub-humid areas, whereas in areas with a short grazing history, the effect would be moderate in semiarid areas and strong in sub-humid areas. The importance of the evolutionary grazing history of the ecosystem as a key factor determining the relationship between grazing intensity and biodiversity was confirmed by Milchunas and Lauenroth (1993), who analysed 236 worldwide datasets. Length of grazing history can be seen as a 'limited operational variable' due to the usually limited information on past grazing (Oesterheld and Semmartin, 2011). However, it has been assumed that in countries and continents with little grazing history by wild or domestic herbivores, livestock grazing would have deleterious effects on the ecosystem (Lunt *et al*., 2007). In a global analysis, Diaz *et al*. (2007) concluded that grazing history, along with climate, was essential for understanding plant trait (i.e. common plant attribute) responses to grazing.

Although widely accepted and referenced, the MSL model has also attracted criticism. In its original form, it assumed a single equilibrium with a single diversity value at a given grazing intensity level, and reversible changes along a successional trajectory. Cingolani *et al*. (2005)

suggested the moisture gradient should be generalized to a productivity gradient, and expanded the MSL model by incorporating the state and transition model (Westoby *et al.*, 1989) that allows alternative equilibria. Several authors then discussed the role of grazers as creators of alternative equilibria or alternative stable stages in grazing ecosystems, thereby affecting their productivity (Milchunas and Lauenroth, 1993; Blinnikov *et al.*, 2011). In tundra ecosystems, the more productive and resilient grassland is created and maintained by large herbivores that increase both the productivity and carrying capacity of the ecosystem (Van der Wal, 2006).

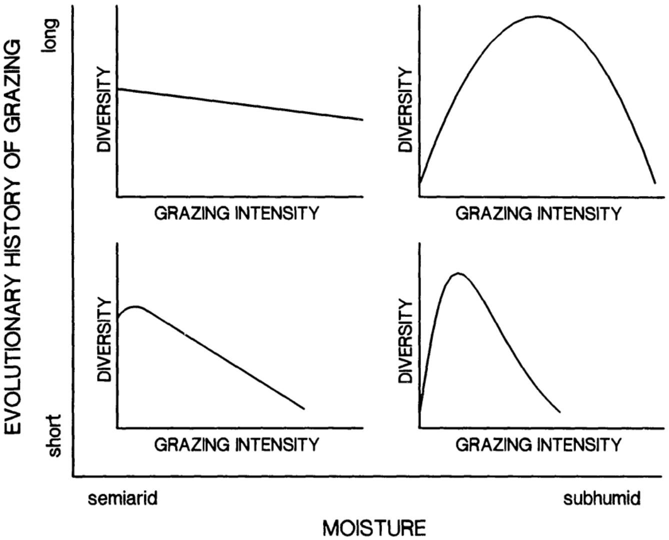

Figure 1. Plant diversity of grassland communities in relation to grazing intensity along gradients of moisture and of evolutionary history of grazing (Milchunas *et al.*, 1988).

Defoliation acting jointly with trampling, seed dispersal, dung deposition and nutrient cycling affects the persistence of species that would not otherwise coexist in homogeneous environments (Bakker, 1998; Adler *et al.*, 2001; Kohler *et al.*, 2006). The MSL model predicts that moderate grazing intensities should benefit grassland biodiversity by creating mosaics of habitats defined by short and tall vegetation patches with contrasting growth forms and competitive interactions. The major underlying process is that sheep, cattle and horses select for short rather than tall grass patches, consistent with the usually higher nutritive value of these short vegetative patches (Dumont *et al.*, 2007; Edouard *et al.*, 2010). Adler *et al.* (2001) coined the term 'patch grazing' to describe this behaviour. Patch grazing stabilizes patch boundaries and can result in some degree of intra- and even inter-annual stability of grazing patterns. The stability of grazing-induced spatial patterns of vegetation was analysed at two spatial scales (25×20 m areas and 1.6×0.8 m grids) in temperate, subalpine and Mediterranean pastures of contrasting productivity. In cattle-grazed pastures, the spatial scale at which inter-annual stability of vegetation patterns was expressed depended on pasture productivity (Dumont *et al.*, 2012). The less fertile grasslands were mainly characterized by fine-scale stability patterns, which were partly explained by variations in the local abundance of low-palatability grasses, e.g. turves of *Nardus stricta* or *Avenula* sp., as these plant species form small but very dense monospecific turves with low spatial extension and strong competitive effect (Herben *et al.*, 1993). Stable large-scale patterns of grazing were more frequent within lightly-grazed productive grasslands (Dumont *et al*., 2012) and resulted in divergent local vegetation dynamics, which could open some opportunity for restoring biodiversity in mesotrophic grasslands (Rossignol *et al*., 2011).

Herbivore size has been suggested to be another important factor determining the intensity of grazing disturbance and patch creation (Bakker *et al*., 2006). Large herbivores (cattle, horses) could increase diversity to a greater extent than smaller, more selective herbivores due to their impact on dominant plant species in the sward. In contrast, small herbivores are assumed to decrease diversity by selecting the most nutritive plant species. In line with this hypothesis, sheep are reported to have a strong and deleterious effect on palatable plant species, compared with that of cattle (Öckinger *et al*., 2006; Sebastià *et al*., 2008; Dumont *et al*., 2011). Plots grazed by sheep showed some level of patch stability between the summer peak of biomass and autumn, but no inter-annual stability, which suggests that sheep may also be less likely to shape sward structure (Dumont *et al*., 2012). The effect of wild herbivores (including small species such as rodents and geese) creating and maintaining open grasslands is often overlooked (Bakker *et al*., 2006; Blinnikov *et al*., 2011). Several authors have shown that the impact of these small grazers is scale-dependent. In the Arctic, greater snow geese increased species richness on a small spatial scale but had little effect on larger scales (Jasmin *et al*., 2008). Small territorial grazers such as pikas (*Ochotona collaris*) feed preferentially close to their burrows. Heavily grazed areas near burrows persist in the long-term, and species richness increases at intermediate distance where grazing intensity was moderate (McIntire and Hik, 2005). Note that the ecological roles of livestock and small grazers are complementary and not functionally equivalent. Large herbivores modify spatial heterogeneity, and thus overall vegetation composition, at large scale whereas small grazers modify spatial heterogeneity and vegetation composition at a local scale (Yoshihara *et al*., 2010).

Grazing affects sward biodiversity, but there is also a mutual/reciprocal effect by which sward biodiversity affects the foraging behaviour of grazing herbivores. To illustrate, two groups of 15 horses grazed Icelandic swards with contrasting levels of biodiversity (11 *vs*. 24 plant species) for 5 years (Thórhallsdóttir *et al*., 2001). Horse movement patterns between feeding stations changed directionally from summer to autumn, with longer feeding times at each station in late season. The horses appeared less motivated to change feeding location with increased plant senescence after the preferred plant items had been selected from the sward, and they grazed longer at each feeding station in the lower- than in the higher-biodiversity sward, thus underlining the importance of being able to select from the sward.

Livestock grazing as a tool to promote pasture biodiversity

Several long-term studies support the MSL model's predicted negative effect of grazing exclusion on biodiversity (e.g. Pöyry *et al*., 2006; Sebastià *et al*., 2008). In Iceland, the effect of grazing exclusion on biodiversity also follows the predicted trend despite a relatively short grazing history. The Husafell farm in south-west Iceland holds one of the island's main birch forests that was exploited for timber, coal and grazing. With changes in agricultural practices in the late 19th and early 20th century, sheep numbers on the farm increased, resulting in overgrazing and forest decline. Sheep were excluded from part of the forest in 1964 and from the whole forest in 1972. Vegetation analyses were taken on both sides of the fence in 1964, 1981, 2001 and 2010 (Thorsteinsson and Thórhallsdóttir, 2011). Birch cover increased by 1.5-2.4% per year and in forest understory the relative abundance of most species or species groups was significantly modified (Table 1). Consequently, plant species richness decreased from 30 to 24 species between 1981 and 2010. In some ecosystems, particularly where natural disturbance is high, grazing can however have aversive effects on sward diversity. For example, grazing in the saline parts of a previously abandoned salt marsh in the Netherlands caused excessive damage to certain plant species, which reduced diversity (Olff and Ritchie, 1998). Austrheim and Eriksson (2001) also suggested that grazing could be negative for plant species richness in low-productive alpine and arctic grassland communities (barren heath, extreme snow-beds). In these strongly resource-limited communities, nutritional input from faecal addition can, however, be of particular importance by creating rich patches that increase biodiversity at a larger scale (Van der Wal *et al*., 2004).

Table 1. Changes in understorey species composition in Húsafell birch forest in western Iceland following grazing exclusion in 1964. The table describes the fate of different species or species groups. Species marked † had entirely disappeared from the study area in 2010 (Thorsteinsson and Thórhallsdóttir, 2011).

Species/species groups showing a significant **increase** in frequency	Species/species groups showing **no significant change** in frequency	Species/species groups showing a significant **decrease** in frequency
Deschampsia flexuosa (*P*<0.01)	*Agrostis spp.*	*Anthoxanthum odoratum* (*P*<0.001)
Empetrum nigrum (*P*<0.05)	*Carex spp.*	*Bistorta vivipara* (*P*<0.001)
Hylocomium splendens (*P*<0.001)	*Equisetum pratense*	*Calluna vulgaris* (*P*<0.05)
Peltigera spp. (*P*<0.001)	*Equisetum variegatum*	*Cladonia chlorophaea* (*P*<0.001) †
Unspecified mosses (*P*<0.001)	*Festuca rubra*	*Erigeron boreale* (*P*<0.001) †
Vaccinium uliginosum (*P*<0.05)	*Galium boreale*	*Festuca vivipara* (*P*<0.001)
	Galium normani	*Juncus trifidus* (*P*<0.01)
	Galium verum	*Kobresia myosuroides* (*P*<0.001) †
	Poa spp.	*Luzula multiflora* (*P*<0.05)
	Taraxacum spp.	*Rhacomitrium spp* (*P*<0.001)
	Thalictrum alpinum	*Selaginella selaginoides* (*P*<0.001)†
		Thymus praecox (*P*<0.05) †
		Trisetum spicatum (*P*<0.001) †

In more productive pastures, the MSL model suggests that in order to maintain high biodiversity value, grazing should remove a fraction of the forage that is available for consumption; the amount depending on pasture productivity. This has led to the classical view that at field scale, grassland management for production purposes usually conflicts with grassland management for conservation purposes (Plantureux *et al*., 2005). However, the way large herbivores affect vegetation diversity in temperate grasslands also depends on background conditions. Dramatic changes in dominance have been reported in fertile grasslands that were taken out of intensive use for biodiversity conservation purposes (Marriott *et al*., 2004). The use of fertilizers to boost grassland productivity, combined with increased frequency of disturbance by either mowing or grazing, favours a relatively small number of competitive plant species and decreases plant diversity (Crawley *et al*., 2005). Consequently, it is unlikely that decreasing grazing intensity will benefit plant species richness in productive grasslands while nutrient levels remain high (Marriott *et al*., 2004). In addition, an impoverished species pool at landscape scale can also impede restoration of their ecological diversity (Poschlod and Bonn, 1998). Conversely, plant species richness was favoured by a reduction of stocking rate on grasslands with moderate to low soil fertility (Kruess and Tscharntke, 2002; Louault *et al*., 2005; Pöyry *et al*., 2006). However, a recent meta-analysis did not find any effect of stocking rate on plant species richness in sheep-grazed temperate pastures (Scohier and Dumont, 2012; Figure 2). In a species-rich upland pasture with low soil nutrient status that was grazed by heifers in central France, forbs and stress-tolerant grasses (*sensu* Grime's plant strategies) increased in abundance following a reduction of stocking rate (Dumont *et al*., 2009). Ten years of applying these contrasting stocking rates did not significantly modify plant species richness (which averaged 57, 57 and 53 species per plot at a high, intermediate or low stocking rate, respectively), which confirms that species richness usually changes more slowly than species abundance (Louault *et al*., 2005; Scimone *et al*., 2007).

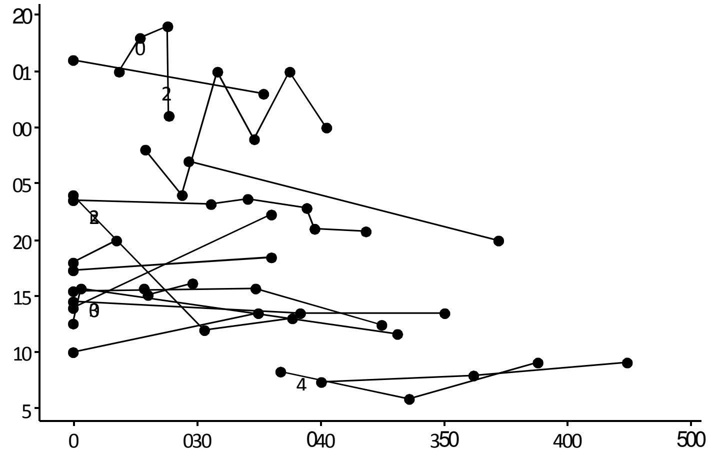

Figure 2. Effect of a stocking rate gradient on plant species richness (y-axis) in sheep-grazed temperate pastures (Scohier and Dumont, 2012).

Irrespective of changes in plant species richness and evenness, lower stocking rates will allow a higher structural diversity of grassland to develop, which will provide a greater number of ecological niches and buffered microclimates for invertebrate species than that of vegetation under more intensive grazing pressure (Pöyry *et al*., 2006; WallisDeVries *et al*., 2007). Lower stocking rates also benefit species that are ill-adapted to frequent disturbance of their habitat, by allowing undisturbed trophic interactions between plants and herbivorous insects (Kruess and Tscharntke, 2002; Pöyry *et al*., 2006; Dumont *et al*., 2009). Ungrazed plants that are allowed to flower provide important resources for nectar- and pollen-feeding guilds of invertebrates (Bergman *et al*., 2008; Scohier *et al*., 2013). Where the grazer selects flowers, as in the case of sheep observed by Öckinger *et al*. (2006), this can be particularly detrimental to butterfly populations. An empirical recommendation for increasing biodiversity in semi-natural grasslands can therefore be to take animals off some of the plots during the main flowering period. This had a positive impact on butterfly populations in an upland pasture that was grazed by cattle at a high stocking rate, where butterfly species richness was 2.5-fold higher and butterfly abundance 2-fold higher than in plots that were continuously grazed at the same stocking rate (Farruggia *et al*., 2012). This observation supports the strategy of temporally varying grazing intensities for grassland biodiversity conservation purposes (also see Fuhlendorf and Engle, 2001; Briske *et al*., 2008). When the same management strategy was tested in sheep-grazed pastures, the benefits of the rotational grazing management were weaker, being mainly evidenced on bumblebee density and species richness, with some additional effects on the local density of butterflies during the exclusion period (Scohier *et al*., 2013). This stresses how important it is to consider differences in grazing styles (and their interactions with grazing period and stocking rate) for management purposes. Recent work also stressed the need to better consider the effects of grazing management during the inactive winter season, as it can cause increased mortality in hibernating butterfly caterpillars (van Noordwijk *et al*., 2012).

Further evidence that different livestock species can have significantly different effects on plant communities comes from studies on the effect of horse grazing on plant communities. Horses have incisor teeth in both their upper and lower jaws, enabling them to graze closer to the ground than cattle. This led them to generate stable large-scale patterns of grazing in both mesotrophic (Dumont *et al*., 2012) and less-fertile semi-natural grasslands (Edwards and Hollis, 1982). Recent results suggest that horses aim to maximize diet quality (Thórhallsdóttir *et al*., 2001), and more specifically digestible protein intake (Edouard *et al*., 2010). Over a 4-year study, Fleurance *et al*. (2010) recorded the species richness and abundance of plants, ground beetles and grasshoppers in a mesotrophic grassland that was continuously grazed by horses at either a high or a moderate stocking rate. Plant species-richness was unaffected by stocking rate, but the creation of relatively stable short patches by horses enabled *Trifolium repens* to compete with tall grasses. Consequently, legume abundance increased at the high stocking rate. A higher structural heterogeneity in moderately-grazed plots favoured ground-beetle and grasshopper populations, especially those species associated with tall swards.

The use of rustic breeds has frequently been recommended as a tool for nature conservation. However, a comparative study conducted in four mesotrophic or semi-natural grasslands across Europe concluded that the narrow differences in diet selection between rustic and commercial breeds (Dumont *et al*., 2007) may prevent any clear management implications from emerging (Scimone *et al*., 2007; WallisDeVries *et al*., 2007). It seems that differences in body weight and feeding experiences at the young age (see also Thórhallsdóttir *et al*., 1990) can explain a large part of the so-called breed effects in temperate grasslands. But is it also the case in more constraining environments, where ability to walk on slopes (VanWagoner *et al*., 2006) or exploit roughages may have a stronger effect? As an example, Sæther *et al*. (2006) showed that in a nutrient-rich vegetation community, the rustic Norwegian dairy breed Blacksided Trønder and Nordland (STN) and the high-yielding Norwegian Red (NRF) cattle showed almost identical grazing patterns, whereas in a less fertile site where plant species distribution was less uniform, the NRF breed seems to cover its higher requirements by grazing in areas with more nutrient-rich vegetation compared to the STN breed. From a semi-natural grassland management perspective, the consequences of differences in grazing style on the vegetation might be different.

The effect of pasture biodiversity on product quality and other ecosystem services

There is increasing awareness of the multifunctionality of agriculture and its positive impacts. In the mid-2000s, the Millennium Ecosystem Assessment highlighted the importance of ecosystem functioning and biodiversity for the welfare of human populations. The ability of ecosystems to provide services is assumed to be linked to the diversity of the resident plant and animal communities (Tilman *et al*., 2006; Mouillot *et al*. 2011). Most semi-natural grasslands are thus recognized as providing a wide range of services. Diversified grazed grasslands affect the nutritional and sensory quality of dairy products. A review by Chilliard *et al*. (2007) found that milk from cows and goats grazed on diversified mountain pasture were richer in fatty acids (FA) considered beneficial for human health (CLA and omega-3 FA) than those grazed on less-diversified lowland pastures. These higher milk FA concentrations could result from the abundance and diversity of dicotyledonous plants that reduce ruminal biohydrogenation. The polyphenol-compounds profile of semi-natural pastures is closely linked to sward diversity (Scehovic, 2000; Reynaud *et al*., 2010). In a diversified mountain meadow, Fraisse *et al*. (2007) identified 170 different phenolic compounds, only 30 of which were common to all plant species. Jeangros *et al*. (1999) showed that total soluble phenolics content was up to 1.7-fold higher in subalpine permanent pastures than in less-diversified pastures. Phenolic compounds have antioxidant properties (Farruggia *et al*., 2008), and milk phenolics seem particularly interesting as they are present in significant amounts and could contain original nutritionally valuable molecules (Setchell *et al*., 2002). For example, Sickel *et al*. (2012) recently showed that Alpine ranges provide fodder with higher levels of α-tocopherol (vitamin E) than lowland cultivated pastures. Finally, the botanical composition of grasslands affects cheese organoleptic characteristics. Gruyère cheese from upland Alpine pastures have a more intense flavour than lowland cheese (Bosset *et al*., 1999). Abondance cheese produced by the same farmers on either lowland or Alpine summer pastures also differed in texture and flavour (Bugaud *et al*., 2001). In both cases, the differences were attributed to the more diversified botanical composition of the upland pastures, but the underlying mechanisms are still not fully understood.

The botanical composition of semi-natural grassland also affects sward productivity and feed value. Their low N and P content is one of the explanations for their species richness but also the reason for a lower productivity than in fertilized meadows. This prompts reluctance among some farmers to use semi-natural grasslands unless they are subsidized. Determining how sward diversity can affect the seasonal dynamics of sward production is a major scientific challenge. Experimental manipulations of plant assemblages (Tilman *et al*., 2006) and modelling studies (Yachi and Loreau, 1999) have suggested that a greater temporal stability of plant production in diversified grasslands could result from variability in plant species responses to abiotic conditions and from the asynchronicity of these responses. In semi-natural pastures, inter-specific differences in maturity and nutritive value led to a difference in feed value between species-rich and species-poor grasslands (Duru *et al*., 2008). The digestibility of species-rich grassland was also more stable (Michaud *et al*., 2012). Species diversity in grassland reduces rumen degradability, which can increase forage protein value when the degradable proteins are in excess in the rumen. By analysing a large database of rumen protein degradability measurements, it was found to be significantly lower in semi-natural grasslands compared with pure grass or pure legume swards (Nozières *et al*., 2006). This could result from between-species differences in protein degradability due to the specific action of plant phenolic compounds (Aufrère *et al*., 2008). Feeding animals on a diverse forage diet can have positive effects on their voluntary daily intake (Farruggia *et al*., 2008). In addition, legumes like *Onobrychis viciifolia* or *Lotus corniculatus* that are rich in condensed tannins are known for their anthelmintic properties against parasitic nematodes (Hoste *et al*., 2006). Relatively high tannin contents have also been measured in some forb species: *Alchemilla* sp., *Taraxacum officinale* and *Achillea millefolium* (Scehovic, 1990) and in maquis shrublands (Farruggia *et al*., 2008).

As grasslands provide different ecosystem services, it makes sense to analyse how they can combine at the farm scale. Production and conservation objectives are not necessarily in conflict at farm scale, because both can benefit from a management strategy based on allocating specific functions to grasslands in relation to vegetation type. For example, Franzén and Nilsson (2008) concluded that 20–50% of semi-natural farmland left ungrazed in May–July to provide abundant nectar and pollen resources could compensate for intense grazing applied on parts of the remaining farm area. Jouven and Baumont (2008) modelled grassland-based beef systems and found that meat production could be maintained by deploying biodiversity-friendly practices on up to 40% of farm area. The practices resulting in the optimal production-biodiversity equilibrium were farming context-dependent. In the upland area of central France, two grassland-based dairy systems were evaluated for their multifunctionality: a continuous grazing system at a low stocking rate in which cows graze species-rich grasslands *versus* a rotational system at a higher stocking rate based on fertile grasslands with lower biodiversity (Farruggia *et al*., 2010). Both systems have advantages and limits in the context of PDO (Protected Designation of Origin) cheese production. Continuous grazing at a low stocking rate on diversified grassland gives better milk performance at the beginning of the grazing season and is compatible with a high level of biodiversity. Rotational

grazing on fertile grasslands allows stable high milk production per hectare and hay stocks, but requires more inputs and involves more complex management, and thus requires forward planning. This can cause grass shortages in summer and discontinuities in production output when the cows change plots, and both these factors are detrimental to farm cheese-making. Grazing system had only a weak influence on cheese sensory and nutritional characteristics.
Beyond these scientific results, there is also a need to produce technical references and management tools to evaluate grassland multifunctionality. A typology-tool to characterize grasslands in the upland areas of central France identified 23 grassland types (Carrère *et al.*, 2012). Each type was characterized based on its agro-ecological conditions and vegetation characteristics, but also according to the ecosystem services provided: milk production, product quality, pollination, habitat preservation and aesthetic value of grasslands. This typology constitutes a useful tool enabling stakeholders to promote grassland-based livestock farming systems that aim to be both productive and biodiversity-friendly.

Conclusion

Views on the preservation of grassland 'wilderness' *versus* the sustainable use of natural resources and on the role of livestock grazing with regard to grassland biodiversity conservation vary between countries and continents. It is clear that different landscape histories, in terms of grazing and different effects of grazing on plant species diversity according to sward productivity, have led to contrasting views on the role of grazing livestock (Lunt *et al.*, 2007; Emanuelsson, 2009). This has spurred the development of different theoretical frameworks such as the production-biodiversity trade-off (Plantureux *et al.*, 2005) which has a background in the competitive exclusion theory (Grime, 1973) and intermediate-disturbance hypotheses (Milchunas *et al.*, 1988), or restoration ecology that is grounded in the basic ecological concepts of succession theory, threshold dynamics and state transitions (Jordan *et al.*, 1990; Kardol and Wardle, 2010). Note that native European grazers such as tarpans (*Equus ferus ferus*) and aurochs (*Bos primigenius*) have become extinct. Attempts to bring these species 'back to life' or at least preserve them (as for the European bison, *Bison bonasus*), though relevant from a conservation standpoint, are unlikely to become reality and would thus have marginal consequences on vegetation. The existing relatively species-poor fauna dominated by browsers or mixed feeders like moose (*Alces alces*), red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus*) is unlikely to maintain the European grassland biodiversity. This leaves us in a situation where cows, sheep and horses now have a real role to play in the preservation of grassland biodiversity and aboveground-belowground linkages (Adler *et al.*, 2001; Kohler *et al.*, 2006; Kardol and Wardle, 2010). It remains relevant to discuss grassland restoration, but even more 'proper management' to maintain existing species-rich semi-natural grasslands and how 'wrong management', like grazing instead of mowing old semi-natural hay meadows, changes vegetation and affects biodiversity. Here, we conclude that grazing livestock, if appropriately managed, play an important role for biodiversity conservation in European grasslands and more generally for the ecosystem services provided by grassland-based livestock farming systems.

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Oral Presentations

Extensive grasslands within the context of the CAP 2013 reform

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Abstract

The prevalent topic of all discussions on agricultural policy is design and impact of the new EU common agricultural policy (CAP) framework from 2014 onwards. Focal issues of debate are the so-called 'greening components' of the first pillar, to what extent the second pillar will be modified (weakened or enforced), the realignment of the boundaries of the less-favoured areas, revision and adjustment of cross-compliance obligations, and the financial ceiling of CAP expenditures in the next programming period. Whereas the proposals of the Commission encompass considerations for slightly improved instruments to address ecological objectives, many stakeholders as well as a significant number of EU member states express heavy opposition to such approaches of greening the CAP, beyond existing benchmarks. It is widely acknowledged that agricultural species-rich grassland systems contribute a major share of desired ecosystem services. Accordingly, all negative impacts on grasslands' quantity and ecological quality have major consequences for political targets. However, necessary support measures so far only exist within the CAP framework. The paper summarizes results of the research project 'The CAP reform 2013 and options for biodiversity and resource management / Federal Agency for Nature Conservation / BfN'. Focus here is on the analysis of the various presented reform models of the CAP and the suspected effects on extensive grasslands.

Keywords: agricultural policy, rural development, grassland, grazing, biodiversity

Introduction and background

Most European ecological heritage refers to landscapes and ecosystems which are characterized by human activities in agriculture and forestry. It is widely acknowledged that agricultural species-rich grassland systems contribute a major share of desired ecosystem services. Benefits of such grassland ecosystems to biodiversity, water, climate, and soils are much more important than indicated by their proportion of the total surface of grassland (EFNCP, 2011). Accordingly, all negative impacts on grasslands' quantity and ecological quality have major consequences for political targets such as those set with the EU-Gothenburg objectives or with the CBD-Nagoya protocol. Most problems are associated with ecologically harmful agricultural practices favoured by inappropriate policies (EEB, 2011). However, necessary support measures can also only be identified within the framework of the common agricultural policy (CAP) of the EU. This policy consists of two pillars: Pillar 1 stands for single area payments (also called direct payments) and is covered to 100% by EU grants. Pillar 2 (also named the rural development programme) encompasses a magnitude of regionalized schemes of various objectives which all have to be co-financed by the EU members. About every seventh year the CAP is newly set for another period. After the McSharry reform (1992) and the Agenda 2000 package (1999) we are now coming to a close with the Luxemburg period, which was implemented in 2005.

Discussion and debate about the programmatic objectives for the period 2014 to 2020 are now at a final stage and again the rural world, and with it associated biodiversity and provided ecosystem services, are at a deciding cross-road as to where to go (Beaufoy and Marsden, 2010; IFAB, 2012; IFOAM, 2012).
Focal issues of debate in the more CAP-critical communities are the so-called greening of the first pillar, the financial ceiling of CAP expenditures, and the share of allocation of CAP budgets to pillar 1 and 2 in the next programming period. Greening entails a concept of ecological and social qualification of the direct payments, and indeed it is the cornerstone of a reformed pillar 1 in the next CAP. This is intended to guarantee that farmers in receipt of financial subsidies shall, in addition to the existing cross-compliance requirements, also generate benefits for nature, environment and climate protection. It is proposed that 30% of the direct payments shall be linked with compulsory greening requirements. Greening measures shall encompass (1) crop rotation; (2) conservation of permanent grassland and (3) implementation of so-called ecological focus areas (EFAs).

Materials and methods

The authors are presently engaged in the research project 'reform of the CAP 2013 and achievement of the biodiversity and environmental goals' which is supported by the German Federal Agency for Nature Conservation (BfN). The objective of this study is to anticipate and judge the expected effects of CAP reform proposals on the biological diversity and to the environment. The basis to achieve such aims is the examination of reports dealing with impacts and efficacy of current CAP measures and of proposals about how the new CAP will look (proposals of EU member states and of various stakeholder groups) in respect of potentially influencing (negatively and positively) high-nature-value farmland with focus on extensive grasslands. This paper presents majors issues to be addressed in the debate of how extensive grasslands might be affected.

Results and discussion

In general it can be delineated that the current CAP pillar 1 includes no mechanisms to target specific support in a way that ensures efficiencies or linkages to EU environmental and ecological policy objectives in respect of extensive grasslands. This is particularly the case in the EU 15 member states where most support is directed to intensively operating farming systems. Though it has to be attributed that in countries where area payments are already fully decoupled – this means that grassland receives the same equivalent as arable – is an appropriate and important tool for supporting basic structures (income) for farms providing high nature value commodities. The crucial problem though that has to be addressed is the question of eligibility of areas with semi-natural grassland vegetation for pillar 1 area payments. This is the case for vast grassland areas (in general extensive grazings) of highest ecological value in the southeast and southwest and also at the north-western fringes of Europe which currently are widely excluded from pillar 1 area payments.
In all member states, pillar 2 programmes are central for the existence and economic viability of extensively working farms, which to a high extent, encompass grassland based livestock regimes. Most important are tailored agri-environment schemes whose positive effects can even be amplified where LEADER grants are available and /or areas are eligible for less-favoured-area payment schemes. But it is also obvious that such good conditions are not common place. The requirement of co-finances almost automatically leads to a vicious circle that only the richer EU member states with, in general, fewer remaining ecological values can afford to implement ambitious but often ineffective pillar 2 rural development programmes.

By using key differences as an indicator the various proposals of how a new CAP shall look can be grouped as follows:
(1) Some member states (e.g. France, Germany, Poland) state that there is no need for a shift of paradigm and rather support the continuation of the current system; in general there is heavy disagreement with the philosophy of CAP greening.
(2) Led by UK opinions, others plead for a general and significant reduction of the CAP budget in general. The logic behind this is that only savings in the largest share of the EU budget would thus allow reductions for those member states being the biggest net payers and this would give options for increased spending in other sectors of the EU budget.
(3) The CAP proposal by the Commission, which is also supported by more CAP-critical groups, and includes demands for substantial and qualified greening components into pillar 1, transfer (modulation) of pillar 1 finances into pillar 2 and generally augmenting finances in pillar 2. At this stage it is remains uncertain which line will be preferred at the end. A striking observation though, is that for the first time ever in CAP history the Commission's concept is much in coherence with the viewpoints of critical NGOs.

Conclusions

Conclusions to be drawn and recommendations to be given for a better and greened CAP are that a qualified greening of pillar 1 and a strengthening of pillar 2, with tailored programmes to support high nature value farmland and farming businesses, are essential to secure the future of European grassland with high ecological values. In respect to permanent grassland, ploughing-up must be generally restricted and should not be allowed up to a lumped ceiling set for a larger region or even for a member state level. Ploughing-up should require official permission and proof that resources (e.g. mineralization, erosion or leaching of nitrate) and conservation interests are not harmed. Bearing in mind how farmers will logically act it will be most detrimental to all existing permanent grassland if a reference area and timeline is set for the future and does not orient to the *status quo*. Thus, a retroactive constraint dating back to at least to the 2012 status should be imposed. More negative consequences will arise when an additional loss by 5% is accepted for a certain time period. The greening requirements should also be extended to include organic farms. Especially in respect to EFAs and permanent pastures, additional positive effects are to be expected. A significant transfer (modulation) of funds (10 to 15%) from pillar 1 to pillar 2 is necessary for an adequate management (e.g. with targeted agri-environment measures) of areas of special ecological interest (e.g. EFAs, Natura 2000 sites, semi-natural extensive pastures). Effective 'dark green CAP measures' in pillar 2 – especially when located in designated areas for conservation – shall be allowed to receive 100% EU grants. This is essential for countries / regions facing economic problems.

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Costs of forage production in disadvantaged mountain areas

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Abstract

Mountain grassland represents an important source of ecosystem services. Its conservation is best achieved by means of a site-specific agricultural use, but less-favoured areas are currently endangered by abandonment because of ongoing socio-economic changes. In order to get a reliable figure of the costs of forage production for the mountain meadows of South Tyrol, an assessment including machinery and personnel costs, as well as the respective working times, was conducted in 2011 for 100 plots on 19 grassland farms. Our results show a greater labour input as the steepness of meadows increases and the dimension of plots declines. Thus, as far as farmers are rational economic agents, specific compensation measures are required for the conservation of extensive grasslands.

Keywords: forage production costs, labour input, natural constraints, ecosystem services

Introduction

Mountain grassland, and extensive grassland in particular, represents an important source of ecosystem services, such as biodiversity, landscape diversity and protection from soil erosion. The conservation of this valuable grassland is best achieved by means of a site-specific agricultural use. There is evidence that in mountain regions of the Alps, extensive grassland is likely to be found in areas characterized by unfavourable climate and topography (Niedrist *et al.*, 2009), which in turn represent a limit to intensification and mechanization. However, such areas are currently endangered by abandonment. This paper focuses on the effects of natural constraints on the costs of forage production in the mountain region of South Tyrol (Italy).

Materials and methods

In order to get a reliable figure for the costs of forage production depending on natural constraints, a survey was conducted in 2011 on 19 farms of the Puster valley. Data were collected in 100 selected grassland fields, encompassing a wide range of altitude, slope conditions and field size (Table 1). These fields are almost exclusively fertilized with the farm's own manure at a loading derived from a mean stocking rate of 1.8 livestock units ha^{-1}. About one-fourth of the investigated area is used for silage. Gross DM yields of between 5.9 and 9.4 Mg ha^{-1} can be expected in this region depending on altitude and management intensity (Kasal *et al.*, 2004). Field area, median of slope and mean altitude of the investigated fields were calculated with the Zonal Statistics as Table-tool and Zonal Histogram-tool of ArcGis 10.0 at a raster resolution of 20 m. Under the supervision of technical personnel, the farmers recorded over the whole year the labour time, the machines and devices used, the personnel involved and all other occurring costs for each operation. Apart from some tasks performed by contractors, the field work is mostly done by farmers and other unpaid family members and/or neighbours. Its economic cost was estimated according to an opportunity cost approach. Among the available methodological alternatives (AAEA, 2000), reference was made to the wages of the collective agreement for agriculture: the unpaid labour of farmers and other operators with an agricultural training was evaluated at the rate of skilled workers, and that of people without an agricultural training at the rate of semi-skilled workers. Labour of children younger than 16 and of elderly people more than 65 years old was also taken into account, although at a minimum wage. The costs of machinery were computed according to Gazzarin (2011), taking amortization costs, repair costs, as well as the fuel costs for towing vehicles into account. Farmers provided information on the purchase price of the machinery and its service life. The yearly working hours of the machines were computed as the sum of the time records of the working steps, further corrected according to estimates of the farmers concerning the use of machinery in other fields not assessed in this project. Given the local context, no opportunity cost of land was considered. The data presented in this paper do not take travelling and transport times into account, in order to make the results independent from the fields' distance to the farm buildings.

Table 1. Summary of site factors and size of the investigated fields (n = 100).

	Minimum	Maximum	Mean ± SD
Altitude (m a.s.l.)	807	2084	1277 ± 290
Slope (°)	2.0	41.2	18.5 ± 10.1
Field area (ha)	0.11	8.26	1.88 ± 1.62

Data were processed by means of a Principal Component Analysis (PCA) using transformed z-scores of altitude, slope and field area. The relationships of the unitary costs and labour input with slope, field area and altitude were described using the Curve Estimation procedure of PASW 17.0.2.

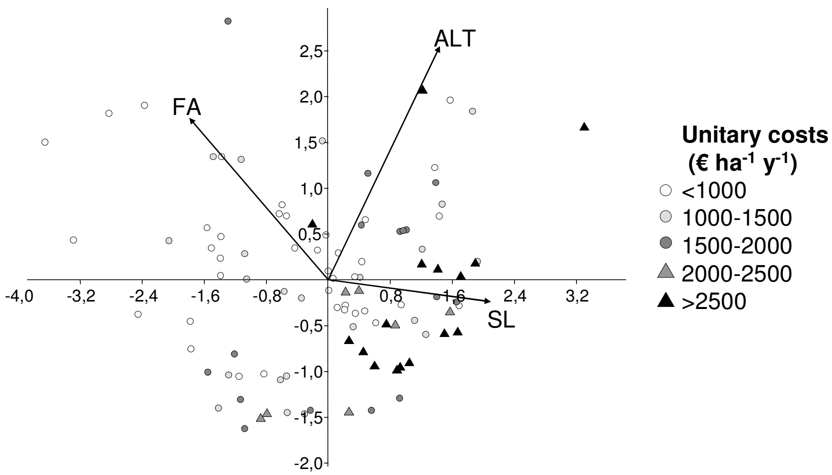

Figure 1. Ordination diagram showing the results of PCA on site factors and plot size (SL = slope, ALT = altitude, FA = field area).

Results and discussion

The first two PCA axes accounted for 83.9% of the total variance (54.2% for the first axis and 29.7% for the second one). Along the first component a rough separation of the two lowest and the two highest cost classes, positively related to slope and to a smaller extent to altitude, was apparent (Figure 1). A negative relation with the field area was also observed. This is consistent with the fact that increasing mechanization constraints occur with increasing slope.

Altitude would be likely to become more relevant if costs per forage yield unit instead of area unit were considered, as climatic constraints are expected to reduce the forage production with increasing altitude. Less expected was the relatively large effect of the field size on the total costs, which may be explained by two different issues: by the needs for machine turning and the larger proportion of field margins in small fields on the one hand, and by the over-proportionally increasing effect of rounding the working times with decreasing field size on the other hand. The incomplete separation of the cost classes suggests that factors other than topography and climate (here roughly described by the altitude) play an important role in determining the production costs, such as the management strategies of the individual farmers, concerning for instance the propensity to renew their machineries.
According to the results of the PCA, unitary costs and labour input were found to increase with increasing slope and with decreasing field area. The regression analysis showed that these relationships are best described by non-linear, highly significant functions, explaining however, the total variance only to a partial extent (Table 2). No significant relation with altitude was found.

Table 2. Effect of slope and field area on production costs and labour input (*** denotes $P < 0.001$).

Dependent variable	Independent variable	R^2	Function
Production costs ($€\ ha^{-1}\ yr^{-1}$)	Slope (°)	0.27***	$y = 1.750\ x^2 - 15.717\ x + 1085.166$
	Field area (ha)	0.26***	$y = 1473.148\ x^{-0.353}$
Labour input ($h\ ha^{-1}\ yr^{-1}$)	Slope (°)	0.37***	$y = 17.53\ e^{0.0451x}$
	Field area (ha)	0.29***	$y = 46.651\ x^{-0.511}$

Conclusions

The present paper provides evidence for increasing production costs and labour input as field steepness increases and the field size decreases. These fields in particular are most likely to be managed extensively and in turn to provide non-marketable, environmental and social ecosystem services. As farmers are rational economic agents, public payments for these services are therefore crucial for ensuring them in the long term.

Acknowledgements

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Resilient and multifunctional-grasslands for agriculture and environmental service during a time of climate change

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Abstract

More than 500 fescue species are dispersed amongst grasslands worldwide with adaptations evolved to their specific location and climate. They provide a vast resource of novel genetic variation for use in grass breeding, and insights into how natural adaptations in grasses have evolved for potential use in future plant breeding to combat climate change. Many fescue species hybridize naturally with ryegrass to generate *Festulolium*, the genomes in combination providing high yields of quality forage together with resilience against abiotic stresses, and compared with ryegrass, enhanced water- and nutrient-use efficiency. These properties have made breeding *Festulolium* cultivars an increased priority in recent years. Specific ryegrass/fescue genome combinations may also lead to improved ruminant nutrition, and others for plant-soil interactions for efficient water and nutrient uptake with modified soil structure for improved hydrology. *Festulolium* are multifunctional and provide environmental service. Their extensive root growth and its subsequent rapid senescence, especially at depth has led to effective soil water retention and potential use in flood mitigation. Deep rooting perennial *Festulolium* cultivars may also be effective in C-sequestration. For a range of foliar and rooting traits in *Festulolium*, it is demonstrated that the *Lolium* genome has a dominant influence in determining trait expression.

Introduction

Grasslands occupy roughly a third of the global land designated suitable for use by agriculture and, more than for any other crop, they comprise a complex ecosystem derived through interactions by a biodiverse species mixture, mainly persisting over sequential years. Their size, persistence and widespread location make grasslands multifunctional, contributing with varying efficiency to a range of environmental services. Due to the impacts of climate change, new understanding of plant-soil interactions and new access to available genomic and phenomic technologies, considerations in plant breeding strategy are now being given to grassland designs for an environmental service. This is reflected in all strategies employed in current forage grass and clover breeding research programmes at IBERS. In the forefront is research on *Festulolium* (hybrids or backcross derivatives from hybrids of a ryegrass (*Lolium*) and fescue (*Festuca*) species). It has been long recognized that *Lolium* and *Festuca* species share complementary traits and, as hybrids, have potential to combine large yields of high quality forage and resilience against climatic stress (Humphreys *et al*, 2006). In practice, elite current nationally listed *Lolium* cultivars are used as parents and are hybridized to specific ecotypes of various *Festuca* species with the objective of providing a specific beneficial trait, traditionally to improve tolerance to an abiotic stress. At IBERS, northern European *F. pratensis* has been a source of genes for improved winter hardiness and frost tolerance, whilst southern European *F. arundinacea, F. glaucescens* or North African *F. mairei* have contributed genes for drought and heat tolerance (Humphreys *et al.,* 2012). An important contributor to the drought resistance of *Festulolium* as compared with *Lolium* ssp. has been their increased root depth to access water more efficiently (Durand *et al.,* 2006). Introgression breeding programmes at IBERS have led to transfers of genes for drought resistance from alternative sites on chromosome 3, a major source of novel genetic variation for improved resistance to severe drought stress for *Lolium* (Humphreys *et al.*, 2006). Both *F. arundinacea* and *F. glaucescens* were sourced as donors for the drought-resistance genes. Recently, as part of a DefraLINK programme (LK0688), through a marker-assisted breeding programme QTL from both *Festuca* spp. for drought resistance were introduced into breeders' lines, firstly into *L. multiflorum*, and subsequently *L. perenne*, achieving in simulated drought-stress conditions up to an 80% improved water-use efficiency (WUE; DMY/unit H_2O consumed) (Humphreys *et al.,* 2012) over both *Lolium* species. The drought resistant *L. multiflorum* line Bb2540 was trialled successfully in field conditions in France when exposed to severe drought stress over three consecutive years. The line has since been entered into UK National Recommended Varieties List Trials. It was demonstrated (DefraLINK LK0688, unpublished) that enhanced water uptake at depth contributed at least in part to the improved WUE. *Festulolium* variety AberNiche, bred by IBERS, is the first to gain entry into the UK National Recommended List. It is an Italian ryegrass-type *Festulolium* and contains *F. pratensis* genes contributing to improved winter hardiness. Earlier, it was demonstrated that *F. pratensis* has a cold-acclimation mechanism that adapts its photosynthetic apparatus to freezing temperatures more effectively than that of *Lolium* with the QTL responsible located to chromosome 4 (Humphreys *et al.,* 2007*)*.

Stress-adaptive mechanisms present in *Festuca* spp. in addition to contributing genes for increased resilience by *Lolium* to climate change, may also contribute other more indirect benefits leading to environmental service. It has been demonstrated that protein protection mechanisms present in *F. glaucescens* have potential to improve ruminant nutrition and reduce harmful greenhouse gas N_2O and NH_3 emissions by livestock (O'Donovan *et al.* 2012). We report herein new examples where *Lolium* and *Festuca* genes interact positively to provide an important environmental service.

Materials and methods

A one-year field trial was undertaken on populations, each of 300 genotypes, of *L. multiflorum* × *F. glaucescens* (*LmFg*); *L. perenne* × *F. glaucescens* (*LpFg*), *L. multiflorum* × *F. mairei* (*LmFm*), and *L. perenne* × *F. mairei* (*LpFm*) (all 2n = 4x = 28). Dry matter yield (DMY), heading date, height at ear emergence, leaf width, plant size, growth habit, tiller density, and disease resistance were assessed throughout. Based on DMY in cut 2 (01/09/2011), the 50 largest plants were selected from each population and these assessed for their forage quality from foliar cuts taken earlier on 27/07/2011 and from the 01/09/2011 cut (%WSC, %DMD, Crude Protein). From each group of 50 genotypes, 10 plants were selected at random and tillers removed and established in 1m deep polythene-lined pipes filled with potting compost to assess root growth, depth, distribution, new and old roots and root turn-over monthly over a full growing season from February - October 2012. The rooting pipes were maintained in a cold glasshouse and supplied with optimal water and nutrients. A Principal Component Analysis (PCA) was employed on all root traits to determine the species' origin for their genetic control.

Results and discussion

In the field trial there was evidence of considerable heterosis between *Lolium* and *Festuca* genomes for DMY, plant tiller number, growth habit and leaf width. Apart from these traits, which in combination generally resulted in a large plant size, it was primarily the *Lolium* rather than that of *Festuca* that determined plant phenotype. All plants comprising the *LmFg* and *LmFm* populations were very tall, erect, and broad leaved, whilst all *LpFg* and *LpFm* plants were extremely prostrate, and narrow leaved. Only for tiller number was the *Festuca* parent species a determinant factor, with more tillers evident in *Fg* × *Lolium spp.* compared to the *Fm* × *Lolium* spp. hybrid combinations. For the 50 genotypes selected for their size from each of the 4 hybrid combinations, the *Lm* hybrids were consistently the larger with a mean DMY/plant (total cuts 1 and 2) of 186g for *LmFg* and 162g/plant for *LmFm*, whilst mean DMY/plant of *LpFg* was 106g/plant and114g for *LpFm*. The forage quality was determined by the *Lolium* rather than the *Festuca* parent for all traits (P<0.05 for %WSC, %DMD, and Crude Protein), with the means identical irrespective of the *Festuca* parent involved. Over 2 cuts, the %DMD mean at 78% was the same for both *LpFg* and *LpFm* (compared with 67% in both *LmFm* and *LpFm*). Similarly the %WSC mean at 24% was the same for *LpFg* and *LpFm* (compared with 21% in both *LmFm* and *LpFm*), and the % crude protein was identical at 14% for both *LpFg* and *LpFm* and was10% for *LmFm* and *LmFg*.

Root growth like foliar growth rate in the hybrids was exceptional, with 1m root growth observed regularly after only 1 month tiller establishment. The *LmFg* and *LmFm* hybrids established and grew more rapidly and more extensively than the *LpFg* and *LpFm* genotypes over the entire growing season providing a more extensive root system throughout the 1m root column. With the exception of *LpFm* in the spring, more new roots (white) rather than old (brown) were evident throughout the growing season in *L. multiflorum*-based *Festulolium* hybrids (*LmFg*, *LmFm*) irrespective of their *Festuca* species origin. The PCA indicated that mean root scores throughout the root columns, and new/old root scores were the principal discriminatory root traits between genotypes with the *Lolium* species origin the primary determinant for specific root trait expression (P<0.05).

The potential use of a *Festulolium* hybrid cultivar for flood mitigation has been demonstrated (Humphreys *et al*. 2012; MacLeod *et al.* 2013) resulting from the rapid turnover of its extensive and deep root system that led to improved soil structure and water retention. An *L. perenne* × *F. pratensis* cultivar reduced runoff by 51% compared with a leading *L. perenne* cultivar, and by 43% compared to *F. pratensis* over a two-year field plot experiment. The current work demonstrates the potential of other *Festulolium* species' hybrids whose extensive, deep root systems may provide an environmental service through C sequestration. The majority of carbon in soils is derived from roots. Deep soil C (below 30 cm) tends to be at lower concentrations than C in the top 30 cm. Deep rooting and highly branched roots such as those present in the *LmFg, LpFg, LmFm, LpFm* populations described herein should increase soil organic matter and enhance the potential for deep soil storage of carbon.

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Anthriscus sylvestris – biology, control and people's perception of cultural landscapes

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Abstract

In a four-year multidisciplinary study, we are combining studies of biology and different control measures of *Anthriscus sylvestris* with studies on people's perceptions of their cultural landscape, with special emphasis on *A. sylvestris*. We studied the effect of cutting the taproots at different lengths in three pot experiments (greenhouse, Ithaca, NY, USA and Ås, Norway and outdoors, Tromsø, Norway). Removal of the upper 4-5 cm of the root may be sufficient for controlling it. Demonstration trials in northern Norway showed that application of herbicides (mecoprop-P, thifensulfuron-methyl) effectively controlled *A. sylvestris*, whereas 2 cuts in spring and summer or 1 cut late in summer did not reduce infestation. Through case studies in coastal communities in Vadsø, Herøy and Sandstrand, northern Norway, we explored how changing cultural landscapes strongly influence people's perception of place. *Anthriscus sylvestris* seems to symbolize a degradation of the cultural landscape, representing a contrast to the living and well-managed landscape that people want to live in.

Keywords: cow parsley, cultural landscape, control measures, people's perception

Introduction

Anthriscus sylvestris is a monocarpic perennial herb, which in recent years has increased in abundance on nutrient-rich road verges and less intensively managed areas and grassland in Northern Norway. It is tall, supresses other species and virtually covers occupied landscapes in white while flowering. *A. sylvestris* propagates by a balance between vegetative and generative reproduction, which makes it difficult to control. The increase of *A. sylvestris* may be associated with the on-going decline and abandonment of agricultural activity in the Norwegian countryside; abandonment or less cutting and grazing of relatively fertile meadows (Hansson, 1994) as well as more humid conditions due to increased precipitation.

In a multidisciplinary study, we have investigated the biology and control measures of *A. sylvestris* and how people perceive cultural landscapes dominated by *A. sylvestris*. In detailed experiments we have been looking at resource allocation in undisturbed plants, translocation of assimilates, sprouting ability from different parts of the taproot and competition with grass. Demonstration trials have been conducted to study mowing at various times and stubble heights, and application of herbicides. Studies on people's perception and demonstration trials have been conducted in Vadsø, Herøy and Sandstrand in northern Norway. Here we present some results from the cases studies, one of the demonstration trials and the experiments with sprouting ability of the taproot.

Materials and methods

A demonstration trial on an abandoned area with large amounts of *A. sylvestris* was established in 2010 in Sandstrand, northern Norway (68.7°N). In plots, 3×16m, treatments with cutting and spraying with the herbicide mecoprop-P and an untreated control, 2 replicates, were applied in 2010 and 2011. The effect was monitored by visually estimating the biomass of the *A. sylvestris* and other species in each plot, and by counting *A. sylvestris* in 4×1.0 m^2 squares in each plot in early summer 2012. The effect of treatments on number of plants was analysed using ANOVA, (Minitab 16). Multiple comparisons were performed with the Tukey test at $P<0.05$ as significant.

In another set of experiments, the various parts of the taproot roots of *A. sylvestris* were cut into different lengths and were planted at various soil depths in Ithaca USA, Tromsø and Ås, Norway. In the Ithaca experiment taproots were collected in autumn 2010, and were cut into 1, 2, 4 or 8 cm lengths from the upper, middle and lower parts of the root in spring 2011 and tested in a greenhouse. In the experiment in Tromsø taproots were collected in June, July and August 2011 and the upper 4 cm, the lower part and intact roots were tested outdoors. The root fragments were planted at 2, 8 and 17 cm soil depth in large pots. In an experiment in Ås, 4-cm root fragments from the upper part, with or without the upper 1 cm, were planted at 2 cm soil depth in pots in a greenhouse in April, May and August 2012. We assessed the percentage emergence from the root fragments at 1-3 months after planting.

Case studies were conducted in Vadsø and Herøy to investigate people's perception of changing cultural landscapes. Through qualitative interviews, we have focused on how actors involved in these coastal landscapes – such as farmers, tourist entrepreneurs, recreationists, agents of public management – make use of, understand and perceive the landscape.

Results and discussion

The demonstration trial in Sandstrand showed that cutting strategies did not reduce biomass of *A. sylvestris* (Figure 1) and this is in accordance with other studies (e.g. Hansson, 1994; Hansson and Persson, 1994). The herbicide treatment with mecoprop-P effectively controlled the species, and the number of small and large rosettes was reduced compared with the control (Figure 1; Table 1). In the other demonstration trials mecoprop-P and also the herbicide thifensulfuron-methyl gave a very good control of *A. sylvestris*. Treatments with cutting of flowers did not reduce the number of small or large rosettes, but there was a trend that seedlings were reduced (Table 1).

Table 1. Effect of 2 years (2010, 2011) of cutting and herbicide (mecoprop-P) strategies on number of seedlings, small and large rosettes of *A. sylvestris* 19 June 2012, in a demonstration trial at Sandstrand, 2 reps.

Treatment	Seedlings $P = 0.061$	Small rosettes $P = 0.020$	Large rosettes $P = 0.003$
	Number of plants m^{-2}		
a) Control	95.3 a	12.0 ab	5.8 ab
b) Cut spring* and July/August, stubble height 5 cm	80.8 a	16.5 a	9.0 a
c) Cut at full bloom, stubble height 70 cm	13.8 a	10.8 ab	8.5 a
d) Cut at full bloom, stubble height 5 cm	16.3 a	11.3 ab	7.0 a
e) Cut late at seed set, stubble height 5 cm	28.0 a	11.5 ab	7.0 a
f) Sprayed mecoprop-P, 2.7 kg a.i. ha^{-1} in spring*	24.6 a	2.9 b	0.5 b
g) Cut in spring*, stubble height 5 cm + July/August sprayed mecoprop-P, 2.7 kg a.i. ha^{-1}	21.8 a	2.1 b	0.0 b

*At stem elongation; results followed by different letters are significantly different (Tukey test $P<0.05$)

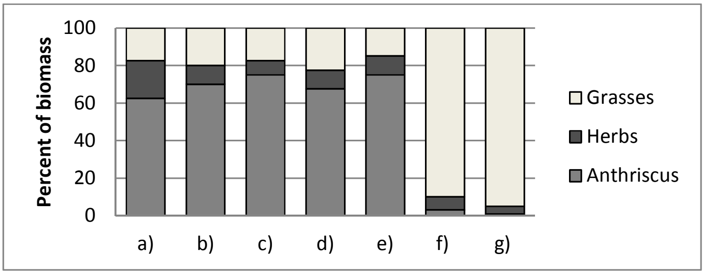

Figure 1. Effects of cutting (5 and 70 cm stubble heights) and herbicide (mecoprop-P; 2.7 kg a.i. ha^{-1}) strategies on biomass of *A. sylvestris*, grasses and other herbs (visually estimated, 2 replicates) at Sandstrand 19 June 2012. For treatments (a-g), see Table 1.

In the sprouting experiments in Ithaca and Tromsø shoots from the upper part of the roots emerged, whereas no sprouting occurred from the lower part of the roots (shown for Tromsø-data in Table 2). Deeper burial resulted in reduced emergence. Emergence was also less where the upper 1 cm of the taproot was excluded (Ithaca, Ås), compared to where the upper 1 cm of the taproot was included (Tromsø, Ås). These results indicate that effective control can be obtained by mouldboard ploughing and in smaller areas by removing the aboveground shoot including the upper 4-5 cm of the root by hand.

Table 2. Percent emergence from various parts of the taproots of *A. sylvestris* tested in Tromsø, Norway 2011. Averaged over 3 test times (June, July, and August) and 10 parallels.

	Burial depth		
Part of taproot tested	2 cm	8 cm	17 cm
	% emergence from root fragments		
Whole root	73	70	17
Upper 4 cm	83	37	23
Lower part	0	0	0

Studying people's perceptions of the cultural landscape showed that cultural landscape is a crucial element of people's perception of place. People and landscapes are closely interwoven through practices (Ingold, 2000), and landscape is part of the dynamic processes of rural transformations. Considering ideals of landscape, place and social practices underlying contemporary cultural landscape management reveals paradoxes; some landscape aspects are considered valuable and worth protecting. At the same time, these landscapes seem to lose their meaning as they are related to dismantling practices. There seems to be a disruption between 'taskscapes' and landscape values; the valued landscape is the traditional, open grazed land, which is disconnected from today's farming practices. The emerging landscape, dominated by weeds like *A. sylvestris*, is perceived as an alienated landscape, characterized by deterioration. Landscape management becomes a struggle of a common identity.

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Establishment of an *Arrhenatherion* meadow through on-site threshing material and green hay transfer

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Abstract

Arrhenatherion meadows are highly abundant in semi-natural grasslands of Central Europe and are regarded as an important resource for the ecological restoration of species-rich grassland. The effectiveness of different restoration methods of former intensively used arable land was tested by means of a field experiment. A donor site was harvested via i) on-site threshing (OST) and ii) fresh hay cutting - green hay (GH). The harvested material was transferred to a receptor site, which was deep ploughed before applying the seed and plant material. Three years after the establishment 20 target species with a transfer rate of 61% were observed for the OST method, resulting in a vegetation cover of target species of 72%. In the same year, the GH method achieved a number of 16 target species at the transfer rate of 52% and a share of target species at the vegetation cover of 74%. The number of target species and also the transfer rate increased over the years of observation. Both establishment methods are, in combination with an adequate site preparation, effective methods for the ecological restoration of *Arrhenatherion* meadows on former arable land. A PCoA analysis showed a trend of increasing similarity of donor site and receptor site over time, but up to now without any statistical significance.

Keywords: receptor site, target species, ecological restoration, transfer rate

Introduction

Semi-natural grasslands are the result of long and sustainable utilization of meadows and/or pastures by humans in Central Europe. *Arrhenatherion* meadows are one of the most common plant communities of these semi-natural grasslands and also an important resource for the ecological restoration of species-rich grassland. Practically relevant restoration of semi-natural grassland has been realized successfully on differing sites for many years in several European countries (Prach *et al.*, 2012; Scotton *et al.*, 2012). Restoration success can be defined as the development towards a natural, self-maintaining habitat with the physical and biological characteristics defined in the restoration project (Scotton *et al.*, 2012). The aim of this research work was to study the transfer rate of target species and the development of the vegetation cover on the receptor site established with on-site threshing material and green hay.

Materials and methods

The donor site (nutrient-poor *Arrhenatherion* meadow) is located in Upper Austria, at 48°18’N, 14°03’E; 310 m a.s.l. The receptor site was an area of plain arable land, located at 47°29’N, 14°06’E, in the Enns valley, at an altitude of 700 m a.s.l. Before establishment, site preparation was done by deep ploughing (80 cm), not only to reduce the nutrient level but also to deplete the soil seed bank of unwanted weeds (Scotton *et al.*, 2012). The soil properties of the donor site and the receptor site are presented in Table 1. The on-site threshing (OST) material was harvested with a plot combine thresher at a cutting width of 1.5 m on 1 July

2009. The harvested material was air-dried and roughly cleaned with a 6 mm sieve. After determination of purity and thousand seed weight of the OST material (described in Haslgrübler *et al.*, 2011) the receptor site was sown with a seed density of 3 g m^{-2} on 25 August 2009. The green hay (GH) was harvested on 1 July 2009 with a hand-operated motor mower early in the morning, because the morning dew enables the seed to stick to the plant. The fresh material was immediately brought to the receptor site to avoid decay and seed losses, as recommended by Scotton *et al.* (2011). The green hay application was implemented immediately after cutting with 3.5 kg m^{-2} on the receptor site, representing a biomass ratio between donor: receptor site of 2:1. In the year of establishment the GH treatment received a cleaning cut after two months in order to control weeds. The experimental trials were established as a complete block design with 3 replicates of 174 m^2 each. The projective cover of the different species on the donor site was surveyed according to Schechtner (1958) in the harvesting year 2009 and on the receptor site twice a year between 2010 and 2012. The development of the different species was assessed over a period of three years under a 2-cut regime. The count of target species was done over all replicates; therefore no statistical tests were possible.

Table 1. Soil properties of the donor and restoration site.

	Gravel %	N % mass	pH $CaCl_2$	P (CAL) mg kg^{-1}	K (CAL) mg kg^{-1}
Donor site (0–20 cm)	44.14	0.52	7.2	15.8	73.9
Receptor site (0–20 cm)	47.83	0.15	6.2	56.0	91.9

A MANOVA and Principal Coordinates Analysis (PCoA) was used for data analysis, done with the statistics language R, Version 2.15.2 with the package vegan, Version 2.0-5 (Oksanen *et al.*, 2012). The analysis was based on a distance matrix focusing on beta diversity calculated with the metric Jaccard index as implemented in vegan (functions 'vegdist' and 'betadisper' in vegan). The grouping variable was year, respectively age of the plot.

Results and discussion

Restoration success depends not only on the number of different species at the donor site and on the transfer rate, but also on site conditions of the receptor area. The OST method showed the highest number of species in 2010, including annual weeds like *Capsella bursa-pastoris* and *Viola arvensis*. The number of target species and also the transfer rate increased over the years. In 2012, twenty target species were observed over all replicates in the OST treatment, and the transfer rate was 60.9% of the total species count. The proportion of target species of the total projective vegetation cover increased over the years and reached 72.2% in 2012. The total number of species in the GH treatment remained stable over the years. The number of target species over all replicates increased slightly between 2010 and 2012. In 2012, the GH treatment reached a total number of 16 target species resulting in a transfer rate of 51.6% of the total species count. The mean relative cover of target species found over all replicates in the vegetation cover was 74.3%. The high amount of biomass/mulch from the GH method in the first year after establishment prevented the germination of forbs and therefore more grasses than forbs were transferred.

In 2012, three years after establishment, more target species were found in the plots of the OST treatments than in the GH plots (Table 2).

The results of the MANOVA (function 'adonis' in vegan) showed no significant differences between the two restoration methods (P = 0.883, R^2 = 0.00754) and years (P = 0.919, R^2 = 0.04828) concerning group (OST vs. GH) variance based on a dissimilarity matrix. The result of the following PCoA for donor and receptor site shows the development of the two methods OST and GH together over three years. The variance (represented by the mean distance from the centroid) within the replicates was, independent of the method in the first two years (2010 to 2011), higher compared to the donor site, and lower in 2012, though without any statistical significance. The receptor site was still rather far from the donor site in terms of its species composition; however, the development of the vegetation seems to be heading in the anticipated direction. Part of the difference might possibly be explained by the distinction of climatic conditions and soil properties of the donor and the receptor site; e.g. the pH value on the receptor site is lower and the soil P content much higher than at the donor site (Table 1).

Table 2. Species number, vegetation cover and transfer rate of target species from on-site threshing (OST) and green hay (GH).

Treatments	Donor site	Receptor site					
Parameter		OST			GH		
Date of survey	6/2009	6/2010	6/2011	6/2012	6/2010	6/2011	6/2012
Number of species	67	38	27	28	30	30	30
Numb. of target species (over all replicates)	48	16	19	20	14	15	16
Transfer rate of target species (% species)	-	50.7	60	60.9	47.7	48.5	51.6
Total cover (%)	97.6	58.7	91.0	85.6	67.0	73.3	86.0
Proportion of target species (% total cover)	83.9	70.1	55.5	72.2	79.0	73.6	74.3

Conclusion

On-site threshing and green hay are suitable harvesting methods and an effective way for an ecological restoration of *Arrhenatherion* meadows on arable land in combination with adequate site preparation, as shown by the results of our project. Four years after establishment of the experimental site there were no significant differences found between on-site threshing and green hay, and therefore it can be stated that the choice of restoration method for establishing an *Arrhenatherion* meadow depends more on the availability of equipment and other practical conditions, such as the accessibility of the sites.

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Volcanic impacts on grasslands – a review

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Abstract

Volcanic eruptions have a wide range of effects on many of the Earth's grassland systems, such as in Africa, the Americas and Iceland. The effects are determined by the severity of the impact, vegetation height and the resilience of the impacted system. Biological soil crusts, often an important component of dryland grazing systems, are severely affected by thin deposition of volcanic ash, whereas taller vegetation with ample nutrient supplies is more tolerant to such disturbances. Redistribution of airborne unconsolidated volcanic materials (tephra, including volcanic ash) in the aftermath of eruptions is often quite harmful to ecosystem survival. The interaction between volcanic events and land-use practices such as grazing is detrimental. Alien plant species in volcanic areas can severely interfere with ecosystem recovery.

Keywords: tephra, volcanic ash, volcanic impact, grazing

Introduction

There are 1545 active volcanoes with >9000 eruptions listed in the current *Smithsonian Catalog of Active Volcanoes* (Siebert *et al*., 2010). About 70 volcanoes are active each year on average, but most eruptions are small. The majority of volcanoes occur at the margins of tectonic plates, which account for 94% of the volcanic activity, with the 'Pacific Ring of Fire' and the African Rift Valley being good examples (Simkin and Siebert, 2000). The activity occurs also in relation to so-called hot spots, which are isolated and transfer heat from the mantle (Bjarnason, 2008), such as on the islands of Hawaii, Galapagos and Iceland, and in Yellowstone, which is an example of intra-continental volcanic hot-spot activity. The volcanic activity affects most of the world's ecosystems as airborne volcanic materials are dispersed over large areas. The impacts vary considerably because of the different nature of volcanic eruptions and the ecosystems being affected. In this paper, the nature of these impacts will be investigated based on published literature with emphasis on grassland ecosystems and volcanic tephra (ash). Over 500 articles were reviewed, but only a few are mentioned here due to space constraints. The full review will be published in Advances in Agronomy (Arnalds, 2013).

Nature of the volcanic ejecta

The most common types of impacts are related to outputs of lavas and airborne volcanic materials. The term for airborne volcanic ejecta is 'tephra', but 'ash' is defined as tephra <2 mm in diameter. Other outputs that can have pronounced effects on ecosystems are: i) pyroclastic flows, which have devastating influences on the affected areas; ii) lahars, which are a mixture of solid volcanic materials and water that also are very destructive, and iii) jökulhlaups which are flood events that result from melting ice during eruptions (see chapters in Sigurdsson *et al*., 2000). The effects of volcanic tephra are dependent on the amount of the tephra, which is commonly reported by the depth of deposition. However, grain size and chemical composition, ranging from basaltic to silicious tephra, also influence ecosystem impacts. The basaltic tephra is more easily weathered, releasing available cations that improve ecosystem fertility, whereas the silicious tephra weathers more slowly and can result in acidic soils. Tephra that falls on snow can, to an extent, be removed by snowmelt, thus decreasing volcanic impacts.

Responses of plants to tephra burial

The ability of vegetation to recover after tephra deposition is in part determined by the height and physiology of the vegetation. Buried plants are deprived of sunlight, and regrowth depends on reserves, with increased elongation of apical meristem, upward growth of apices, rhizomes and shoots (Maun, 1998). Plants that succeed have a chance to utilize reserves otherwise not available to them (Gilbert and Ripley, 2008). The plants vary in their response: some die, some are little affected, while others may show positive responses (Maun, 1988), and this difference results in changes in botanical composition upon impacts. The low-growing lichens, mosses and biological soil crusts are extremely vulnerable to disturbance, as is seen in Iceland (Aradottir *et al*., 2010). Many of the grassland species can tolerate tephra deposition of similar depth to the vegetation height. Thin deposition (<1-2 cm) on low-growing dryland vegetation, especially crust-dominated systems, can be detrimental. Recovery rates for grasslands under relatively dry climatic conditions can be expected to reach decades where burial exceeds the vegetation height (as judged from a review of the literature). Subtle volcanic inputs (<1 mm) can, however, be quite beneficial by recharging soils with plant-available nutrients, influencing areas at great distances from the origin of the tephra.

Tephra redistribution

Volcanic tephra affects the physical properties of the surface, such as infiltration rates and run-off, and this depends much on the nature of tephra. Reduced infiltration rates, with increased run-off, are more often reported than enhanced rates. Redistribution of tephra to depths exceeding the vegetation height can be enormous, both by wind and water. While redistribution of tephra can reduce the tephra depth, it can cause severe damage as well. Water erosion can reach staggering proportions of >100,000 t km^{-2} yr^{-1} (e.g., Pinatubo, Philippines; Eyjafjallajökull, Iceland), with deflation rates exceeding 10 mm yr^{-1}. Wind distribution can also be severe with negative effects such as reoccurring dust pollution long after initial tephra inputs, as witnessed after the 1991 Hudson eruption (Chile; Wilson *et al*., 2011) and the 2010 Eyjafjallajökull eruption (Iceland).

Volcanic eruptions, grazing and ecosystem resilience

Recovery of grasslands after severe volcanic disturbances is dependent on the ability of the vegetation to stabilize the tephra, which depends not only on vegetation height and composition, but also on the available nutrient reserves. Systems with tall-growing species and ample nutrient reserves provide resilience against eruptions. Grazing affects vegetation height, which can be quite low in degraded systems. This is exemplified by the communal grazing lands in Iceland where grazing is a detrimental factor for survival after volcanic impacts. Low-growing vegetation facilitates redistribution of the tephra, often with harmful effects on neighbouring ecosystems. Wind transport of tephra from bare erosion spots also leads to enhanced tephra thickness elsewhere. Dryland grassland systems, where biological soil crusts are important ecosystem components, suffer severely from volcanic disturbances, as is witnessed after the 2010 Eyjafjallajökull eruption in Iceland. It can be concluded that the ability of Icelandic ecosystems to recover from volcanic impacts has been greatly reduced over the >1100 years since Iceland was settled.

Exotic/alien species

Land use and the introduction of new species may influence recovery of grassland ecosystems after tephra deposition. Many such plants are invasive in nature and their spread may become facilitated after eruptions, which has compounded ecosystem recovery in many volcanic areas. It is clear that the use of exotic invasive species should be avoided in volcanic areas unless their effects on recovery and ecosystem development after eruptions are fully understood.

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Poster Presentations

Forage production and essential oil content of *Psoralea bituminosa* and *P. morisiana* accessions

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Abstract

Fifteen accessions of *Psoralea bituminosa,* belonging to the botanical var. *albomarginata*, *crassiuscula* and *bituminosa,* and *P. morisiana* of Spanish and Sardinian origin were studied for their forage yield and essential oil content and composition. The accessions showed different total and seasonal forage production, reaching the maximum value of 500 g fresh weight per plant in *P. bituminosa* 'Calnegre'. The essential oil content ranged from 0.003% to 0.054% of leaf fresh weight. The essential oil composition of accessions varied in function of the species, the botanical variety and their origin. The number of identified oil components was 66 in *P. bituminosa bituminosa*, 47 in *P. bituminosa albomarginata*, 51 in *P. bituminosa crassiuscula*, and 59 in *P. morisiana*, representing at least 92% of total components in each oil. Caryophyllene oxide and β-caryophyllene, Z-β-farnesene e D-germacrene and long-chain alcohols were the most abundant components. The good forage production and low essential oil content are positive factors for the use of *Psoralea* as forage.

Keywords: *Psoralea*, forage production, essential oil

Introduction

Psoralea bituminosa L. (pitch or bitumen trefoil) and *P. morisiana* Pign. et Metlesics are considered potential forage species for their ability to maintain green leaves in late spring and summer, and also in drought conditions, thanks to their deep rooting system. However, some secondary metabolites, among them essential oils, can limit forage intake and decrease palatability as they may contribute to the strong smell of bitumen in *P. bituminosa.* Nevertheless, they can be useful for pharmacological applications. A study was carried out to verify the forage yield and the essential oil content and composition of 15 accessions of *Psoralea* spp.

Materials and methods

In 2010 an experimental field was established in Alghero (Sardinia, 40°35'N, 8°22'E). The site has a Mediterranean climate with an average annual rainfall of 540 mm, and an alluvial soil with a high limestone content and neutral pH (6.9). Seven Spanish accessions (belonging to *P. bituminosa*) and eight Sardinian accessions (belonging to *P. bituminosa* and *P. morisiana*) were arranged in 3 randomized blocks. Three plants per plot were cut in October and December 2010, March, October and December 2011, only if plant height exceeded 15 cm from soil. Fresh and dry weight of forage were determined. In October 2011, 200 g of fresh leaves of each accession were distilled in a Clevenger-type apparatus according to the Italian Pharmacopoeia Standards. The yield and the composition of essential oils were determined. Essential oils were analysed in a Hewlett Packard GC 5890 and in a mass spectrometer Hewlett Packard G1800B GCD System. Each oil analysis was repeated 4 times. Oil components were identified on the basis of the retention times for GC and the comparison of GC-MS analysis with the NIST library.

Results and discussion

Forage yield was significantly different among accessions and seasons, ranging from 3.3 to 109 g $plant^{-1}$ (Table 1). In the year of establishment, most of the bitumen trefoil accessions showed higher yields after summer and a faster autumn regrowth than *P. morisiana*. Smaller differences in terms of forage yield were observed in the second year. Nevertheless, Albo-H-Tolfrio and Crassiuscula showed a higher yield stability during the course of the experiment. With the exception of Famara, Tenerife and Loculi among *P. bituminosa*, and Burcei among *P. morisiana*, all accessions showed a satisfactory forage production, even though not comparable to that found by other authors under a different plant management system (Mendez *et al*., 2006).

Table 1. Forage yield from *P. bituminosa* (P.b).and *P. morisiana* (P.m.) accessions during 2010 and 2011. The same letters in the same column indicate values that are not statistically different at $P \leq 0.05$ (LSD test). 1 = var. *albomarginata*; 2 = var. *crassiuscula*; 3 = var. *bituminosa*.

Species	Bot. var.	Accession	Forage yield (g $plant^{-1}$)				
			Oct 10	Dec 10	Mar 11	Oct 11	Dec 11
P. b.	1	Albo-H-TolFrio	81.8ab	77.1ab	-	93.6a	44.2ab
		Famara	18.8ef	-	34.4de	22.1bc	-
	2	Crassiuscula	74.0a-d	78.2ab	-	54.0ab	56.8a
		Vilaflor	80.8ab	46.7bc	-	58.3ab	-
	3	Calnegre	109.2a	93.8a	-	3.3c	-
		Llano del Beal	63.9a-e	-	51.9cde	66.9ab	-
		Tenerife	50.7b-f	55.7bc	-	21.8b-e	22.0c
		Loculi	10.4f	-	25.7f	32.3bc	-
		Monte Rosello	76.1abc	39.2c	-	57.3ab	26.0c
		Siniscola	37.1b-f	-	60.3cd	50.2abc	-
P. m.		Monte Gonareddu	32.1c-f	-	97.9a	42.8bc	33.2bc
		Punta Giglio	40.1b-f	-	70.7bc	39.4bc	-
		Siliqua	36.4b-f	-	90.0ab	57.4ab	-
		Bitti	30.3d-f	31.0d	-	69.8ab	30.9bc
		Burcei	21.3ef	-	33.3e	22.3bc	-

Steam distillation of leaves yielded between 5.4 and 107 mg, a very low amount of essential oil. About 96 compounds were identified in the essential oils of *P. bituminosa*; specifically 66 in var. *bituminosa*, 47 in var. *albomarginata*, 51 in var. *crassiuscula*, and 59 in the species *P. morisiana*, and belonging to 10 main chemical groups (Table 2). The most abundant compounds in the oils of all accessions were sesquiterpenes, ranging from 45.82% in Famara and 75.37% in Siliqua. The most abundant compound in *P. bituminosa* was β-caryophyllene, followed by z-β-farnesene in Spanish accessions and caryophyllene-oxide in Sardinian accessions; Z-β-farnesene predominated in *P. morisiana*, followed by β-caryophyllene. *Psoralea* accessions also showed a relatively high content of long chain alcohols, in agreement with findings reported by other authors (Pecetti *et al*., 2007). These alcohols act as carriers in biological systems and their relation with furocoumarins, a class of compounds responsible for photosensitization when ingested by animals, needs to be investigated. Furocoumarins are abundant in *Psoralea* leaves (Martìnez *et al.*, 2010).

Table 2. Composition of essential oils of *P. bituminosa* and *P. morisiana*. The numbers refer to the percentage of class of compounds respect to the total. 1 = alcohols; 2 = sesquiterpenes and oxygenated derivatives; 3 = hydrocarbons; 4 = furocoumarins; 5 = other terpenes; 6 = aldehydes; 7 = Sulphurated compounds; 8 = esters; 9 = acids, and 10 = others.

Accessions	1	2	3	4	5	6	7	8	9	10
AlboHTolFrio	39.68	47.30	0.67	0	3.21	0.73	0	0	1.12	0
Famara	32.54	45.82	0.73	0	5.52	1.44	0	3.54	4.23	0
Crassiuscula	21.59	53.28	1.42	0	8.98	0.99	0	4.17	7.32	0
Vilaflor	24.49	46.41	1.76	0.65	11.76	2.14	0	2.38	6.74	0
Calnegre	29.56	50.56	1.38	0	8.65	1.12	0	0.34	1.15	0
Llano del Beal	6.51	66.09	0	0	20.61	0.32	0.39	1.56	0.59	0.39
Tenerife	24.63	52.12	0.77	0	7.28	1.11	0	2.89	2.65	0
Loculi	13.60	56.09	2.01	0	21.62	0.43	0	1.96	1.11	0.48
Monte Rosello	10.82	62.73	0.94	0	16.76	0.54	0	1.27	0.93	0.35
Siniscola	12.78	65.70	0.33	0	16.10	1.22	0.30	0.67	1.29	0
M. Gonareddu	21.91	70.40	0	0	1.10	0	0	0	0	0
Punta Giglio	26.42	70.71	0	0	0.30	0	0	0	0	0
Siliqua	18.67	75.37	0	0	2.56	0	0	0	0	0
Bitti	25.92	68.91	0	0	0.87	0	0	0	0	0
Burcei	21.95	72.01	0	0	0.83	0	0	0	0	0

Conclusions

Psoralea bituminosa showed a good forage yield under conditions when most forage plants are not suitable to be grazed or mown. Its leaves contain a low amount of essential oils and this is a positive attribute for the use of this species as green foliage for animal feed. Some oil compounds, such as long chain alcohols, may enhance the absorption of other secondary potentially toxic metabolites and their interaction needs to be investigated further.

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Potential of red vetchling (*Lathyrus cicera*) for forage production

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Abstract

Red vetchling (*Lathyrus cicera* L.) is being evaluated by the Institute of Field and Vegetable Crops and the Faculty of Agriculture of the University of Novi Sad. A small-plot trial was conducted in 2009 and 2010 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi. It included eleven red vetchling accessions of diverse geographic origin and was aimed at assessing the possibility of the use of red vetchling as a forage crop. The accession MM 06/13 had the highest two-year average forage dry matter yield (7.2 t ha^{-1}), while the accessions ACC 127 and K-1271 had the lowest two-year average forage dry matter yield (3.5 t ha^{-1}). The two-year average forage dry matter crude protein content ranged from 188 g kg^{-1} in the accession SA22083 to 213 g kg^{-1} in the accession ACC 327.

Keywords: forage dry matter yield, fresh forage yield, *Lathyrus cicera*, red vetchling

Introduction

Red vetchling (*Lathyrus cicera* L.), also known as chickling pea and chickling vetch, originated in the Mediterranean centre of diversity. Today, it is used mostly in animal feeding in South-Western Europe, West Asia and North Africa (Fernández-Aparicio and Rubiales, 2010). It is suited to poor soils, tolerates both length and intensity of low temperatures in the Mediterranean areas and is highly resistant to spring droughts (Vaz Patto *et al.*, 2006).
There are about 30 vetchling species present in the wild and agricultural flora of Serbia and other Balkan countries. Some wild vetchling species are considered to have a considerable potential for becoming cultivated (Mihailović *et al.*, 2011). Others are crops that have become largely neglected and underutilized (Mikić *et al.*, 2011), such as grass pea (*L. sativus* L.) and red vetchling. For this reason, an extensive evaluation of numerous vetchling species is being carried out jointly by the Institute of Field and Vegetable Crops and the Faculty of Agriculture of the University of Novi Sad, with a main goal of their (re)introduction to wide production. This research was aimed at assessing the potential of a set of red vetchling accessions for forage production in the agroecological conditions of Serbia.

Materials and methods

A small-plot trial was carried out in 2009 and 2010 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi. It included eleven red vetchling accessions of diverse geographic origin, maintained within the *Lathyrus* collection at the Institute of Field and Vegetable Crops.
In both trial seasons, all eleven accessions were sown in early March, with a rate of 150 viable seeds m^{-2} (Mihailović *et al.*, 2006-2007), a plot size of 5 m^2 and three replicates. Each

accession was cut in the stages of full flowering and first pods, as a balance between forage yield and quality, namely in the first half of May in both years.

The number of days from sowing to first flowering was recorded for each accession. The forage dry matter yield (t ha^{-1}) was determined on the basis of fresh forage yield and forage dry matter proportion in the green forage samples taken after cutting and then dried until constant mass at room temperature. The same forage dry matter samples were used for separating stems from leaves, adding flowers and first pods into the latter, and calculating leaf proportion in forage dry matter. Forage dry matter crude protein content (g kg^{-1}) was determined on the basis of forage dry matter nitrogen content (g kg^{-1}) × 6.25.

Results were processed by analysis of variance (ANOVA) applying the Least Significant Difference (LSD) test using the computer software MSTAT-C.

Results and discussion

The average two-year number of days from sowing to first flowering ranged between 59 days in ACC 127 and MM 06/13, and 72 days in K-1271. The average length of red vetchling growing season was shorter in comparison to that in common vetch (*Vicia sativa* L.) accessions tested in the same agroecological conditions, with 85 days (Mikić *et al.*, 2013).

With 7.2 t ha^{-1} and 6.5 t ha^{-1}, respectively, the accessions MM 06/13 and K-1703 had significantly two-year average higher forage dry matter yield in comparison to all the other nine tested red vetchling accessions (Table 1). On the other hand, the accessions ACC 127 and K-1271 had the lowest two-year average forage dry matter yield (3.5 t ha^{-1}). Overall, the agronomic performance, in terms of forage dry matter yield, of the tested red vetchling accessions was inferior to that of the grass pea accessions in the same agroecological conditions, with a range between 3.9 t ha^{-1} and 9.0 t ha^{-1} (Mihailović *et al.*, 2011).

Table 1. Two-year average values of forage dry matter yield (t ha^{-1}), forage dry matter proportion, forage dry matter stem proportion and forage dry matter crude protein content (g kg^{-1}) in red vetchling accessions at Rimski Šančevi in 2009 and 2010.

Accession	Number of days from sowing to first flowering	Forage dry matter yield	Forage dry matter proportion	Forage dry matter leaf proportion	Forage dry matter crude protein content
Argos	61	4.2	0.22	0.54	198
SA22083	67	4.5	0.21	0.55	188
ACC 127	59	3.5	0.25	0.54	201
ACC 327	64	4.1	0.22	0.43	213
CPI37712	65	5.6	0.21	0.59	195
K-1271	72	3.5	0.26	0.57	197
K-1420	68	5.8	0.22	0.57	199
MM 06/13	59	7.2	0.21	0.56	201
K-1703	62	6.5	0.25	0.59	205
K-1372	64	5.1	0.22	0.57	202
K-368	71	4.8	0.20	0.46	209
$LSD_{0.05}$	5	0.8	0.02	0.07	9

The average two-year forage dry matter proportion in the tested red vetchling accessions varied from 0.20 in K-368 to 0.26 in K-1271, with significant differences (P<0.05) among many of them (Table 1). It was similar to the forage dry matter proportion of the grass pea accessions in a preliminary trial at Rimski Šančevi (Mikić *et al.*, 2010).

On average, the highest leaf proportion (0.59) in forage dry matter was in the accessions CPI37712 and K-1703, while the lowest one (0.43) was in the accession ACC 327. The tested red vetchling accessions had a lower forage dry matter leaf proportion in comparison with that of yellow vetchling (*L. aphaca* L.) accessions in the same agroecological conditions, with a range between 0.54 and 0.61 (Mikić *et al.*, 2012).

There were significant differences in the two-year average values of forage dry matter crude protein content among most of the tested red vetchling accessions. It ranged from 188 g kg^{-1} in SA22083 to 213 g kg^{-1} in ACC 327, demonstrating that our red vetchling had higher forage dry matter crude protein content than common vetch in Turkey, with 180.3 g kg^{-1} (Yucel and Avci, 2009).

Conclusion

Some of the tested accessions of red vetchling demonstrated a good agronomic performance in terms of forage dry matter yield, leaf proportion and crude protein content. The next steps will comprise a more detailed study on forage yield components, forage chemical composition and stress resistance. This offers a solid basis for improving this neglected crop by breeding, and opens a possibility of developing its first cultivars for forage production.

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Comparison of *Phleum phleoides* versus *Phleum pratense* species for forage potential

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Abstract

The agricultural importance of timothy (*Phleum pretense* L.) has led to many studies of feeding value and production, whilst Boehmer's cat's-tail (*Phleum phleoides* (L.) H. Karst.) has never been systematically evaluated for agronomic traits. This study examined and compared the agronomically important traits of *P. phleoides* and *P. pratense*. The experiment was carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry in 2008 and 2009. Plant height, number of tillers, earliness, dry matter yield from 1st harvest and regrowth after 1st harvest were evaluated along with crude protein (CP), neutral detergent fibre, water soluble carbohydrates content and dry matter (DM) digestibility at full heading stage from the 1st harvest. *Phleum phleoides* fell behind in yield parameters compared to *P. pratense*. However, the ability of *P. phleoides* to resist drought stress, combined with high CP content and DM digestibility shows the agronomic potential of this species, which could be improved by plant breeding.

Keywords: timothy, Boehmer's cat's-tail, feeding value, agronomic potential

Introduction

Breeding activities in forage species have mainly directed selection towards increasing economic yields, but not for adaptation and survival to environmental stresses (Židonytė, 1992). Highly developed mechanisms to resist water stress in Boehmer's cat's-tail (*Phleum phleoides* (L.) H. Karst.) allow this species to develop and grow in unfavourable areas or under adverse conditions (Hubbard, 1992; Židonytė, 1992). Timothy (*Phleum pratense* L.), however, being one of the most productive grass species in terms of the first-cut yield, has a low aftermath growth under dry conditions (Lemežienė *et al.*, 2004). The study of (Jonavičienė*, et al.*, 2012) demonstrated different water stress responses in *Phleum* species. *Phleum phleoides* plants maintain higher leaf relative water content (RWC) and show less reduction in quantum efficiency of photosystem II (F_v/F_m) under water stress, compared with *P. pratense*. However, timothy is known for its good palatability, nutritional feed value and thus is a major forage crop in northern latitudes, while the information regarding the nutritional value of Boehemer's cat's-tail is limited (Casler, 2001). The aim of the present study was to determine the variation in forage quality of *P. pratense* versus *P. phleoides* species and to evaluate their forage potential.

Materials and methods

Plant material comprised two Lithuanian cultivars of hexaploid *Phleum pratens*e (cv. Jauniai and Žolis) and two wild ecotypes of diploid *Phleum phleoides* (No. 2718 and 2754). Experimental material was evaluated at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (55°19'N, 23°54'E) in 2008 and 2009. Seeds were sown in pots with soil mixture and grown in a greenhouse (16 h light/8 h dark; temperature: 20°C ± 5°C). In late spring, 15 randomly selected plants per accession were transferred to the experimental field and planted at 50×50 cm distances in carbonate gleyic, moderately heavy drained brown soil. The plough layer was 25-30 cm; pH 7.2-7.5; humus 1.9-2.2%; total N 0.14-0.16%; mobile phosphorus was 201-270 and potassium 101-175 mg kg $^{-1}$ of soil. The following agro–morphological traits were estimated following standard protocols (Tayler, 1985): plant height (tillering stage) (PH); number of branches (NB); heading time (time of 50% inflorescence emergence) (EA); dry matter yield from the 1st harvest (DMY); regrowth after 1st harvest (RG). Analyses of crude protein (CP), neutral detergent fibre (NDF), water soluble carbohydrates (WSC) and DM digestibility (DMD) were performed at grass full-heading stage from the 1st harvest. The samples were dried, ground by a mill with 1 mm sieve and analysed by NIR spectroscopy (Butkutė *et al.*, 2003).

Results and discussion

Accession × year interactions were significant (P<0.05) for all 5 traits and variation among accessions was significant at P<0.01. All studied traits demonstrated variation amongst the two *Phleum* species (Table 1).

Table 1. Mean and standard deviation (SD) and coefficient of variation of traits of *P. pratense* and *P. phleoides*. PH - plant height, NT - number of tillers, EA – heading time, RG – regrowth after 1st harvest, DMY – dry matter yield.

Variable	PH		NT		EA		RG		DMY	
	Mean±SD, cm	CV%	Mean±SD	CV%	d-o-y±SD	CV%	Mean±SD, cm	CV%	Mean±SD, g plant^{-1}	CV%
P. pratense										
Jauniai	36.5±5.52	15.23	126±43.58	34.49	155±0.63	28.21	25.4±4.95	33.77	58.0±13.01	22.43
Žolis	25.5±7.24	28.56	119±39.26	39.16	157±1.79	32.78	23.6±2.91	30.32	65.9±29.59	44.94
Mean	31.0±6.38	21.90	123±41.42	36.83	156±1.21	30.5	24.5±3.93	32.05	61.9±21.30	33.69
P. phleoides										
2718	11.2±4.81	40.76	76±14.53	18.87	153±0.52	32.27	23.1±2.09	16.77	32.1±9.92	7.86
2754	17.7±4.01	18.61	72±14.44	19.50	154±1.03	36.89	21.7±4.28	22.35	32.2±5.83	2.51
Mean	14.5±4.41	29.69	75±14.85	19.19	153±0.77	34.58	22.4±3.19	19.56	32.1±7.88	5.19

The accessions of *P. phleoides* were 2.5 days earlier and had nearly identical regrowth after the first cut, compared with average *P. pratense* accessions. The greatest differences between *P. phleoides* and *P. pratense* were NB, PH and DMY, supporting the previous reports that diploid and tetraploid timothy species have lower forage yield than hexaploid *P. pratense* (Joachimiak and Kula, 1997). The DMY mean for *P. phleoides* (32.1 ± 7.88 g plant^{-1}) was nearly twice lower than that for *P. pratense* (61.9 ± 21.30 g plant^{-1}). However, the results of resistance to drought stress experiments report that *P. phleoides* was more vigorous under conditions of limited water availability (Jonavičienė *et al.*, 2012) suggesting it may have utility in less-favoured areas.

Accession × year interactions were significant (P<0.05) for 2 of 4 chemical composition parameters (except for NDF and WSC) and variation among accessions was significant at P<0.01. During the experimental period the herbage chemical composition varied among *P. pratense* and *P. phleoides* species for all nutritive value parameters (Table 2).

Herbage quality had low variation within species, whereas the variation between species was much higher. The DMD of *P. phleoides* accessions was similar to that of *P. pratense*, whilst NDF concentration in *P. pratense* was 12% higher than in *P. phleoides*. The greatest differences between *P. pratense* and *P. phleoides* species were obtained for CP and WSC. *Phleum phleoides* accessions had 37% more CP than *P. pratense*, whereas WSC was 72% higher in *P. pratense* that in *P. phleoides*. CP ranged from 84 to 108 g kg^{-1} for *P. pratense* and from 122 to 178 g kg^{-1} for *P. phleoides*, and WSC ranged from 201 to 233 g kg^{-1} and from 47 to 76 g kg^{-1}, respectively. The extremely low WSC concentration of *P. phleoides* limits its usefulness as a forage crop, but the CP content being almost two-fold higher than in *P. pratense* seems to be a promising feature.

Table 2. Mean (g kg^{-1}) and coefficient of variation of quality characteristics of *P. pratense* and *P. phleoides* species. CP – crude protein, NDF - neutral detergent fiber, WSC – water soluble carbohydrates, DMD – dry matter yield.

Variable	CP		NDF		WSC		DMD	
	Mean±SD, g kg^{-1}	CV %	Mean±SD, g kg^{-1}	CV %	Mean±SD, g kg^{-1}	CV %	Mean±SD, g kg^{-1}	CV %
P. pratense								
Jauniai	90±9.36	5.71	581±21.45	5.40	222±12.38	13.99	603±71.10	9.38
Žolis	103±2.01	10.09	546±9.98	1.75	210±7.76	18.53	650±32.55	9.34
Mean	97±5.69	7.9	564±15.71	3.58	216±10.07	16.26	627±51.82	9.36
P. phleoides								
2718	141±10.20	13.30	647±19.32	6.86	53±6.23	1.65	597±15.96	8.45
2754	165±7.85	8.45	644±14.87	4.27	68±8.59	2.82	601±16.01	7.68
Mean	153±9.02	10.88	646±17.09	5.57	60.5±7.41	2.24	599±15.99	8.07

Conclusions

Some agro-morphological traits of *P. phleoides*, such as NB, DMY, PH are inferior to *P. pratense*; however, EA, RG, as well as CP and NDF content, indicate some potential for agronomic adaptation. *Phleum phleoides* may be a promising species for utilization in grass mixtures under changing environmental conditions.

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Festulolium (×*Festulolium* Asch. & Graebn.) and hybrid ryegrass (*Lolium* × *boucheanum* Kunth.) seed yield components and their contribution to yield

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Abstract

The objective of this research was to study the correlations between seed yield and its formative elements of *Festulolium* spp. and *Lolium* × *boucheanum* cultivars. Seed production is an important part of grassland farming. The grass seed yield components such as the number of generative tillers, the number of spikelets per flower head, the flower head length and weight, 1000 seed weight, and the influence on seed yield of several agronomic traits such as plant height, above-ground biomass, lodging resistance and winter hardiness were examined over three sowing years. Analysing relationships between seed yield and evaluated traits, significant correlation, at least in one of the trial years, was established for the number of generative tillers, ear length and the spikelet number per ear. The spikelet number per ear had a significant (P<0.05) positive effect on the seed yield in all trial years. The generative tiller number had a positive significant (P<0.05) effect on seed yield in one trial year, but not in the other two. Cultivars characterized by greater ear weight and above-ground biomass showed a tendency of forming greater seed yield, but these correlations were not significant.

Keywords: *Festulolium, Lolium* × *boucheanum*, seed yield, seed yield components

Introduction

Festuloliums have recently been introduced in many Europe countries. For seed producers, new genotypes should have good seed production, because seed multiplication has to be profitable. Therefore, seed yield has become an important objective of research programmes (Nosberger and Staszewski, 2002). Seed yield is a highly complex trait which is influenced directly or indirectly by a number of genetic and agronomic traits as well as by agricultural practices and environmental factors. Grass seed yield components, such as the number of generative tillers, the number of spikelets per flower head, flower head length and weight and lodging resistance, have been recognized as important traits affecting seed yield (Hebblethwaite *et al*., 1980; Sliesaravičius, 1997; Bumane and Bughrara, 2003; Marshall and Wilkins, 2003).

Materials and methods

Field trials were conducted in Latvia on a calcareous sod – gleyic soil, fine sandy loam (20 to 30 cm deep arable layer, soil pH_{KCl} 7.2, high plant available phosphorus and good potassium content, humus content 31 g kg^{-1}). Cultivars *Festulolium braunii* 'Perun' *(Lolium multiflorum* × *Festuca pratensis),* 'Punia' *(L. multiflorum* × *F. pratensis), Festulolium loliaceum* 'Saikava' *(L. perenne* × *F. pratensis), Festulolium pabulare* 'Lofa' *(L. multiflorum* × *F. arundinacea)*, hybrid ryegrass 'Tapirus' and 'Ligunda' *(L. multiflorum* × *L. perenne)* were sown without a cover crop, in a randomized block design with four replications and a plot area of 8 m^2. The seeding rate was 600 viable seeds m^{-2}. Trials with the same design were sown in the three following years. The seed yield was investigated in the first production year of each sowing year (cycle) trial. Plots were harvested at the onset of seed ripening. Prior to seed harvest, samples were taken from an area of 0.05 m^2 of each trial plot and analysed for yield structure. The following traits were examined: number of generative tiller m^{-2}, the number of spikelets per ear, ear length (cm) and weight (g), 1000 seed weight (TSW), plant height (cm) and above-ground biomass (kg ha^{-1}). The following were assessed visually (using a scale 1-9): lodging resistance (9=sowing is free from lodging; 1=completely lodged) and winter hardiness (9=plants have fully survived; 1=completely winterkilled). Each year data were statistically analysed independently using analysis of linear regression and correlation for significance (P<0.05).

Results and discussion

Significant correlations (at least in one of the trial years) were found between seed yield and the generative tiller number, ear length and the spikelet number per ear in loloid type festulolium and hybrid ryegrass cultivars (Table 1). The spikelet number per ear had a significant positive effect on the seed in all the three years of trials. This suggests that the spikelet number per ear could be one of the determinant factors of seed yield formation for hybrid ryegrass and loloid type festulolium cultivars. Cultivars characterized by greater productive tiller number showed the tendency of forming greater seed yield; however, the relationship was only significant in one of the three trial years. Relationships between ear length and seed yield in all trial years were characterized by a positive linear correlation; however, this relationship was only significant in one trial year.

Table 1. Correlation among seed yield in each year and its components.

	First trial year			Second trial year			Third trial year		
Traits	Correlation coefficient	R^2	P-value	Correlation coefficient	R^2	P-value	Correlation coefficient	R^2	P-value
Number of generative tillers	0.76	0.57	0.004	0.61	0.37	0.062	0.59	0.35	0.070
Number of spikelets per ear	0.75	0.56	0.005	0.66	0.44	0.037	0.91	0.83	0.000
Ear length	0.44	0.20	0.148	0.28	0.08	0.428	0.65	0.43	0.040
Ear weight	0.17	0.03	0.599	0.51	0.26	0.131	0.53	0.28	0.117
TSW	0.26	0.07	0.421	-0.24	0.06	0.506	-0.34	0.12	0.334
Plant length	0.29	0.08	0.364	0.64	0.42	0.044	-0.07	0.00	0.855
Above-ground biomass	0.50	0.25	0.097	0.19	0.03	0.609	0.32	0.10	0.370
Lodging resistance	-0.07	0.01	0.824	0.13	0.02	0.728	-0.26	0.07	0.460
Winter hardiness	0.46	0.21	0.131	-0.18	0.03	0.616	-0.55	0.30	0.100

A significant linear relationship between plant height and seed yield was established in only one trial year, and correlative relationships were inconsistent between the remaining trial years. Such fluctuations indicate that plant height does not influence seed yield. Seed yields tend to increase with increasing above-ground biomass of cultivars, as indicated by a positive coefficient of correlation. However, this relationship was not significant. Cultivars characterized by greater ear weight showed the tendency of forming greater seed yield in all trial years as indicated by positive coefficients of correlation. However, these relationships were not significant.

Analysis of mutual correlative relationships between seed yield components showed significant correlations, mainly between the spikelet number per ear and the ear length and weight. Significant correlations were present between the length of ear and weight of ear for all three trial years (r = 0.88; r = 0.68 and r = 0.76). With the increase of the spikelet number per ear the weight of ear increases; however, the relationship was only significant in two (r = 0.72 and r = 0.71) of the three trial years. Cultivars characterized by greater ear length showed the tendency of forming more spikelets per ear in all the three years, although this was significant in only two (r = 0.58 and r = 0.67) of the three trial years. Cultivars characterized by greater productive tiller number showed the tendency of forming greater spikelet number per ear; however, the relationship was significant in only one of the three trial years. For the other studied traits (TSW, lodging resistance and winter hardiness) there was no correlation with seed yield.

Conclusions

Seed yield was found to be mainly dependent on spikelet number per ear. Cultivars characterised with higher above-ground biomass, greater productive tiller number, greater ear length and weight showed the tendency of forming greater seed yield.

Acknowledgements

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How do non-adaptive grasses control growth cessation during autumn in high-latitude regions?

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Abstract

Use of perennial ryegrass (*Lolium perenne*) and ×*Festulolium* is expected to increase in high-latitude regions due to milder winters and a prolonged growing season. One main challenge is inadequate growth cessation in autumn to allow sufficient cold hardening. Possible mechanisms behind this were examined by measuring photosynthetic activity and growth rate in triple-replicate field trials at two sites in Norway: Fureneset (59°N) and Vågønes (67°N). Ten entries, including meadow fescue (*Festuca pratensis*), were investigated weekly from mid-September to early December 2011 using selected leaves (n = 10×3) of the regrowth after the third cut. Leaf growth rate (LGR) decreased with time and was significantly higher at Fureneset than Vågønes. Photosynthetic acclimation before winter also differed at the two sites. A clear relationship between LGR and changes in photosynthetic apparatus was observed. In the south, relative light energy excess appeared to act as a signal triggering growth cessation, whereas in the north LGR was limited by the energy available for photosynthesis. Differing responses were also observed in northern- and southern-adapted meadow fescue cultivars.

Keywords: growth cessation, photosynthetic activity, leaf growth rate, perennial ryegrass, ×*Festulolium*

Introduction

A prolonged growing season and milder winters are expected in high-latitude regions as a result of climate change. Therefore, use of perennial ryegrass (*Lolium perenne*) and ×*Festulolium* may increase due to their high regrowth capacity and feed quality. The main challenge for these non-adapted species is inadequate growth cessation in autumn to allow sufficient cold hardening, a situation that is exacerbated by increased temperatures in autumn resulting in hardening occurring later and at lower light radiation. Growth cessation is a prerequisite for cold acclimation, although the underlying mechanisms, comprising a complex interaction between light and temperature, are unclear (Huner *et al*., 1993). Changes in the relative redox state of photosystem II (PSII) determine increasing frost tolerance and acclimation of photosynthetic apparatus to avoid low-temperature photoinhibition. Rapacz *et al*. (2004) demonstrated that the capacity for photosynthetic acclimation to cold in *Lolium* × *Festuca* hybrids is correlated (r = 0.7) with genotypic differences in winter survival.
This study therefore investigated leaf growth rate and photosynthetic activity and examined possible relationships between the two. A high correlation would mean that photosynthetic activity measurements could act as a proxy for growth cessation when selecting for this character in breeding programmes for high-latitude regions.

Materials and methods

Two field trials with entries of perennial ryegrass (5), ×*Festulolium* (4) and meadow fescue (*Festuca pratensis*) (2) were established in 2010 at two coastal sites in Norway: Fureneset, Fjaler (61°34'N, 5°21'E, 10 m a.s.l.), and Vågønes, Bodø (67°17'N, 14°27'E, 35 m a.s.l.). In 2011 the trials were fertilized according to local norms and cut three times. The third and final cut was taken on 29 August at Fureneset and 6 September at Vågønes. In the regrowth after the third cut, 10 plants in each of three replicate plots were individually marked and leaf growth measured on predetermined leaves weekly to determine cumulative leaf growth during the period. Close to the marked plants, photosynthetic activity was observed on 10×3 plants per entry using a HandyPea fluorimeter (Hansatech Ltd., Kings Lynn, UK). The recordings continued until the days became short in early December.

Results and discussion

Autumn of 2011 was mild at both sites, particularly in late autumn. Mean air temperature in November was 6.3°C at Vågønes and 8.1°C at Fureneset, while the long-term average for the sites is 0.9°C and 4.5°C, respectively. Leaf growth rate (LGR) decreased with time and was significantly higher at Fureneset (2.85 mm day^{-1}) than at Vågønes (2.1 mm day^{-1}) during the study period (Figure 1). ×*Festulolium* Felopa (*F. pratensis* × *L. multiflorum*) had significantly higher accumulated leaf growth (ALG) than the other entries and was the only entry with equal ALG at the two sites. Generally, the populations reacted fairly quickly to changes in temperature, as was the case in late October at Fureneset and early November at Vågønes, when a warm spell gave increased leaf growth. The meadow fescue entries had significantly lower ALG than the other entries and accounted for much of the difference between the two sites, since ALG in the southern-adapted meadow fescue cultivar tested (cv. Fure) was almost twice that in the northern-adapted cultivar (cv. Norild). There were no consistent differences between cultivars of perennial ryegrass and ×*Festulolium*.

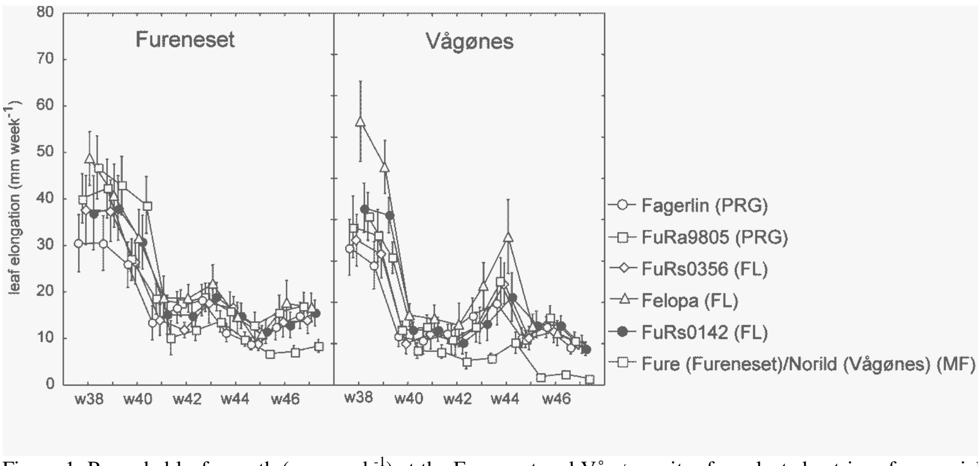

Figure 1. Recorded leaf growth (mm $week^{-1}$) at the Fureneset and Vågønes sites for selected entries of perennial ryegrass (PRG), ×*Festulolium* (FL) and meadow fescue (MF) during the period September (week 38) – early December (week 48) 2011.

Photosynthetic acclimation before winter differed at the two sites, irrespective of entry. The main difference was that plants grown in the north did not show the increase in photochemical activity of PSII that was observed during autumn in the south (Figure 2). This may be associated with a general decrease in metabolic activity in the north. A clear relationship between LGR and photosynthetic apparatus was observed (Table 1). In the south, the relative light energy excess absorbed (ABS) and trapped (Tro) in PSII reaction centres acted as a signal triggering growth cessation, whereas in the north LGR was limited by the energy available for photosynthesis (photosynthetic electron transport, ETo).

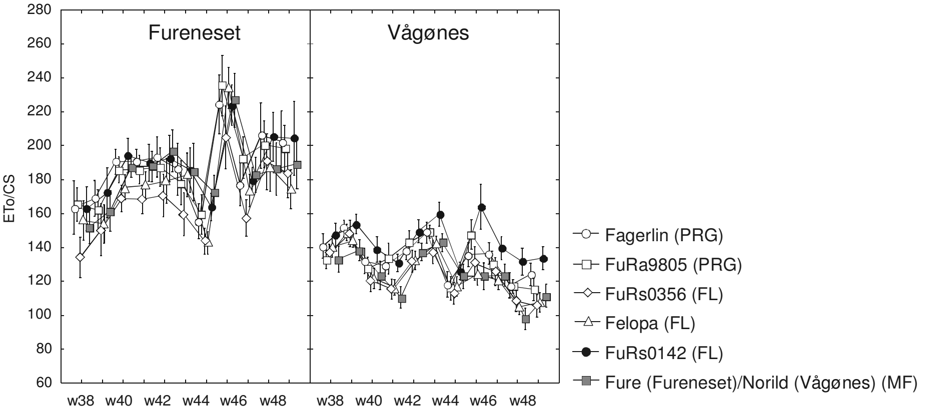

Figure 2. Phenomenological flux of energy used for electron transport (ETo/CS) at the Fureneset and Vågønes sites during the period September (week 38) – early December (week 48) 2011 for selected entries of perennial ryegrass (PRG), ×*Festulolium* (FL) and meadow fescue (MF), as recorded by fluorimeter.

Table 1. Linear correlation coefficients between LGR and chlorophyll fluorescence parameters: phenomenological energy fluxes for light energy absorption (ABS), trapping (TRo), photosynthetic electron transport (ETo) and energy dissipation (DIo) calculated for leaf cross-section (CS) and single active PSII reaction centre (RC).

	ABS/CS	TRo/CS	ETo/CS	DIo/CS	ETo/RC	TRo/RC	DIo/RC
Fureneset	-0.558*	-0.58*	-0.343	-0.437*	0.149	-0.317*	-0.3*
Vågønes	-0.111	-0.115	0.277*	-0.076	0.677*	0.099	0.099

* statistically significant, $P<0.05$

Conclusions

Assuming growth cessation to be a prerequisite for cold acclimation, the clear relationship observed here between photosynthetic apparatus and LGR indicates that fluorescence recording might be a valuable tool for early selection of perennial ryegrass and ×*Festulolium* populations for high-latitude regions.

Acknowledgements

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Leaf spectroscopy: a surrogate measurement of autumn growth cessation of non-adapted grasses at high latitudes

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Abstract

Currently there is an interest to extend the cultivation of perennial ryegrass (*Lolium perenne* L.) into high latitude regions. However, present cultivars show insufficient growth cessation in the autumn, thus hampering cold acclimation and sufficient cold hardening. Here we investigate whether ground-based NDVI (Normalized difference vegetation index) measurements can be used as a surrogate measurement for autumn growth cessation when screening valuable breeding material of perennial ryegrass and *Festulolium* hybrids. We measured NDVI 18 times in the period 18 September to 30 November 2012 in experimental plots with perennial ryegrass and *Festulolium* cultivars as well as a northerly adapted cultivar of meadow fescue (*Festuca pratensis* Huds.) for comparison. Changes in NDVI values with time differed both between species and cultivars and seem to correspond with the ability to acclimate before winter sets in. Our preliminary conclusion is therefore that NDVI is a promising tool for assessing autumn growth cessation.

Keywords: NDVI, acclimation, winter survival, perennial ryegrass, high latitude

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most common forage species in large parts of Europe but has been used with limited success in the Nordic countries north of 60°N. It has superior productivity and feed quality compared with timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.), which are the most common forage grasses in the region, and the challenge is to expand its range of cultivation northwards into the high latitude regions. One of the main limitations for growing perennial ryegrass in these areas is insufficient growth cessation in the autumn, thus hampering cold acclimation and adequate cold hardening. The result is poor winter survival and lack of persistence. *Festulolium* hybrids are also of interest for high latitude areas as they have good regrowth potential and herbage quality but their winter survival is similarly inadequate.

Breeding for increased adaptation of these species entails an understanding of the mechanisms triggering growth cessation in autumn. However, for the screening of potential material it would be valuable to have a simple system, which could act as a surrogate measurement for autumn growth, such as leaf expansion rate, which both is a laborious and time consuming measurement. It is well known that cold stress affects photosynthesis and changes in properties of the lipid membrane may have immediate effects on chlorophyll fluorescence (Berry and Björkman, 1980). Hyperspectral reflectance spectroscopy, using imaging sensors, can be applied to estimate chlorophyll fluorescence and the normalized difference vegetation index (NDVI) has been developed to relate leaf chlorophyll content and crop biomass to spectral reflectance features (Jones *et al.*, 2010).

The aim of the current study was to investigate whether ground-based NDVI measurements can be used as a surrogate measurement for autumn growth cessation when screening valuable breeding material of perennial ryegrass and *Festulolium* hybrids under field conditions in Iceland.

Materials and methods

A field trial with 20 entries of perennial ryegrass (10), Hybrid ryegrass (1), Festulolium hybrids (7), tall fescue (*Festuca arundinacea* Schreb.) (1), and meadow fescue (1) was established at Korpa Experimental Station (64°09'N, 21°45'W) in a complete randomized block with three replicates on 8 July 2011 under the auspices of the NOFOCGRAN NordForsk Network. In 2012 winter damage was recorded on 3 May and the trial was fertilized according to standard practice and harvested on 21 June and 11 August. Fixed plots for NDVI measurements were established on 18 September and a total of 17 measurements were subsequently taken in the period 18 September to 30 November 2012 (see Figure 1) using a hand held SKR 1800 Two channel Light Sensor (Skye Instruments, Llandrindod Wells, UK) 2 m above ground level around midday. NDVI is calculated based on the reflectance in the near infrared (NIR 780-900 nm) and red (660 nm) wavebands using the formula: NDVI = (NIR − Red)/NIR + Red). Values range between 0 and 1 for vegetated surfaces with high positive values indicating an abundance of photosynthetically active vegetation (Eidenshink, 1992).

Results and discussion

The NDVI index started at 0.89 (mean over entries) on 18 September and gradually decreased during the autumn reaching the minimum value of 0.67 on 30 November. There were significant differences ($P<0.05$) between entries after the first two sampling dates and differences tended to increase with time. Here we only present NDVI results for five cultivars; two cultivars each of perennial ryegrass and *Festulolium*, which differed most in mean NDVI values over the whole period, and the meadow fescue cultivar Norild for comparison (Figure 1). NDVI values decreased most rapidly for Norild, which reached the lowest minimum value of 0.56 by 30 November. The perennial ryegrass Birger followed Norild until early November after which it had significantly higher values. Figgjo, on the other hand, had consistently higher values than Birger throughout and ended with the highest value overall of 0.71 in January. NDVI values for the *Festulolium* hybrids were mostly comparable to Figgjo and it was only at the end of October that Perseus had significantly lower values than both Figgjo and Paulita.

It is quite obvious that the NDVI index is sensitive enough to detect the response of these cultivars to short term changes in temperature, as slight increases or decreases in temperature prior to measurements show up clearly in the index values (Figure 1). This is not surprising since the photosynthetic light-harvesting apparatus is often the first point of damage under cold stress and this influences the chlorophyll fluorescence (Jansen *et al*., 2009).

It is clear from the NDVI values that growth cessation occurs earlier for Norild and the photosynthetic activity of Norild is significantly lower by 30 November than for the other entries. Norild suffered no winter damage during the first winter in the experiment so it can be safely assumed that growth cessation clearly influences cold acclimation and hence winter survival. This is not surprising as Norild is a well-known northerly adapted cultivar. The *Festulolium* hybrids, on the other hand, suffered severe winter damage and the rapid drop in NDVI values between 15 and 30 November indicates that these cultivars had undergone insufficient acclimation when hit by a sudden cold stress. The ryegrass cultivars suffered limited winter damage during the first winter, as is commonly the case here, but interestingly though Figgio both suffered more winter damage and had higher NDVI values than Birger. We have to wait, however, untill spring 2013 to be able to observe the exact relationship between the NDVI index and winter survival after this winter.

Conclusions

Changes in NDVI values with time differed both between species and cultivars and they seem to correspond with their ability to acclimate before winter sets in. Our preliminary conclusion is therefore that NDVI is a promising tool for assessing autumn growth cessation.

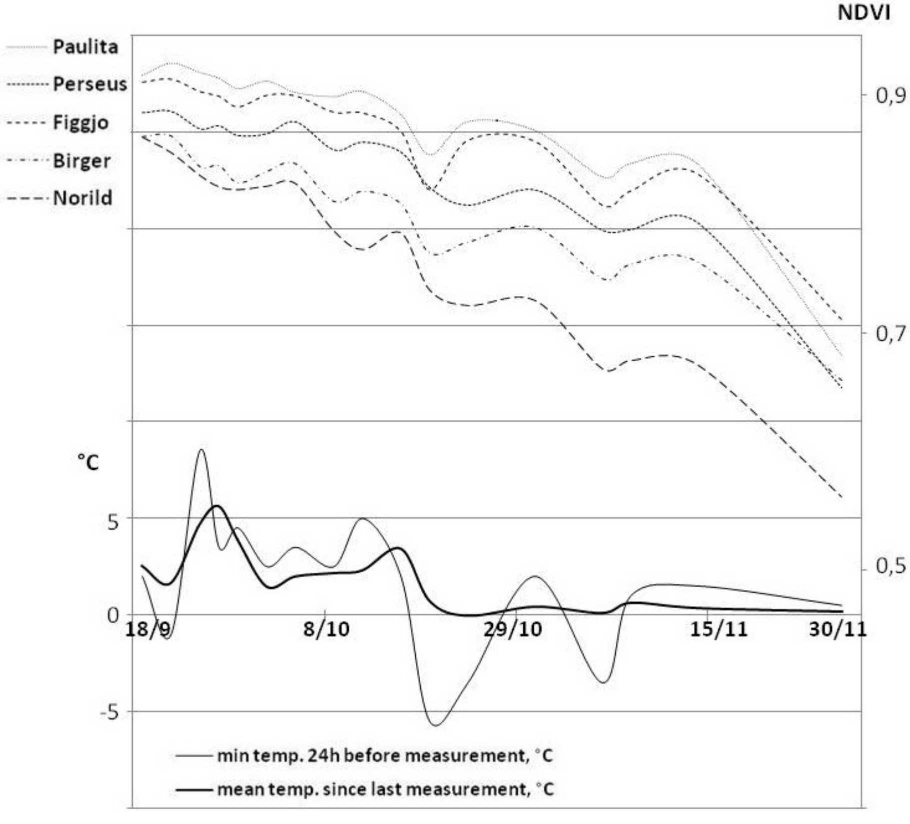

Figure 1. NDVI values (right axis) for selected cultivars from 18 September to 30 November 2012 at Korpa Experimental Station together with the minimum temperature 24 h prior to the measurement and the mean temperature since the last measurement until 30 November.

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Monitoring of farming landscape changes (1999-2009) in a natural park of eastern France

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Abstract

The present paper deals with a monitoring of farming landscape changes during the decade 1999-2009, focusing on grassy habitats. The 'Parc Naturel Regional de Lorraine' (PNRL) is a territory of 2200 km² located in north-eastern France, dominated by agricultural areas. The preservation of landscapes and natural habitats is one of these purposes. PNRL and the Laboratoire Agronomie & Environnement developed a GIS methodology in order to set up a long-term landscape observatory. A representative sample of the variety of landscape contexts was designed, giving an 80 km² monitored area in the PNRL. The main significant results of this 10-year study concern changes of grassy habitats: grassland area decreased and grassland fragmentation increased, which confirms the same global pattern at the European scale. In the same way, heterogeneity of landscape globally decreased. All of these changes are known to have negative impacts on biodiversity. On the other hand, we show some positive changes: area of grass buffer-strips along rivers increased, due to new CAP rules.

Keywords: landscape observatory, monitoring, farmland, agriculture, grassland, GIS

Introduction

European human-dominated landscapes have changed for several decades. Agricultural activity is recognized as one of the main drivers on landscape changes. The major modifications deal with large increase of cultivated areas and fragmentation of uncultivated features such as forests, natural meadows and hedgerows (Robinson and Sutherland, 2002). Many other driving factors such as urbanization and land planning may cause damage on landscape structure and composition. In France, Regional Natural Parks set goals and guidelines for protecting landscapes and natural heritage in a sustainable way. These Parks also foster ecological research programmes and public education in the natural sciences. In that way, the Lorraine Regional Natural Park (in French: 'Parc Naturel Régional de Lorraine' or PNRL) initiated a long-term observatory of landscape changes, on the basis of aerial photography interpretation. In this study, we present the landscape monitoring we carried out for the decade 1999-2009, the first period that we have focused on.

Materials and methods

This study took place in the Lorraine Regional Natural Park (PNRL), in North-Eastern France (Figure 1). From within the total area of the Park, which is more than 2200 km², we choose to design a spatially stratified sample in order to follow the landscape changes. We focused our study on a sample of 80 squares (1 km² each), representative of the landscape-condition diversity in the Lorraine Natural Park. Some of them were located on Natura 2000 areas, others on 'ordinary' areas. The analysis of landscapes was made by digitizing aerial photographs (BD Ortho® from IGN – French National Institute of Geography) as vector shapefiles for extracting percentages of the different land-use. The typology of land-use we choose to work on can be summarized as four main types: (i) herbaceous habitats (grassland + grass buffer strips along rivers, i.e. 'good agricultural practice' of the European Common Agricultural Policy), (ii) woody habitats (wood + hedgerow), (iii) crops, and (iv) other minor types, e.g. roads or buildings (Figure 1). In order to monitor landscape changes over the period of a decade, we digitized aerial photos taken in 1999, 2004 and 2009. Then we extracted from these GIS data of the percentage of the different land-use as well as structure indexes such as fragmentation and heterogeneity (Burel and Baudry, 2003).

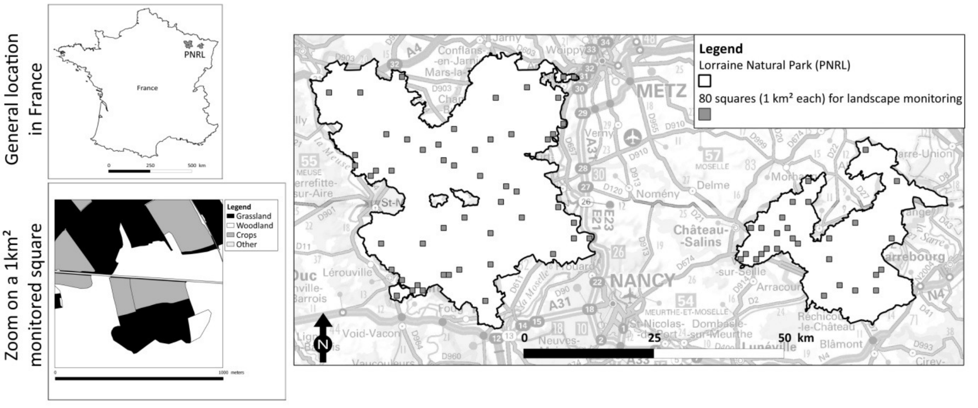

Figure 1. Location of the Lorraine Natural Park and cartography of the 80 studied squares (1 km² each) focused for landscape monitoring.

Results

The composition of the Lorraine Natural Park is 34.95% woodland, 33.55% crops, 23.28% herbaceous habitats (mainly grassland) and 8.23% other habitats. Lorraine Natural Park presents a higher proportion of herbaceous habitats (mainly grassland) than the administrative region (Lorraine) in which it is located (23.28% vs. 15%).

The comparison between Natura 2000 areas and 'ordinary' areas gives a significantly higher percentage of grasslands in Natura 2000 squares (39.5% ± 9 vs. 20.4% ± 2) (P=0.03271) and a significantly smaller percentage of crops (12.9% ± 6 vs. 37.1% ± 5) (P=0.00872).

In the temporal study of landscape changes from 1999 to 2009 (Figure 2), herbaceous habitats are the main impacted land-use: grassland significantly decreased (P=0.00265) whereas grass strips significantly increased (P=0.03224).

In the same period, we observed than fragmentation of herbaceous habitats increased (P=0.02615) and heterogeneity of the whole landscape decreased (P=0.00261).

Discussion

The studied landscapes in Lorraine Natural Park present a typical pattern of European farming landscape with a mosaic of 3 main types of land use: forests, grasslands and crops. Nevertheless, in comparison with the region in which it is located (Lorraine), the Lorraine Natural Park displays a landscape pattern with more semi-natural habitats (grassland, woodland), which shows a potentially better landscape quality for hosting biodiversity (Duelli *et al.*, 2003). This is particularly confirmed in the Natura 2000 areas where crops are highly dominated by semi-natural habitats (e.g. grassland).

The striking result of this study deals with the decrease of grassland areas over the decade of 1999-2009, and their fragmentation. This result in consistent with general European large-scale tendencies in farming landscapes (Le Roux *et al.*, 2003) and is known to be a threat for

numerous floral and faunal taxa, e.g. butterflies and birds (Billeter *et al.*, 2008). The decrease of landscape heterogeneity leads to similar consequences: homogenization of landscape and habitats could negatively impact on biodiversity (Le Roux *et al.*, 2003).
A positive change is the increase of grass buffer strips in landscapes. These have been established as a direct result of the 'good agricultural practice' measures of the EU's Common Agricultural Policy (CAP) from the middle of the 2000s.

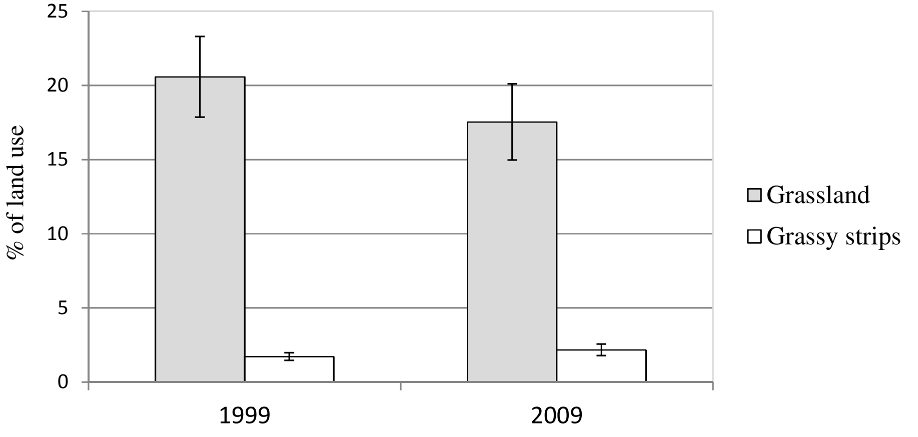

Figure 2. Herbaceous habitat (grassland + grass strips) − changes over the decade 1999–2009.

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Payment-by-results agri-environmental support for grasslands in Europe: Lessons learnt for future agri-environmental support in Wales

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Abstract

Grasslands are the main agricultural land use in less favoured areas in Wales. They receive considerable financial support through standard action-based agri-environmental schemes (AES). Ecological success of action-based approach has been criticised for not delivering value for money. Evidence from Europe suggests that grassland Payment by Result (PBR) approaches improve environmental outcomes and are more cost-effective than action-based schemes. Successful PBR approaches have built on existing action-based schemes, engage farmers in scheme construction, develop easily measurable indicators, and involve extensive trial periods. A PBR approach was rejected for the Glastir AES in Wales but, we argue, it would be possible to implement a PBR approach modelled on European successes. Simplicity of PBR components would encourage buy-in to PBR approach and foster acceptance by farmers and administrators.

Keywords: payment-by-result, grassland biodiversity, less favoured areas, Wales

Introduction

Grasslands are the main agricultural land use in less favoured areas (LFAs) in Wales and receive considerable financial support through standard action-based agri-environmental schemes (AES). However, the ecological success of the action-based approach has been repeatedly criticised for not delivering 'value for money' (e.g. Wrbka *et al.*, 2008). Alternative payment approaches such as payment-by-results (PBR) have increasingly received attention and a number of examples of PBR AES have been implemented in Europe (Burton and Schwarz, 2013). Early ecological assessments have been positive, indicating this approach has potential to improve environmental outcomes and the cost-effectiveness of schemes supporting ecosystem service benefits from grasslands in LFAs (Burton and Schwarz, 2013). In the context of a recent rejection of the PBR approach by the Welsh Government in the development of their Glastir AES, the paper synthesizes key aspects of PBR scheme design that contributed to the successful implementation of existing examples in Europe and derives general guidelines for the implementation of future PBR payments.

Barriers to introducing the PBR approach in the Glastir AES in Wales

Glastir was introduced in 2009 to deliver a wider range of environmental goods and services, including carbon and water management, not included in previous Welsh schemes. In addition, Glastir was intended to deliver greater value for public money and move away from a culture of subsidy-payments to farmers. The Welsh Government was thus interested in the possibility of shifting the payment model from an action-based to a PBR model. However the current scheme design has stopped short of this shift, and only involves the discursive reframing of scheme outcomes as 'ecosystem goods and services' rather than attaching payment to measurable results (Wynne-Jones, 2013). Civil servants involved in the design and implementation of Glastir argued that the implementation of a PBR approach would require a break from the costing of payments upon income foregone and additional costs (required under WTO rules and EC regulation 1698/2005) and that they were thus unable to make this change. However, given that other schemes discussed in this paper have been able to implement a PBR model, it is evident that there are opportunities within the existing regulations. A barrier to adoption of PBR is that Glastir is intended to address a broad range of issues (WG, 2012a). This complexity is further enhanced by the scheme's remit to address environmental issues across the whole of Wales, rather than in more confined locations. Beyond these barriers, it is evident that Glastir has had additional difficulties in its introduction, including critiques of levels of payment, weaknesses in administration of the scheme and poor communication of the scheme's aims (WG, 2012b). At the heart of many of these problems is the lack of a pilot project. This failing has meant that many simple issues arising with the scheme not addressed until a full review was undertaken in 2011 (Roberts, 2011). This created a long period of uncertainty, which meant farmers were often unwilling to enter a legally binding contract. Many farmers are now waiting for a period of policy stability before committing to Glastir.

Key aspects of successful PBR agri-environmental payments

In contrast to Glastir, PBR AES in Europe are considered relatively successful in terms of promoting the conservation of grasslands (Burton and Schwarz, 2013). Evidence from German and French examples shows that the direct targeting of grassland biodiversity outcomes, improved flexibility for farmers and close cooperation between farmers and conservationists improve the diversity and abundance of grassland indicator plant species of high conservation concern (e.g. de Sainte Marie, 2010). Matzdorf and Lorenz (2010) observe that the number of participating farms and the area registered in the MEKA II scheme in Baden-Württemberg almost trebled from 2000 to 2005. Positive uptake developments have also been reported from the 'Flowering Meadows' contracts in France (CNO, 2011). Similar schemes exist in the RDPs of other German federal states, with these representing some of the longest running schemes in the EU (Matzdorf and Lorenz, 2010). What aspects of their implementation have made them successful in comparison to Glastir? First, the schemes do not involve radical changes but instead complement existing action-oriented schemes. Consequently, the additional risk of result-oriented provision is reduced (Aviron *et al.*, 2010). Second, the majority of existing PBR schemes went through relatively extensive trial periods with farmers. For example, before a PBR component was implemented in the NAU/BAU programme in the RDP in Lower Saxony, a number of trials were conducted in collaboration with research organisations and the farming community. Feedback into the design was obtained, and training courses were run for farmers (Haaren and Bathke, 2008). Close collaboration between regional nature parks and local farmers is a key factor in the success of the French PBR contracts (de Sainte Marie, 2010). In contrast, the MEKA II scheme was based on an existing action-based scheme, but carried on an existing good relationship between farmers and administrators (Matzdorf and Lorenz, 2010). Third, one objective of the existing grassland PBR schemes has been to keep the indicators of success that lead to payment as simple as possible (Schwarz *et al.*, 2008). Fourth, in the German and French cases there is evidence of substantial effort being made to develop appropriate indicator systems for grasslands (e.g. Oppermann and Gujer, 2003). Care was taken that indicators were scientifically robust but also measurable and identifiable by farmers themselves and did not conflict with agricultural goals (Matzdorf *et al.*, 2008).

Way forward for future attempts to implement PBR grassland payments in Wales

Concerns expressed by the civil servants involved in the design and implementation of Glastir that a shift to a PBR approach would not conform to WTO rules are not justified. The implementation of a number of examples in the national and regional RDPs emphasises that the approach is in line with current policy regulations in the EU. Currently, PBR payments are calculated either based on a standardized land management scenario assumed to deliver the environmental outcome or the application of auctions. However, one of the main challenges for the implementation of PBR schemes is to achieve a balance between the complexity of targeted ecological outcomes and the simplicity of the scheme design to foster acceptance of this approach by administrations and farmers. The experience from the rejected PBR approach in Wales and the successful examples in other EU countries suggests that the shift to PBR AES needs to be incremental, adding simple and comparably low risk PBR payments to existing action-based AES. That way, skills in environmental provision can increase over time and new networks and co-operations will emerge. Such a general approach for the implementation of PBR payments would also allow time for the infrastructure required for more complex and higher risk schemes (e.g. voluntary advisory bodies) to develop around the new 'industry' of producing environmental goods. Applying this approach to the Welsh situation requires (in the first instance) the addition of a PBR grassland payment to the existing Glastir options, instead of trying to transform the whole scheme to a PBR approach in one step.

Conclusions

The implementation of the PBR approach has improved the effectiveness of AES targeted at grassland biodiversity. Successful examples have started as simple PBR payments and build on existing AESs by adding a PBR component to mainstream action-based AESs. The relative simplicity of adding PBR components to existing mainstream AESs such as Glastir in Wales would encourage buy-into the PBR approach and foster acceptance by farmers and administrators of future attempts to establish more complex PBR schemes.

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Higher price for meat from extensive grassland?

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Abstract

About 350,000 ha of semi-natural areas are protected by the Nature Protection Law in Denmark (§3-areas). This comprises dry -, moist - and wet grassland, salt meadows and heath. About 300,000 ha of these semi-natural areas need management to obtain a good nature quality status. Most of the managed areas get EU subsidy, but management of semi-natural areas is not attractive to farmers any longer. Only 45,000 ha of §3 areas in DK had stewardship management in 2010. Grazing could be more attractive to farmers if they could get a higher price for meat produced on semi-natural areas, as an added value connected to a documentation of nature responsibility. It is estimated that the 300,000 ha of §3-areas could deliver 20% of the Danish consumption of beef meat. However, to pay added value, consumers must be assured that they support areas with good nature quality. From data gathered on 9 Danish cattle farms we suggest a botanical nature quality score and an index showing the % of §3-area of total land managed for forage production included in a system for claiming trustworthy meat from protected semi-natural areas.

Keywords: pasture, meat, nature quality, consumer information, management documentation

Introduction

In Denmark about 350,000 ha of semi-natural areas are protected by §3 in the Nature Protection Law (§3-Areas). These comprise dry, moist and wet grassland, salt meadows and heath land. A substantial part of these areas have never been fertilized and sprayed with pesticides and are therefore supposed to have high nature quality or a potential for good nature quality. About 300,000 ha of these semi-natural areas need management by grazing and/or mowing to obtain a high nature quality status.

Some meadow birds can be found on more productive but unfertilized and managed pastures (Laursen and Hald, 2012). Unfortunately, a high botanical nature quality is mostly combined with a relatively low biomass production, making their management less attractive to the farmers. The areas of high botanical quality therefore have the highest risk of abandonment, as seen in Vejle County where only one-third of 188 formerly known areas of high botanical quality were still managed in 2005 (Vesterholt and Levesen, 2006), and this figure has dropped further since.

Most of the managed areas in Denmark get EU subsidy. Management of semi-natural areas that have a high number of naturally occurring grassland species get an EU subsidy of 2000 DKR per ha (2012 rate) when managed by grazing. Grassland areas with low nature quality and dominated by cultural forage species can achieve an EU subsidy of 2200 DKR per ha plus 1650 DKR for management by grazing (2012 rate). Therefore, management of low productive semi-natural areas with high nature quality is not attractive to farmers, and only 45,000 ha of §3-areas had stewardship management in 2010 (Nielsen *et al.*, 2011).

Grazing could be more attractive to farmers if they received a higher price, i.e. an added value, for meat produced from semi-natural areas. The 300,000 ha of §3-Areas with a need of management could deliver 20% of the Danish consumption of beef meat (Hald, 2009). However, to pay added value, consumers must be assured that they support nature quality. This could be documented as the farmer's nature responsibility effort. In this paper we suggest two responsibility variables for a system to claim trustworthy meat from protected semi-natural §3-areas. Variable No.1 is the nature quality score of the vegetation. Variable No. 2 is the percentage of §3-Area managed in relation to total land needed by the farmers herd for feed.

Materials and methods

With the aim to characterize the farmer's nature responsibility, 9 cattle farms in Jutland were chosen to describe the farm grazing system and nature values as measured by a botanical characterization of grazed grassland areas outside rotation (i.e. areas protected by the §3 of the Nature Protection Law). Some of the areas were subdivided into nature type units. Plant species were listed and the occurrence of the species was quantified on a scale from 1 to 10 (Hald, 2011). A nature quality score of the vegetation was calculated using the quantitative plant list and the national nature character value for each plant species (Fredshavn and Ejrnæs, 2007). This nature quality score is independent of area sampled. Variable No. 1 was calculated as quality score weighted by the area it represented. The nature quality score was compared with the national mean score for dry and for moist pasture (Fredshavn *et al.*, 2009). As variable No. 2 we suggest the percentage of hectares of semi-natural grassland maintained in relation to total land needed by the farmers herd for feed, not only as grazing but also the concentrates. Based on the use of 1 Feed Unit (FU) as concentrate, we assess this as 1.85 m^2 of land, seeing that 1 FU in concentrate is equal to 1 kg barley, and the mean yield of barley in Denmark was 5.4 t per ha in 2011. When including land for concentrates it is possible to calculate percentage § 3-areas managed of total land needed to feed the herd. The nature responsibility taken by the farmer is proposed as graduated on a 3-level scale (1 to 3 stars). A score of two stars is aimed at as a mean national score (Table 1). So the farm can be a low (1 star), medium or a high (3 stars) nature-responsibility farm, measured by different variables.

Table 1.Variable range for responsibility level (1-3 stars) according to botanical nature quality score and percentage of §3-area managed in relation to the total land needed to feed the farmer's grazing herd.

Responsibility stars	*	**	***
Variable 1. Area weighted nature quality score	<2.5	2.5-3.5	>3.5
Variable 2. % semi-natural areas of total needed land [1)]	0-25	25-75	75-100

[1)] In Denmark beef cattle herd can normally manage semi-natural areas during 6 summer months, i.e. they get up to 50% of their feed from these areas but if forage from the semi-natural grassland areas is also used in winter by winter grazing or hay they may get up to 100%

Results and discussion

With regard to the proposed variable No.1, the 9 case-farms obtained one or two stars for nature quality (Table 2). Therefore, they were at low or medium responsibility level for variable No. 1. None of the case farmers had a better botanical nature quality than the national mean. The proposed responsibility levels of variable No. 2 are shown in Table 1. However, data to calculate this variable are not yet complete for the 9 case farms.

In conclusion, to get a high nature responsibility score and thereby added value to the meat, the system encourages the farmer to manage protected areas with high nature quality and low productivity. It also encourages the farmer to use winter feed harvested on protected areas. The system needs to be expanded with other variables and a simple control system has to be assessed.

There is a need to develop a variable for responsibility per kg of meat sold, i.e. the hectares of protected nature areas managed per kg of meat sold. In an on-going project we also consider proxy variables for landscape and fauna, as well as public access, to get a more complete system.

Table 2. Weighted nature quality score of protected semi-natural areas managed by the 9 case farmers. They are classified as moist (meadow) or dry (commons) grassland. The weights are ha area each 'n' score represents in the calculated means. n: Number of subareas. National mean scores are from Fredshavn *et al*. (2009).

Farm	Variable no. 1. Mean score moist (n)	Variable no. 1. Mean score dry (n)	Nature quality responsibility (stars out of 3 stars)
Farm 1	2.33 (1)	1.98 (1)	*/*
Farm 2	2.77 (7)	-	**
Farm 3	3.14 (5)	2.77 (3)	**/**
Farm 4	2.19 (2)	-	*
Farm 5	2.07 (2)	-	*
Farm 6	3.05 (2)	-	**
Farm 7	2.54 (5)	-	**
Farm 8	1.85 (7)	2.58 (4)	*/**
Farm 9	2.50 (4)	3.20 (3)	*/**
National mean	**2.66 (5200)**	**3.19 (2078)**	**

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Economic evaluation of farms participating in the agro-environmental programmes in terms of their development

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Abstract

In 2007 a farm study was conducted on 30 grassland-based livestock farms that participated in the Rural Development Programme (RDP). Mean farm area was 19.69 ha (range 2.2-182.0 ha) and farms were divided into 4 groups: 1-10, 10-20, 20-50 and >50 ha. The mean proportion of permanent grasslands was 53.8%. Crop production comprised bulk feeds and feed grain (oats), cereal mixtures, triticale and barley. Cereals accounted for 78.5% of crop area, root crops 9.4% and legumes 2.1%. The highest livestock (cattle, pigs, horses, poultry) density per ha of arable land (AL) (mean 0.5 LU) was on farms in the 20-50 ha category. Both the farm investments in fixed assets and average direct costs of crop and animal production were low. The revenue from agricultural production was medium and low. The proportion of subsidies from the RDP was high (17%). Gross margin was medium and low, and its value per ha of AL and per capita increased with the increase of farm area (except in the 20.1-50.0 ha size category). The effectiveness of fixed assets was high: its index ranged from 0.39 to 0.58 with a mean of 0.43. Only 23% of surveyed farms had a chance of further development.

Keywords: crop structure, livestock density, gross margin, efficiency of fixed assets

Introduction

Subsidies to farms participating in the Rural Development Programme (RDP) have increased farmers' interest in methods that protect valuable grassland habitats and water on agricultural land (AL). Farms with a substantial share of grasslands (GL) may breed beef and dairy cattle without large inputs, to supply the Warsaw market. Sosnowski *et al.* (2006) suggested that low-input beef cattle breeding is an alternative use for abandoned meadows. This facilitates the development of the agricultural landscape and the use of biological functions of GL (Gajda *et al.*, 1994). Subsidies from the RDP affect agricultural development in the study region of Poland (Harkot and Lipińska, 2003; Mickiewicz *et al.*, 2010). The aim of this study was to assess the farms participating in the RDP in 2004-2006 in terms of farm development.

Study methods

The study was performed in 2007 using direct inquiry methods on 30 selected farms (Mazowieckie province) that participated in the RDW in 2004-2006. Farms were oriented to animal production based on their own GL. They were divided into 4 size groups: 1-10, 10-20 (most frequent), 20-50 and >50 ha. AL structure, livestock (mainly ruminants), crop structure and soil class were considered in the questionnaires. The main economic evaluation criterion was the farm gross margin (GM). Production value was the sum of plant and animal production and any increased reserves. Direct costs were calculated for the whole farm and separately for the crop and animal production.

Results

The mean area of the studied farms was 19.69 ha (relatively large for Poland). Farms included typical family farms and those oriented to a specific production, particularly ruminant breeding (Table 1). The share of AL varied from 58 to 98%. In farms < 20 ha, grasslands dominated in the AL structure, whereas larger farms were dominated by arable land. The mean share of GL in the AL structure was 53.8% (mean for Poland is 21%), which facilitated ruminant livestock breeding. High plant diversity (from sedges to legumes to protected plants) was a requirement for farming in agreement with the rules of environmental protection.

Table 1. Land use and AL structure in studied farms (mean and range).

Group	Farms ha	Farms No	Surface area ha	AL in the total area %	Arable lands in the AL structure %	GL in the AL structure %	Forests and other, ha
I	1-10	11	5.7 2.2-8.9	90.74 70.1-96.6	38.0 0.0-74.9	62.1 25.1-100.0	0.6 0.2-2.7
II	10-20	13	14.4 10.0-19.1	87.3 70.0-98.0	48.3 22.2-96.4	51.8 3.6-77.8	1.8 0.3-3.3
III	20-50	5	21.8 23.2-39.6	87.3 58.0-95.8	52.1 25.8-82.6	47.9 17.4-74.2	4.5 1.0-16.1
IV	>50	1	182.0	77.2	81.0	19.0	41.4
	Total	30	19.7 2.2-182.0	88.2 58.0-98.0	46.2 0.0-96.4	53.8 3.6-100.0	3.1 0.2-41.4

Crop structure (Table 2) depended on the demand for feed, soil type and farmers' choice (vegetables). It was, however, subject to the production of bulk and grain feed, particularly of oats, cereal mixtures, triticale and barley for livestock and partly for the market. As in the whole country, the share of cereals increased (from 57.1 to 96.5%) with increasing farm area, while that of root crops decreased in the same order (from 13.8 to 3.5%), apart from one farm in group IV on rich soils, where wheat, cereal mixtures (70.7%) and sugar beet (15.1%) were grown. The share of legumes grown for grain ranged from 0% in group III to 14.3% in group IV of selected farms.

The main crops were potatoes and oats, rye and triticale, wheat, cereal mixtures, barley and lupins. Some farmers grew vegetables, mainly root crops and onion on one farm. Cattle (alone) were bred on 8 farms, and cattle with pigs on a further 6 farms. Three farms in 2 groups each bred: pigs alone, cattle and poultry. On two farms horses were bred alone and on two others there were horses and poultry. Four farms had no animals and some kept someone else's horses for recreation. Variability of animal production on farms was an effect in the vicinity of the Warsaw agglomeration.

Table 2. Selected elements of crop structure (%) and livestock in studied farms (mean and range).

Group	Farms ha	Cereals	Legumes	Tuber crops	Total livestock LU ha^{-1} AL including: cattle	pigs	horses	total
I	1-10	57.1 0.0-100.0	1.9 0.0-20.8	13.8 1.0-100.0	0.2 0.0-0.6	0.04 0.0-0.4	0.1 0.0-0.6	0.4 0.0-0.8
II	10-20	90.4 69.7-100.0	2.0 0.0-16.8	7.6 1.0-18.1	0.2 0.0-0.6	0.2 0.0-0.9	0.1 0.0-0.3	0.5 0.0-0.9
III	20-50	96.5 83.4-100.0	0.0	3.5 0.0-16.7	0.3 0.0-0.6	0.5 0.0-2.4	0.02 0.0-0.1	0.8 0.0-2.4
IV	>50	70.7 70.7-70.7	14.3 14.3-14.3	15.05 15.1-15.1	0.0	0.7 0.04-2.4	0.3 0.1-0.6	0.5 0.0-2.4
	Mean	78.5 0.0-100.0	2.1 0.0-20.8	9.4 0.0-100.0	0.3 0.0-0.6	0.2 0.0-2.4	0.1 0.0-0.6	0.5 0.0-2.4

Animal stock (Table 2). The greatest diversity of species and the largest livestock density (0.8 LU ha^{-1} AL) was on farms in group III; slightly smaller (0.5 LU) in groups II and IV (large numbers of pigs on a leased farm). Livestock numbers were low (below national mean) and diverse among size groups of farms. Farmers kept mainly ruminants, and providing good feed for animals was one of the most important factors in effective management.

Selected elements of economic characteristics. Mean value of fixed assets excluding land, i.e. ratio of gross margin to the value of fixed assets (dimensionless) on farms in 2007 was 13913 PLN ha^{-1} of AL (from 0 – machines rented – to 40846 PLN). The highest value of fixed assets was on farms in group I, and the lowest in group III (Table 3).

Table 3. Gross margin in farms of various size and investment (mean and range).

Farms ha	Farms No	Area of AL, ha	Value of fixed assets PLN/ha AL	Index of soil quality	Employ-ment	Gross margin GM, in PLN per ha AL	Gross margin GM, in PLN per person	Effect of fixed assets*)
1-10	11	5.1	15327	24.5	27	1909	9509	0.39
		2.0-7.5	0-29314	0.0-32.6	11-50	492-23246	1040-23246	0.12-1.01
10-20	13	12.7	15373	32.2	11	3169	28193	0.47
		7.5-18.0	18851-40846	20.9-55.4	6-16	1406-16649	10513-114130	0.10-1.37
20-50	5	27.3	6751	30.5	7	2513	40210	0.58
		21.8-37.9	1002-12252	23.9-48.5	4-13	821-5205	15546-82636	0.07-1.10
>50	1	140.6	11895	59.3	4	5945	168578	0.50
Mean	30	16.6	13913	30.4	19	2692	28024	0.43
		2.0-140.6	0-40846	0-59.3	4-50	492-16649	1040-168578	0.07-1.37

*) the ratio of gross margin to the value of fixed assets

Mean GM was 2692 PLN ha^{-1} AL (from 1909 PLN in group I to 5945 PLN ha^{-1} AL in group IV). Across all farms it ranged from 492 to 16649 PLN. The value of GM per ha increased in farms up to 20 ha, decreased in group III, and increased in the largest farm to 5945 PLN ha^{-1}. GM per person increased with farm size (9509 PLN in group I to 168 578 PLN in group IV. The effectiveness of fixed assets was high, its index varied from 0.39 to 0.58 (mean 0.43). Economic analysis demonstrated that c. 23% of studied farms had an economic size above 40-50 thousand PLN and a chance for further investments and development.

Conclusions

Farms showed a low and medium level of investments in fixed assets, low direct costs of crop and animal production (i.e. low intensity). They had also medium to low incomes from that production: c. 17% of incomes came from subsidies within the RDP.

Gross margin (GM) per ha of AL increased with increasing farm size (except the group of 20-50 ha) and per person it increased up to a farm area larger than 50 ha AL. Production costs were not always satisfactorily compensated by incomes. Only 23% of studied farms had a chance for further development and investment.

The index of the effectiveness of fixed assets was high – mean 0.43 (range 0.39 to 0.58).

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The economic and ecological effects of drying permanent grasslands on peat-muck soils near very weak and medium arable soils

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Abstract

The study aimed to assess economic and ecological effects of the drainage of permanent grasslands (PG) 50 years ago (1954) in the Kuwasy peatland, Biebrza River valley, situated on peat-muck soils near very weak and medium arable soils (in 15 villages). The status after drainage was evaluated in 1962 (common agricultural census) and 2007 (representative census). Drainage and grassland management were the factors intensifying agricultural production. Yields from PG increased almost threefold; those from agriculture land (AL) – by 50-150% (mainly cereals). Animal stock increased by more than 80% in the first period (1954-1962) and by 200% to 2007. Total production in cereal units per ha and per capita increased fourfold. Mean surface area of farms increased by 1.8 ha. The share of AL in the agricultural area decreased and that of PG increased, particularly on weak soils. The share of cereals and fodder crops in the crop structure increased except in villages that had better soils. Drainage indirectly increased the level of plant production (which has recently declined again due to socio-economic reasons) and the intensity of animal production, particularly in villages of weak soils in AL surrounding PG. Such areas drained long ago should be modernized and rationally used to fully utilize the potential that appeared after regulation of water and air relations in peat soils.

Keywords: grasslands, drainage, crop structure, yields, livestock, global production

Introduction

The level of management of permanent grasslands (PG) in Poland has declined in recent years and some PG have been entirely abandoned (Jankowska-Huflejt *et al.*, 2011) due to the run-down of drainage structures and worsening of soil-water conditions. The question arises whether to restore the drainage systems for further intensive meadow utilization or to leave meadows to natural succession.

Materials and methods

Production and economic factors were studied in farms from 15 villages that used (for c. 50 years) PG situated on peat-muck soils in the Kuwasy peatland drained and managed in 1954. The study area is in the Biebrza River valley near very weak and medium soils of arable lands (AL). The status after drainage was evaluated in 1962 (common agricultural census) and in 2007 (representative census). Economic effects of PG drainage were: changes in land use and crop structure, yield increments in PG and AL, increased livestock and global production in cereal units (c.u.). Steered interviews, monographic method, tabular statistics and the point method of Kopeć (1968) were used to assess the intensity of agricultural production.

Results and discussion

Land use and crop structure. Agricultural areas (AA) decreased to the benefit of forests and other land-uses (roads, ditches, barren lands) during the study period. The share of AL in AA decreased and that of PG increased, particularly in villages of weak soils (from 62.5 to 70.1%;

Table 1). The mean farm area increased by 1.8 ha: in villages of weak soils to 14.1 ha and in villages of medium soils to 11.4 ha. The share of farms larger than 10 ha increased from 47.8 % to 83.0% and that of farms of 5-10 ha decreased from 32.4 to 14.5%. Equipment in machinery (tractors, harvesters and balers) improved. The inputs of both mineral fertilizers (by ca. 100 kg NPK ha^{-1}) and organic fertilizers (due to larger livestock) increased (Prokopowicz, 1974). The contribution of cereals and tuber crops decreased in the crop structure in favour of fodder crops (Table 1). The share of the latter increased to 44.3% (maize, clovers with grasses and grasses alone) in villages of medium soils and to 9.0% in villages of weak soils.

Table 1. Land use structure (% AA) and crop structure (%).

Villages of soils	Land use structure, % AA						Crop structure, %								
	Arable land			Permanent grassland			Cereals			Tuber crops			Fodder crops		
	1954	1962	2007	1954	1962	2007	1954	1962	2007	1954	1962	2007	1954	1962	2007
Medium	51.0	54.0	48.1	47.6	44.6	46.3	61.9	55.9	54.0	23.1	27.0	1.0	8.0	11.0	44.3
Weak	36.2	41.7	24.7	62.5	56.2	70.1	59.3	51.1	86.7	25.8	33.7	3.5	5.8	8.4	9.0
Mean	37.5	47.0	34.9	56.4	50.8	59.3	61.0	53.4	67.0	24.4	30.3	2.0	6.9	9.6	26.7

Permanent grasslands. Before drainage, PG yielded 1.0-1.5 t ha^{-1} of less-valuable plants (sedges, *Molinia* spp., red fescue, bent grass, horsetails or, less frequently, reed canary grass, timothy grass or tussock grass) (Niewiadomski, 1954). After drainage the meadows became dominated by tussock grass, red fescue and meadow fescue. By 2007 secondarily bogged meadows were overgrown (65% by sedges, rushes, herbs and weeds and 35% by grasses). Moderately to intensively drained meadows are covered in 66% by grasses, with 4.0-10.2% sedges and rushes and 17.0-13.2% herbs and weeds.

Yields. After drainage in 1954-1962, the yields of cereals increased by 16%, those of potatoes by 44%, and sugar beet by 58% (Table 2). Through the entire 54-year period the yields of cereals doubled, those of potatoes remained unchanged and the growing of sugar beet was abandoned due to limitations on sugar production. Hay yields from PG doubled during 1954-1962 (4 t ha^{-1}) and almost tripled (5.8 t ha^{-1}) by 2007. In general, cereal and potato yields were higher by 0.2-1.6 t ha^{-1} and hay yields by 1 t ha^{-1} in villages of medium soils than in villages of weak soils. Meadows were fed with runoff rich in mineral components flowing from higher elevated arable lands.

Table 2. Yields of crop plants in t ha^{-1} (Prokopowicz, 2008).

Villages of soils	Cereals			Potatoes			Sugar beet			Meadow hay		
	1954	1962	2007	1954	1962	2007	1954	1962	2007	1954	1962	2007
Medium	1.2	1.6	4.2	10.7	17.0	24.0	18.4	29.0	-	2.0	4.0	6.3
Weak	1.0	1.4	2.6	10.7	17.0	19.6	-	-	-	2.0	4.0	5.4
Mean	1.1	1.5	3.6	10.7	17.0	21.0	18.4	29.0	-	2.0	4.0	5.8

Animal production. Livestock numbers and the efficiency of animal production increased after drainage of PG. Total livestock (without horses) increased from 23.9 LU 100^{-1} ha AA in 1954 to 76.4 LU 100 ha^{-1} AA in 2007. Cattle increased from 16.5 to 75.8 LU 100 ha^{-1} AA (Table 3) due to higher yields from PG and AL, higher demands for milk and beef and the concentration of animal breeding (30-50 heads in a farm). In 1962 the milking efficiency was higher by 22% (to 2500 L) but in 2007 it was already 4750-5600 L due to better genetic quality of animals and specialization of some farms. In 2007 the total livestock and the cattle stock was slightly higher in villages of weak soils.

Table 3. Livestock of farm animals (LU 100 ha^{-1} AA).

Villages of soils	Livestock in total			Cattle			Pigs		
	1954	1962	2007	1954	1962	2007	1954	1962	2007
Medium	24.6	43.0	76.0	16.2	35.8	74.5	6.7	5.2	0
Weak	23.2	44.8	76.8	16.8	33.9	76.8	5.3	7.4	0
Mean	23.9	43.4	76.4	16.5	33.3	75.8	6.0	8.7	0

Agricultural production increased from moderately intensive in 1954 to highly intensive in 1962 and remained such in 2007 (Table 4). The intensity of plant production in 2007 decreased below the level of 1954. Intensity of animal production, mainly dairy cattle, increased, however, over 200 points. The intensity of plant production was higher in villages of medium soils, while the intensity of animal production showed a reversed relationship; the same was true for animal, mainly cattle, stock.

Table 4. The intensity of agricultural production (Prokopowicz, 2008) point method of Kopeć (1968).

Villages of soils	Determinants of the intensity of production								
	Plant			Animal			Total		
	1954	1962	2007	1954	1962	2007	1954	1962	2007
Medium	99.8	118.5	72.1	79.7	131.4	160.0	179.4	250.0	230.1
Weak	80.1	111.7	66.9	82.0	131.4	233.0	162.1	243.1	299.9
Mean	89.9	115.1	68.9	80.9	131.4	200.0	170.8	246.6	268.9

Total production (Table 5) increased twofold in the first decade. Plant production increased less but animal production increased nearly threefold. For the entire study period, total production increased to 76.0 c.u. per ha AA including plant production increase to 31.3 and animal production increase to 44.1 c.u. Per capita total production increased from 49.1 to 98.9 c.u. in 1962, and to 200.5 c.u. in 2007. This was an effect of increased agricultural production and the decreased rural population. Despite smaller yields from PG, production and farmers' incomes increased markedly.

Table 5. Total production (cereal units per ha AA and per capita).

Villages of soils	Total production in cereal units per ha AA									Per capita cereal units		
	Plant			Animal			Total					
	1953	1962	2007	1953	1962	2007	1953	1962	2007	1953	1962	2007
Medium	14.8	24.7	39.1	5.21	12.8	45.6	20.0	37.4	84.6	51.3	95.9	217.0
Weak	13.3	22.1	24.9	4.5	15.0	42.2	17.8	37.0	67.0	49.9	103.9	189.5
Mean	13.8	23.8	31.3	4.7	13.5	44.1	18.5	37.2	76.0	49.1	98.9	200.5

Conclusion

Drainage and grassland management indirectly and directly intensified agricultural production. Yields from PG increased almost threefold and those from AL by 50-150% (mainly cereals). Animal stock increased by >80% in the first period (1954-1962) and by 200% to 2007. Total production in cereal units per ha and per capita increased fourfold. Mean surface area of farms increased by 1.8 ha. Land use structure, particularly in villages of weak soils, changed in favour of AL. Recently, these proportions have turned in favour of PG. The share of cereals and fodder crops in the crop structure increased at the expense of tuber crops. The share of cereals decreased in favour of fodder plants only in villages with better soils.

Reclamations indirectly increased the level of plant production; this has recently declined again. The intensity of animal production increased, particularly in villages with weak soils in AL surrounding PG. Such areas drained long ago should be modernized and rationally used to fully utilize the potential that appeared after regulation of water and air relations in peat soils.

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Vegetation heterogeneity and functional structure drive forage production of grazed wet grasslands

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Abstract

This work investigates the production capacity, together with the forage quality produced, in grazed wet grasslands, taking into account the heterogeneity in the soil and vegetation patterns. The effect of the soil nitrogen resources, grazing intensity, and vegetation functional structure for the quantity and quality of the forage was investigated. It was shown that heterogeneity in the functional structure drives forage quantity, with a negative effect of the functional diversity. Its effect on the forage stability during the long-lasting grazing season of the studied coastal wet grasslands remains to be tested.

Keywords: plant traits, heterogeneity, ecosystems services, diversity

Introduction

Coastal grasslands cover hundreds of hectares along the French Atlantic coast and they have shaped both landscape and agriculture activities, as well as biodiversity. The periodic flooding of the grasslands and the locally salted soil constitute high constraints for their agronomic use. In wet grasslands, grazing is one of the main agriculture practices and their agronomic value is critical for grazing maintenance, together with the associated biodiversity and social functions. The agronomical values of wet grasslands have been poorly investigated, but they are generally considered to be low.

In this work, this view has been challenged by measuring the forage production, as characterized by its quantity and quality over a range of plant patches; i.e. at the scale of local assemblages. We investigated whether vegetation performance depends on (i) contrasts in plant community structure, considering both community-weighted traits (CWM) and functional diversity (FD), and (ii) soil resources.

Two plant community types were studied: the meso-hygrophilous and the mesophilous communities, spreading along a topographical gradient (Amiaud *et al.*, 1998). This grassland type is characterized by the heterogeneity of the vegetation, which is related to (i) variation in the flooding duration (Violle *et al.*, 2010), (ii) contrast conditions in soil nitrogen resources (Rossignol *et al.*, 2011), and (iii) spatial contrasts in the intensity of grazing (Loucougaray *et al.*, 2004) as plant communities are selectively grazed by cattle and horses. Altogether, the grassland vegetation comprises a variety of plant patches organized as a mosaic (Marion *et al.* 2010).

The relationships between, on one hand, the functional structure and soil resources and, on the other hand, forage quantity and quality, were therefore investigated.

Materials and methods

The studied wet grasslands are situated in the Marais poitevin on the French Atlantic coast. These grasslands are extensively grazed by cattle or horses from April onwards every year. For each patch, the soil water content (%) of the soil layer, the soil mineral nitrogen net content (µg g^{-1}dry soil) and the grazing intensity exerted from April to June (Rossignol *et al.*,

2011) were measured. Five above-ground traits and four below-ground traits were determined using standardized procedures. Peak standing biomass was measured for the ten patch types, and the biomass sorted into species. The community-weighted mean (CWM) trait values for each trait were calculated for each of the *in-situ* vegetation plots studied. The functional diversity in each plant patch was approached by the Rao's functional diversity index and can be interpreted as the average trait dissimilarity of two randomly chosen individuals. The relationship between the vegetation functional structure and the biomass produced was investigated by linear regressions, as suggested by Diaz *et al.* (2007), to test the biomass-ratio (Grime, 1998) and diversity hypothesis (Tilman, 1997).

Results and discussion

Vegetation patches showed contrasts in terms of their floristic composition (Marion *et al.*, 2010) and for above-ground and below-ground traits (Chanteloup and Bonis, 2013). Grazing management impacts on vegetation structure and diversity depend mainly on the species of grazing herbivore (cattle or horses), whereas stocking rate showed only a limited effect (Loucougaray *et al.*, 2004).

Along the range of plant patches occurring within the grazed wet grasslands, the biomass at the peak standing crop varied from 174 g m^{-2} to 795 g m^{-2} and ANPP range was from 2.13 to 7.04 g m^{-2} day^{-1}. These wet grasslands thus provided both poorly and highly productive plant patches, without any fertilization input. Regarding the forage quality, as approached by measurement of the *in vitro* digestibility, it varies from 55.3% to 69.15% and thus remains of modest level throughout.

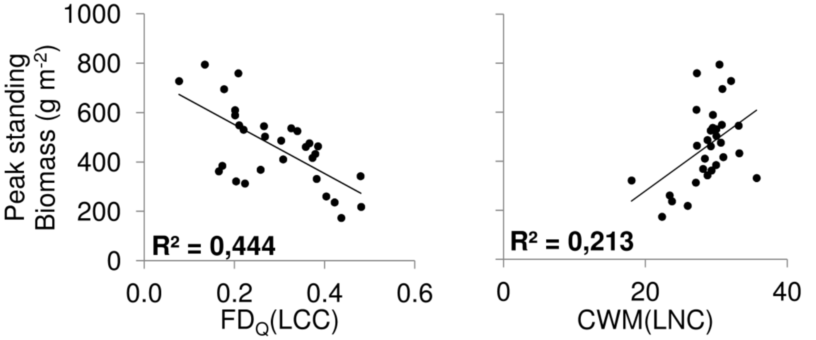

Figure 1. Peak standing biomass as a function of Rao's index of functional diversity for leaf carbon content, FD_Q (LCC), and community weighted mean values for leaf nitrogen content, CWM (LNC).

Unexpectedly, the mineral nitrogen content in the soil, which ranged from 49.6 to 71.8 µg N g^{-1}, did not significantly impact on the biomass production. The relatively high soil fertility status of the studied grasslands may have buffered the effect of the observed variation in soil nitrogen; the nitrogen soil variations thus remain too small to boost the herbage production as there are no mineral fertilizers are applied to the paddocks.

Biomass production was found to be positively related to the community-weighted mean trait values for reproductive height, leaf dry matter content, specific leaf area and specific root area, and negatively related to CWM for root tissue density (Figure 1). The traits showing a positive linked to biomass are those that are positively correlated to the growth rate (i.e., SLA, Hrep and SRA) and those negatively correlated with biomass are negatively correlated to plant growth rate.

The functional diversity effect on ecosystem services, and especially biomass production, is usually expected to be positive in relation to the diversity hypothesis (Tilman, 1997). It was thus unexpected to find a negative relationship between the level of functional diversity and biomass production. We suggest that the increase in functional diversity in the fertile grassland studied may result in a 'dilution' effect of the assemblage's growth capacity, compared with that of poorly diversified assemblages, as a possible explanation for this result. The forage quality was found to be negatively related to the leaf dry matter content and plant height average value (i.e. CWM). By contrast, a positive effect of the functional diversity in the leaf carbon content has been found, with a R^2 of 0.7. In agreement with the 'diversity hypothesis', this result has seldom been reported previously.

Conclusions

Marginal wet grasslands were shown here to provide locally high primary production of good quality despite the constraints of flooding and soil salt. This production service does not depend to fertilization and it appears to be highly sustainable from both an economic as well as ecological points of view. The agricultural services provided are based on an efficient ecological functioning in which soil and plants interact. Both dimensions of the functional structure of the vegetation, i.e. average trait values and diversity, intervene on different facets of their agronomic values. The spatial heterogeneity in vegetation structure constitutes a constraint but it may also be an opportunity for a more stable provision of forage during the length of the grazing season. Testing this hypothesis constitutes an on-going perspective of this work.

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‘Natura 2000 habitats 6230’ in siliceous substrates of the Lagorai mountain range (Trento, NE Italy)

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Abstract

Species-rich *Nardus* grasslands on siliceous substrates in mountain areas (and submountain areas, in Continental Europe) have been indicated as 'Natura 2000 habitats 6230' by the Council Directive 92/43/CEE. Over the years, habitats 6230 have been described more accurately for the European Member States and bioregions. Italian authors stated that habitats 6230 can be found up to sub-alpine environments and are characterized by dominance of *Nardus stricta* or its presence along with other acidophilic species. The objective of this study was to investigate the contribution of these referential characteristics to the identification of habitat 6230. The experiment was performed in the Lagorai mountain range (Trento, NE Italy), on meadows and pastures situated on siliceous substrates. During the summer 2010, sixty botanical surveys were carried out on 100 m^2 plots, in a 250 ha research area (1470-2100 m a.s.l.). Among the plots that fitted the description of habitat 6230, the total number of species ranged between 25 and 62, cover of *N. stricta* was between 5 and 70%; other acidophilic species were 7 to 18, with cover values ranging between 5 and 70%. Except for specific biodiversity, the traits studied did not consistently present high values; therefore, they appeared to be unsuitable for the identification of habitat 6230.

Keywords: *Nardus stricta*, specific biodiversity, pasture, meadow, phytosociology

Introduction

The Council Directive 92/43/EEC defined the habitats 6230 as 'Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in Continental Europe)'. Over the last two decades, European documents have described the habitats 6230 more accurately, indicating their geographical distribution, soil characteristics and altitude (European Commission, 1999, 2003, 2007; Galvánek and Janák, 2008). Italian manuals and reports indicated that habitats 6230 occur up to subalpine areas and they are characterized by *Nardus stricta* dominance (Lasen and Wilham, 2004; Lasen, 2006; Poldini *et al*., 2006; Italian Ministry for Environment, 2010), or its prevalence along with other acidophilic species (Masutti and Battisti, 2008). In contrast, Litterini *et al*. (2012) found large variability in terms of specific biodiversity, cover of *N. stricta*, and number of other acidophilic species across habitats 6230 located on calcareous substrates. The present study was carried out to investigate the relationships between these traits in habitats 6230 situated over siliceous substrates of the Lagorai mountain range (Trento, NE Italy).

Materials and methods

The work was carried out on a site of around 250 ha in the area of 'Desene di Sotto', 'Desene di Sopra', 'Prese' and 'La Portella mountain pass', located on the south-western slopes of the Lagorai mountain range (Trento, NE Italy). The area (at 1470-2100 m a.s.l.) was entirely situated below the altitudinal limit of arboreal vegetation and the herbaceous coenosis were managed as meadows or pastures on a regular basis. The site was characterized by Paleozoic and Mesozoic fine-grained siliceous rocks and the substrates were: brown podzolic, Humo-Ferric podzol, mountain podzol, brown ranker, ranker, protoranker, litosoil and emerging rocks. Meteorological parameters were collected from the closest weather stations located in the survey area (Sant'Orsola, 930 m a.s.l.), having an annual precipitation ≈ 911 mm, mostly distributed from April to November. The long-term (69 years) recorded average temperature was 9.3°C, with an average minimum temperature below 0°C for up to 6-8 months per year. In May 2010, sixty experimental plots measuring 10×10 m were established across the area of study. The plots were selected in order to cover uniformly the area of study and to have diversity in appearance between plots. For each plot, botanical surveys were subsequently performed in June, July and September 2010 to evaluate the total number of species and their percent cover (Pirola, 1970). Data from the three surveys were averaged to obtain one value and the specific biodiversity was subjected to multivariate analysis of variance using MULVA-5 (Wildi and Orlóci, 1996). The clusters were subjected to phytosociological analysis (Mucina *et al*., 1993) and the data of clusters corresponding to habitats 6230 (Lasen, 2006) were used to investigate the correlation between cover of *N. stricta* and the number of other acidophilic species, their cover, and specific biodiversity (total number of species), using SAS Proc Corr (ver. 9.2; SAS Institute, Cary, NC).

Results

In the area of study, 41 plots were identified as '6230 habitats' as there were species of the associations *Polygalo–Nardetum strictae* (3 plots), *Homogyno alpinae–Nardetum strictae* (12 plots) and *Sieverso montanae–Nardetum strictae* (26 plots). The remaining plots were sited on nutrient-rich soils and/or were characterized by higher fertilization applications in comparison with those identified as 6230 habitat. Among these plots, phytosociological analysis indicated that eight (meadows, at 1470-1590 m a.s.l.) constituted the association *Pastinaco–Arrhenateretum elatioris*, while 11 (pastures, at 1780-2100 m a.s.l.) had a floral composition intermediate between the association *Crepido–Cinosuretum cristati* and *Crepido–Festucetum commutatae*. Among the plots fitting the description of habitat 6230, cover of *N. stricta* ranged between 5 and 70%, with an average of 39% (Table 1). The number of acidophilic species (except *N. stricta*) ranged between 6 and 18, with an average of 12, and cover values ranging between 5 and 70% (average = 30%). Similar values were detected for each of the three *Nardetum* associations, but also across the 19 plots that were not recognized as habitats 6230 (Table 1).

Table 1. Specific biodiversity, cover of *Nardus stricta*, number of other acidophilic species, and their cover in pastures and meadows of the Lagorai mountain range (Trento, NE Italy). Values in parentheses indicate the number of experimental units surveyed per each vegetation type.

	Number of species		Cover of *N. stricta* (%)		Number of acidophilic species		Cover of acidophilic species (%)	
Vegetation	δ	μ	δ	μ	δ	μ	δ	μ
Polygalo–Nardetum strictae (3)	42-47	45	35-55	45	13-18	15	32-42	38
Homogyno alpinae–Nardetum strictae (12)	35-62	49	5-40	30	8-18	12	15-58	31
Sieverso montanae–Nardetum strictae (26)	25-61	42	8-70	42	6-18	12	5-70	29
Habitat 6230 (average, 41)	25-62	44	5-70	39	6-18	12	5-70	30
Pastinaco – Arrhenateretum elatioris (8)	31-75	48	2-15	7	3-11	7	5-24	14
Crepido–Cinosuretum cristati / Crepido–Festucetum commutatae (11)	28-51	39	4-70	39	5-16	12	8-57	31

δ, Range; μ, Average

A high number of species was found in all the plots (Table 1) but the correlation analysis revealed no relationships between specific biodiversity and cover of *N. stricta* or other acidophilic species (Table 2). A significant but weak correlation was detected, instead, between total number of species and acidophilic species (except *N. stricta*).

Table 2. Correlation coefficients relating specific biodiversity, cover of *Nardus stricta*, number of other acidophilic species, and their cover in habitats 6230 of the Lagorai mountain range (Trento, NE Italy).

Parameter	Number of species	Number of acidophilic species	Cover of acidophilic species
Cover of *N. stricta*	- 0.24 ns	- 0.30 ns	- 0.32 *
Number of species	—	0.37 *	- 0.21 ns
Number of acidophilic species	—	—	0.46 **

*, **, Significant at the 0.05 and 0.01 probability level, respectively;. ns, not significant at the 0.05 probability

Discussion and conclusions

In meadows and pastures located on siliceous substrates of the Lagorai mountain range, the majority of coenoses were identified as habitats 6230. With the exception of specific biodiversity, the traits measured in these vegetations (i.e., cover of *N. stricta*, number of other acidophilic species and their cover) did not consistently present high values. As such, none of these traits appeared to be suitable for the identification of habitat 6230. The results of this study agreed with Litterini *et al.* (2012), who reported a weak correlation between cover of *N. stricta* and specific biodiversity in habitats 6230 located on calcareous substrates. Moreover, the abundance of species recurred also in other phytosociological associations of the area, which further invalidates the use of this characteristic to discriminate for habitat 6230. In conclusion, habitats 6230 do not show univocal identification characteristics, other than their presence below the altitudinal limit of arboreal vegetation (European Commission, 2007) and their phytosociological classification.

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Effect of insect predators on plant size and seed production of *Pulsatilla pratensis* subsp. *Bohemica*

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Abstract

Pulsatilla pratensis subsp. *bohemica* (Ranunculaceae) is an endangered species of dry grassland in the Czech Republic. Flowers can be predated by the insect *Dasineura pulsatillae* (Cecidomyiidae), as well as by a new and probably not yet scientifically described species from the family Anthomyiidae. Both insects lay eggs into flowers and upon hatching the larvae will feed on them. In 2012 an experiment was established which aimed to investigate the effect of insect predation on plant size and seed production. The experiment took place in well preserved acidophilous dry grassland where, starting in early spring, some plants were protected against insects using synthetic cloth bags. With this design we compared predated versus non-predated plants. In unprotected plants, 31% and 57% of the flowers were predated by *Dasineura* and the Anthomyiid species, respectively. Predated flowers produced seeds with decreased seed weight. There was also a significant difference in the length of the longest leaf. The Anthomyiid species completely destroyed 21% of seeds in predated flowers, equating to 4% of seeds in the locality. However, over the studied locality there were sufficient viable seeds produced despite insect predation, signifying that predation does not pose a major threat to the stability of the *Pulsatilla* population.

Keywords: *Dasineura pulsatillae*, gall midge, Anthomyiidae, pasque flower, predispersal predators, dry grasslands

Introduction

Pulsatilla pratensis subsp. *bohemica* (Ranunculaceae) is an endangered early-spring grassland community species (Procházka, 2001). It is found only in central Europe, specifically Germany, Czech Republic, Poland, Austria, Slovakia and Hungary. Most of the localities in the Czech Republic are situated in central and northwest Bohemia, as well as in the southern part of Moravia (Skalický, 1988).

Pulsatilla can be predated by many insect predators. Some of these are monophagous – larvae that can feed only on *Pulsatilla*. One such predator is *Dasineura pulsatillae* (Diptera: Cecidomyiidae), first found in the Czech Republic in 1972, at one locality in central Bohemia. The next occurrence of *D. pulsatillae* was recorded in 2009, in the acidophilous dry grassland reservation Na horách (Jiras *et al*., 2010), where this study was conducted. In addition, another insect predator from the family Anthomyiidae (Diptera) was found in the locality, and this is probably a new species yet to be described. Larvae of *D. pulsatillae* and the Anthomyiid species developed within the flowers of *P. pratensis*, feeding on the sap from developing seeds and receptacles. Larvae of Anthomyiid species, moreover, create apertures in seeds (Jiras *et al*., 2010).

The aim of the present study is to investigate the influence of these rare insect species on the growth and seed production of endangered *P. pratensis* subsp. *bohemica* plants.

Materials and methods

The experiment was established in the dry grassland nature reservation called Na horách, which is situated in the southern part of Central Bohemia, Czech Republic (49°48'07''N; 13°57'09''E) on a south-facing slope of Cambrian shale. The slopes are covered with xerophilous vegetation of *Hyperico-Scleranthion* and *Koelerio-Phleion* alliances. Six plant species occurring at the locality are legally protected plants in the Czech Republic, including a rich population of *P. pratensis* subsp. *bohemica* (Hlaváček and Karlík, 2010).

In early spring 2012, one hundred and eighty plants were chosen at random. One half of the flowers were covered with a synthetic cloth bag (45% polyester, 55% nylon), aiming to protect them against females of *D. pulsatillae* and the unidentified Anthomyiidae species laying eggs within the flowers. Due to a lack of information about the term of oviposition in the two studied species, it was necessary to cover the flowers very early (before the opening of buds, plant sizes 5-10 cm). To prevent the autogamy of *Pulsatilla pratensis*, which may lead to limited seed production (Torvik, 1998), all the covered plants were pollinated by hand with a small brush two weeks later.

Several fitness indicators were compared between non-predated flowers, flowers predated only by *Dasineura*, flowers only predated by the unidentified species, and flowers predated by both species. Indicators that are focused on are the seed weight (average weight of one seed, counted from the weight of one hundred seeds), the number of leaves, the length of the longest leaf, and the stem height. For statistical analysis we used a one-way ANOVA, Kruskal-Wallis non-parametrical test and Tukey HSD test.

Results and discussion

In unprotected plants, 27%, 34% and 25% of the flowers were predated by *Dasineura*, the Anthomyiid species, and both of them, respectively. In flowers with presence of Anthomyiid species only 79% of viable seeds remained. This means that 21% of seeds were completely destroyed in predated flowers, representing 4% of seeds in the locality. Only seeds that were not destroyed by Anthomyiid species were included in the statistical analysis.

There was a significant difference in the average seed weight and in the length of the longest leaf between predated and non-predated plants (see Table 1). The effect of predation on stem height and number of leaves was found to be non significant.

Table 1. Tests of significance of differences between plants with different status of predation and their fitness indicators. Using of Tukey HSD test, differences among predation categories with the same letter were not significantly different. F – results of one-way ANOVA; H – results of Kruskal-Wallis non-parametric test; SD – standard deviation; *P* – significance value; n.s. - not significant; *** *P* <0.001; * *P* <0.05.

Trait	*Dasineura pulsatillae* (n = 22)		Anthomyiid species (n = 32)		*Dasineura* and Anthomyiid species (n = 23)		No predation (n = 103)		Results of tests		Significance
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	H	*P*
Seed weight (mg seed^{-1})	1.6^{a}	0.47	1.6^{a}	0.46	1.5^{a}	0.33	2^{b}	0.63	7.9	-	***
Number of leaves	2.59	1.30	2.63	1.34	2.61	1.20	2.75	1.07	-	3.28	n.s.
Longest leaf (mm)	128	31.4	132	28	147	34	144	30.7	2.7	-	*
Stem height (mm)	248	52.9	267	40.3	279	43.9	259	48.6	1.78	-	n.s.

Dasineura larvae feed on the *Pulsatilla* receptacles and developing seeds, but the next step of their development takes place in the soil. Even if *Dasineura* larvae appeared absent within the flowers, it is possible they affected the investigated fitness parameters before having left the flower. Therefore it is difficult to differentiate between the effect of *D. pulsatillae* and the Anthomyiid species on seed production and plant growth.

Conclusions

Although it was recorded that plants predated by *D. pulsatillae* and the Anthomyiid species produced lighter seeds than the non-predated plants, the population of *P. pratensis* can prosper due to the large amount of seeds and longevity of *Pulsatilla* plants. Therefore it can be concluded that further research is necessary to determine, if any, the differences in germination of those seeds affected by *Dasineura* and the Anthomyiid species.

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Tree and grazing influence on herbaceous pasture diversity in the Dehesa agroforestry system

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Abstract

Mediterranean Dehesas are one of the European natural habitats associated with high diversity and they provide important natural resources and ecosystem services. In our research, we study tree contribution and grazing influence over pasture alpha diversity in a Dehesa in Central Spain. We analysed Richness and Shannon-Wiener (SW) indices within the herbaceous layer under sixteen holm oaks (*Quercus ilex* ssp *ballota* (Desf.) Samp). We established 64 sampling units distributed in two directions and at two distances from the trunk. The sampling units were located in four different grazing management zones, depending on which grazing livestock species and at what stocking rate. We analysed floristic composition by species or morphospecies, and species abundance for each sample unit. We used linear mixed models (LMM) and generalized linear mixed models (GLMMs) to study relationships between alpha diversity measures and independent factors. Edge crown influence showed the highest values of Richness and SW index. No significant differences were found between orientations under tree crown influence. Grazing management had a significant effect on Richness and SW measures. We preliminarily quantify and analyse the interaction of trees and grazing management over herbaceous diversity in a year of extremely dry conditions.

Keywords: Mediterranean grazing, richness, Shannon-Wiener index, Generalized linear mixed models, Linear mixed models, tree-grass interaction

Introduction

Mediterranean Dehesas are one of the European natural habitats assigned as 'Sites of Community Importance' (43/92/EEC Directive) and, currently, they are a magnificent example of High Nature Value Farmland (Hoogeveen *et al.*, 2004). Dehesas are associated with high diversity and they are providers of important natural resources and ecosystem services. The presence of the tree plays an important role within these ecosystems. Within their canopy and roots influence area, trees can change grassland species composition, herb layer structure, spatial distribution and biomass (Scholes and Archer, 1997). Trees create a softer microclimate (less thermic and humidity variation) beneath their crowns (Ludwig *et al.*, 2001). In addition, they provide shelter for livestock and wildlife that concentrate around trees. This fact plays an important role in fertilizing soil through dung deposition. Furthermore, this fertilization process is different according to grazing type (Treydte *et al.*, 2009). It is important to consider management decisions over tree-grass interaction in ecosystem dynamics and functional studies, whilst also recognizing that Dehesa management also depends on the socioeconomic realities of the environment and policies. In our research, we attempt to analyse how tree contribution (through the study of the below-crown herbaceous species) and grazing influence (considering different grazing species and stocking rates) modify alpha diversity in a Dehesa.

Materials and methods

The study was conducted within a typical Dehesa in central Spain (39°N, 5ºW), 350 m a.s.l. The area has a continental Mediterranean climate. Soils are sandy (>80% of sand), acidic, and poor in organic matter (<1%). Sixteen holm oaks (*Quercus ilex* ssp *ballota* (Desf.) Samp). were selected for the study, distributed in two different grazing management zones (with cattle or sheep as grazing species) and two different stocking rates (medium or intensive). The average canopy radius (standard deviation) of trees is 6.8 m (1.3). At each tree, we located four sampling frames (50×50 cm), considering two positions according to the proximity of the trunk (Distance: 0.5 radius or 1 radius -edge of the crown-) in two directions (Orientation: north-east and south-west). In total, 64 sampling units were studied. We analysed floristic composition by species or morphospecies, and species abundance for each sample unit. We calculated Richness and Shannon-Wiener (SW) indices to measure alpha diversity. We used generalized linear mixed models (GLMMs) and linear mixed models (LMM) to study relationships between alpha diversity measures and independent factors (grazing species, stocking rate, distance and orientation). The model approach in Zuur *et al*. (2009) was used for the alpha measure. Table 1 shows selected models. For data processing, analysis and presentation of results we used R programming environment (Version 2.14.1, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org).

Table 1. Models with the best Akaike's Information Criterion (delta <2) of Richness and SW index related with other variables in the analysis of alpha diversity. AIC=Akaike's Information Criterion.

Alpha measure	Explanatory variables	AIC (top models)	Delta
Richness	Distance + Management + (1|Tree)	57.21	0.00
	Distance + Management + Orientation + (1|Tree)	57.73	0.52
Shannon Wiener	Distance + Management, random =~1|Tree	112.01	0.00
	Distance + Management + Orientation, random=~1|Tree	113.85	1.84

Results and discussion

The sampling year was extremely dry (298.1 mm). Rainfall was 50% lower than the average rainfall of the last 20 years on the farm, 596.7 mm (Lopez-Carrasco *et al.*, 2012). Therefore, this fact determined a very low herbaceous production, and consequently a reduction in the species number. The holm oaks selected for the study have a mean (standard deviation) canopy radio of 6.8 m (1.3). Treydte *et al*., (2009) show that only large trees have an effect on the understorey. In our case, tree size was sufficient to result in differences in the herbaceous composition under the crown. Edge crown zone showed the highest values of Richness and SW index (P=0.0178 and P=0.0108) (Figure 1). Edge crown has higher light availability than the zones below the crown and allows the presence of light-demanding herbaceous species (Marañón and Bartolome, 1993). Given the hard climatic conditions of our study, we found no significant differences between orientations under the influence of tree crown. In our study area, the cattle zone had a significant effect on Richness and SW measures (P=0.00185 and P=0.0107) (Figure 2), providing higher herbaceous diversity below the canopy than was found in the sheep zone. Furthermore, sheep grazing at low stocking rate gave less diversity (P=0.0071). We did not find differences between stocking rate levels at the cattle grazing zones.

The tree random effect scarcely modifies the alpha diversity average among trees.

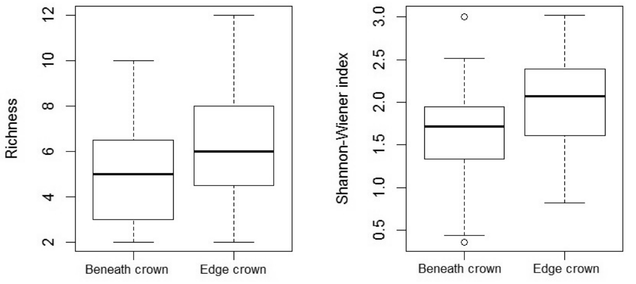

Figure 1. Alpha diversity variation according to distance to the trunk.

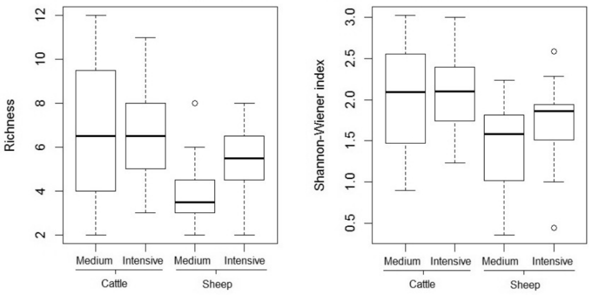

Figure 2. Alpha diversity variation according to grazing species and stocking rate.

Conclusions

We quantified and analysed the tree effect on herbaceous species alpha-diversity in a typical Dehesa of Central Spain in a very dry year. Richness and SW index were higher at pastures under the edge crown, at any orientation. Management practices, through the combination of grazing species and stocking rate, also had a significant influence on alpha-diversity.

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Effects of management on vegetation structure in horse pastures

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Abstract

Horses are known to create and maintain heavily grazed short patches within a matrix of avoided tall patches. However, there is little systematic research in horse pastures under practical farming conditions. Data on grassland management were recorded for 70 horse-farms in Germany. Soil-chemical factors, species richness and cover were measured for short and tall patches (each 25 m²) within 280 sites. We found distinct differences in soil nutrient concentration and species composition between short and tall patches. This heterogeneity within sites was found to enhance plant diversity at paddock scale. Stocking methods as well as sward maintenance measures affected heterogeneity and plant species composition.

Keywords: horses, pasture, floristic composition, heterogeneity

Introduction

Grazing by large herbivores has been reported to lead to a mosaic of patches of different sward height and species composition (SC). This heterogeneity is assumed to enhance plant diversity (Marion *et al.,* 2010). Horses are known to create distinct sward heterogeneity. They avoid grazing close to faeces spots and therefore establish latrine patches with a tall sward where faeces are concentrated, and short sward patches where they graze. This results in a shift of nutrient concentration between grazed and avoided patches (Archer, 1973; Oedberg and Francis-Smith, 1976). Two sets of incisors enable horses to graze selectively and close to the soil; thus, overstocking is likely to result in sward degradation. Nutrient concentration and selective grazing are known to affect species competition and vegetation heterogeneity in pastures and these effects are much stronger on horse pastures than on cattle pastures. However, there has been little in-depth research on the vegetation structure of horse pastures in farming practice. In this study, we focus on (1) how short and tall patches differ in soil-chemical factors and SC, and (2) how this heterogeneity is affected by grassland management.

Materials and methods

We collected data on farm structure, grassland management and vegetation on 70 horse farms in Germany. Paddocks were selected following a stratified random design. On each farm two paddocks were grazed only, two further paddocks were grazed and cut (mown-pasture) or cut only (meadow). Management factors such as stocking method (continuous, rotational, strip stocking) and stocking density, cutting regime and sward maintenance measures (N-fertilization, harrowing, use of herbicides) were detected for each paddock by standardized questionnaire. Top-soil pH and plant available nutrients (P and K) were measured and botanical relevées (plant species, yield percentage) were carried out in two 25-m² subplots. We chose a grazed short and an avoided tall patch for pastures, for meadows a central short and a fringe tall sward patch. Species richness (SR) and yield percentage of functional groups [grasses (G), forbs (F), legumes (L)], and also cover of nitrophilous species (W) and High-Nature-Value (HNV)-indicator species (Matzdorf *et al.,* 2010) were obtained for each subplot. Paddocks were classified according to the HNV-concept (Matzdorf *et al.,* 2010) using indicator species. Sørensen's similarity coefficient was calculated for each site. Canonical constrained ordinations (CCA) were performed in Canoco 4.5 focusing the relationship between management and vegetation (therefore, we used longitude, latitude and local site factors as covariables without taking them into further consideration). ANOVAs with short and tall patches as repeated measures were calculated with stocking methods, cutting regime and sward maintenance measures as fixed factors. Significant effects were tested using Tukey's test for unequal sample sizes. In the case of variance heterogeneity, ANOVAs were repeated with nonparametric tests (Friedman‘s ANOVA). Also linear regressions were performed on continuous variables. All univariate statistics were performed in Statistica 9.0.

Results and discussion

Indicating the potential of horse pastures to maintain species-rich grassland, 42% of the recorded sites were estimated as HNV-grassland. Soil chemical factors differed significantly between short and tall patches within sites. Short patches showed lower values for P and K (both P=0.001) than tall patches (Table 1). This is concomitant with the highly significant (P=0.002) patch-effect, which explained more variance in SC on pastures than any other variable. The gradient of K in particular is strongly positive correlated with the vegetation composition of tall patches (Figure 1). SC of pastures was significantly different (P<0.05) compared to the more homogenous meadows. This confirms the distinct nutrient cycling and heterogeneity induced by grazing horses (Archer, 1973).

Table 1. Mean values of soil and vegetation parameters for different site management.

Type of management		Var.	P	K	Species	HNV	G	F	L	W	Sørensen
	Stocking	Patch	Mean								
Pasture and mown-pasture (N = 240)	Continuous (N = 90)	Short	17.7	15.2	12.7	2.2	75.8	17.1	1.2	5.0	0.597
		Tall	24.1	27.6	13.4	2.1	67.5	28.0	0.9	16.6	
	Rotational (N = 126)	Short	16.1	14.9	12.7	2.5	74.4	19.1	1.1	5.0	0.586
		Tall	21.1	21.2	13.2	2.5	63.7	32.0	0.8	17.4	
	Strip (N = 24)	Short	14.8	16.5	11.8	1.5	68.1	23.1	0.9	4.2	0.673
		Tall	16.4	24.5	12.8	1.6	77.4	19.0	1.1	7.2	
Meadow (N = 40)		Short	15.9	12.4	11.7	2.5	78.1	16.5	1.0	5.7	0.602
		Tall	18.2	20.9	11.9	2.2	72.5	20.7	0.8	9.7	
Management			-	-	-	+	*	*	-	+	**
Patch			***	***	+	-	*	***	***	***	
Management × Patch			*	*	-	-	***	**	**	*	

Results of ANOVAs with site management (pastures with mown-pastures: continuous stocking, rotational stocking and strip grazing; meadows) as fixed factor, patch (short and tall) as repeated measure for soil nutrients (P and K, mg $100g^{-1}$ soil DM) and number of species and HNV-species, cover of plant functional groups. Significance level of mean differences is shown as * P<0.05, ** P=0.01, *** P=0.001 (+ P<0.08)

SR did not differ significantly between short and tall patches (Table 1) but yield percentage of plant functional groups did. On tall patches, abundance of F and W, like *Rumex obtusifolius* and *Urtica dioca*, increased significantly compared to short patches (Figure 1). In contrast, on short patches *Festuca pratensis, Lolium perenne* and *Trifolium repens* were more abundant. Fleurance *et al*. (2010) also found an increase of legumes on heavily grazed plots. Short patches are also characterized by rosette hemicryptophytes like *Bellis perenne* (Marion *et al*., 2010), which grow below the grazed sward horizon. Sørensen's similarity coefficient reflects this local contrast in SC. For continuous and rotational stocking, we found lower (P=0.01) similarity in SC and differences in cover of plant functional groups than for strip stocking or meadows. SR and richness of HNV-indicator species were not affected by the stocking method but occurred less frequently (P=0.01) under higher stocking density. Sørensen's coefficient was negatively correlated with stocking density (P=0.001). Therefore, distinct heterogeneity occurring under high stocking density might not generally favour plant diversity and SR; rather, it seems to indicate overstocking and sward degradation. Sward maintenance measures such as N-fertilization, harrowing and the use of herbicides showed negative (P<0.05) effects on SR, percentage of F and HNV-species at the paddock scale, but only harrowing significantly (P=0.001) affected heterogeneity between short and tall patches by reducing distribution of P. However, sites that have been estimated as HNV-grassland showed lower (P=0.01) Sørensen's similarity coefficients than others.

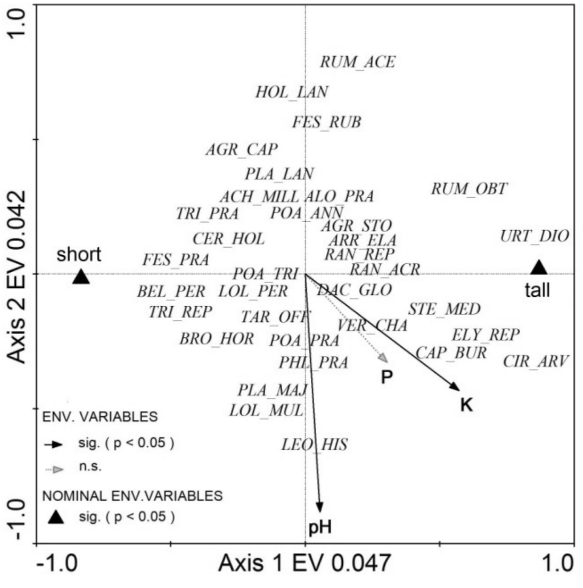

Figure 1. Canonical constrained ordination biplot, for 240 pastures and mown-pastures including 36 best fitting species and environmental variables: short patch (short, P=0.002), tall patch (tall, P=0.002); pH (P=0.002); P (P=0.188) and K (P=0.002). Significant effects obtained by Monte-Carlo-Permutation test (499 permutations).

Conclusions

Horses establish distinct nutrient shifts and heterogeneity in species composition. Except in cases of overstocking or intensive use, this might lead to sward diversification and be an opportunity to preserve species-rich grassland, particularly in regions of intensive agriculture.

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A Grass Use Intensity index to be used across regions and grassland managements

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Abstract

The widely used concept of intensity of grassland management can be defined according to the fertilization level and defoliation frequency for mown meadows or to the stocking rate for pastures. Many questions arise for comparing situations over a large gradient of pedo-climatic conditions, or grazing, mowing and mixed grass utilizations. We therefore propose an index of intensity of grassland management, combining grazing and mowing, and considering regional differences in biomass productivity. A model predicting the percentage of grass eaten by the animals as a function of the stocking rate was developed based on field measurements. The index can sum this percentage of grass defoliated by grazing to that defoliated by cutting, considering that one cut defoliates 100% of the vegetation. Regional differences in biomass productivity are taken into account by dividing the sum of defoliations by the biomass productivity of grassland at the regional level, which is estimated from remote sensing images. This index could be used to roughly estimate management intensity when field measurements are not available and a large range of situations have to be compared.

Keywords: mixed grassland, number of cuts, remote sensing, stocking rate

Introduction

Intensity of grassland management is a key parameter for assessing the effects of agriculture on grassland biodiversity. Difficulties arise when it has to be quantified over a broad range of grassland types. First, comparing mown and grazed plots or estimating the intensity of grassland management when plots are alternatively mown and grazed require estimating the proportion of standing biomass removed by the grazing events (Lienin and Kleyer, 2012). However, this proportion is rarely known. Second, the effects of the number of defoliations on grassland vegetation depend on the length of vegetation period and of other abiotic factors influencing plant growth. Quantifying management intensity in different regions therefore requires accounting for differences in the potential of biomass production across regions. Herzog *et al.* (2006) proposed to normalize the mowing and grazing intensity by the maximum of these two intensities at the regional scale. This approach is very sensitive to the determination of these maxima and thus to definition of regions and sampling effort. Our aim is here to propose a methodology for calculating an index of intensity of grass use that could be used over a large gradient of pedo-climatic conditions and plot utilization. We use the term 'Grass Use Intensity index' (*GUI*) and not 'intensity of grassland management' because the level of fertilization is not aggregated to the frequency of defoliation in the proposed index.

Materials and methods

The percentage of vegetal cover defoliated (*%D*) at each mowing or grazing cycle is first estimated to calculate a defoliation index. It is considered that 100% of the vegetation is defoliated at each mowing event. For grazing, *%D* was estimated from the stocking rate based on sward height measurements in a field experiment comparing the effects of different grazing intensities in France, UK and Germany (Dumont *et al.*, 2007). At the French site, the percentage of grazed patches was also recorded, which showed that the percentage of vegetation below 12 cm would be a good indicator of the percentage of grazed grass (data not shown). This percentage was thus used to determine the effect of the stocking rate (LU days ha^{-1}) on *%D* by grazing in the three sites. The defoliation index was then obtained by summing the percentage of defoliation in successive plot utilizations along the grazing season. We propose to use the Normalized Differenced Vegetation Index (NDVI) from remote sensing images to estimate potential grass production (P_{pot}) at each site (Paruelo *et al.*, 1997). NDVI values were obtained from MODIS satellites images (250 m·250 m pixel, one image every 16 days) and were filtered using the protocol of Taugourdeau *et al.* (2010). A model to estimate grass production from the yearly dynamic of NDVI was constructed using production data from 217 grasslands in France and Switzerland. To facilitate the interpretation of the absolute values of the *GUI*, the index could be scaled with a reference yield and corresponding number of cuts for an intensive utilisation system, for instance 5 cuts (Σ *%D* = 500) for a production of 12 t DM ha^{-1} yr^{-1}. The *GUI* is thus calculated as:

$$GUI = \frac{\sum \%D}{P_{pot}}\left(\frac{12}{500}100\right)$$

Results and discussion

The relationship between the number of LU days ha^{-1} during one grazing cycle and the percentage of vegetation below 12 cm was found to be: *%12cm* = 16.28*ln* (LU days ha^{-1}) – 10.3 ($R^2 = 0.70$; Figure 1) for the less productive site in France (F), and *%12cm* = 20.60*ln* (LU days ha^{-1}) – 44.6 ($R^2 = 0.69$) for the more productive sites in Germany (G) and the UK. The difference between sites is probably due to the grazing behaviour of cattle that were shown to increase their selectivity for short vegetative regrowths in the most productive grasslands (Dumont *et al.*, 2007). This could explain the lower percentage of plot cover that was considered as being grazed for a given stocking rate in G and in the UK compared with F.

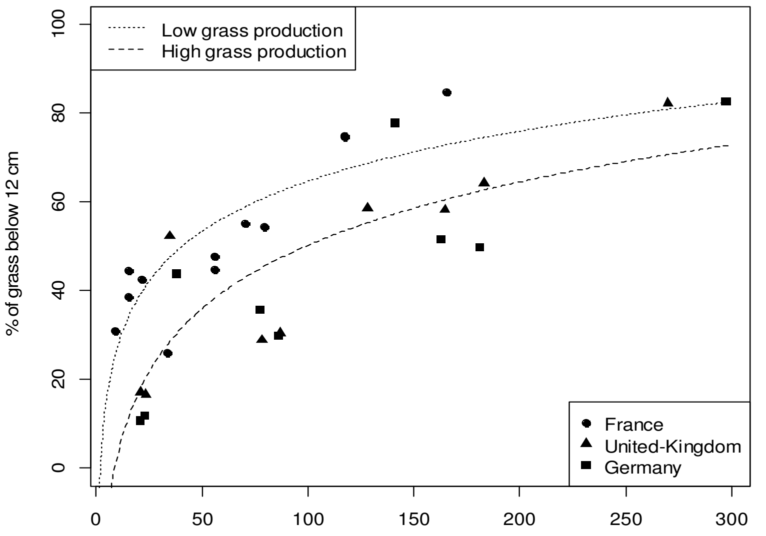

Figure1. Relationships between stocking rate and percentage of cover below 12 cm for each grazing cycle based on three sites in F: France, U: United-Kingdom and G: Germany.

Productivity of the 217 grasslands with yield data was best predicted from the dynamic of the NDVI of the pixels corresponding to the locations of the grasslands by the equation: $P_{pot} = 11.9NDVI_{feb} + 6.9NDVI_{sep} - 14NDVI_{nov} + 4.8$ ($R^2 = 0.38$). Examples of *GUI* calculated for some scenarios using the above proposed equations are given in Table 1. For a grassland situated at a location with a P_{pot} of 7.2 t DM ha^{-1} yr^{-1} (e.g. upland areas), 3 cuts per year correspond to a *GUI* of 100, while for the same number of cuts at a location with a P_{pot} of 12 t DM ha^{-1} yr^{-1} (lowland) the *GUI* would be of 60. Grazing 770 LU days (which corresponds to a grass consumption of 10 t DM) in 7 grazing cycles at the location with P_{pot} =12, yield a *GUI* of 73, although this corresponds to an intensive grazing system. The lower *GUI* calculated for intensive grazing than for intensive cutting is due to the fact that the percentage of cover defoliated by grazing animals never reaches 100% even in the most intensive systems.

Table 1. Calculated Index of Grass Use Intensity (*GUI*) for locations with different potential of production (P_{pot}) under different agricultural management.

P_{pot} (t DM ha^{-1})	Cuts	LU days	Graz. cycles	$\Sigma\%D$	*GUI*
12.0	5			500	100
12.0	3			300	60
12.0		770	7	366	73
12.0	3	330	3	457	91
7.2	3			300	100
7.2		440	4	265	88

This paper proposes a methodology for calculating an index that could quantify grass use intensity over a large gradient of pedo-climatic conditions and for different types of utilization. Combining information from remote sensing images to estimate the potential grass production across regions with an empirical model to estimate the proportion of plot cover defoliated during grazing events allows such comparisons. The relationship between stocking rate and percentage of defoliated cover as well as the estimation of the potential grass production from NDVI are based on a small number of data, and will have to be validated before the proposed *GUI* can be widely used.

Acknowledgements

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Conservation with extensive grazing – highlighting a conceptual approach and recommendations for the new CAP to give support to such systems

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Abstract

Grazing is still a feature of many cultivated landscapes in Europe, and multiple benefits are associated with the grazing of semi-natural grasslands. Such regions harbour a particularly rich biodiversity, provide income to farmers, are important for tourism and recreation, and help maintain vital rural communities. The extensive use of grassland in the form of large-scale, near-natural grazing systems could make a considerable contribution to the protection of species diversity, water resources, soil, and climate stability. The retention of extensively grazed grassland is thus an important task for future agricultural policy and rural development. But such sustainable or high nature value (HNV) systems as these are increasingly being exposed to pressures from intensive types of agriculture and inappropriate and unsupportive EU agricultural policy. The authors are engaged in a research project that examines such detrimental factors on the base of on-site farm surveys and by analysing the current agricultural policy status that affects extensive livestock grazing. Focus of the research is the situation of German farms. This paper summarizes the arguments and requirements to maintain large-scale extensive grazing systems which have to be addressed in the current process of developing the new common agricultural policy (CAP) framework for the period from 2014 to 2020.

Keywords: agricultural policy, rural development, grassland, grazing, biodiversity

Introduction and background

European cultural landscapes have been shaped by farming activities for thousands of years and harbour long-term co-evolutionary processes. Such ecosystems are a major contribution to the world heritage of biodiversity. Important in this context are the extensively grazed grassland ecosystems which are characterized by rich biodiversity, with diverse communities of plants and animals maintained by extensive grazing. Some still encompass a considerable diversity of livestock species as well (Veen *et al.*, 2009).

Agro-ecosystems with sustainably high biodiversity have also been labelled 'high nature value' (HNV) farming systems. The prevailing characteristic of HNV farming is its low intensity, which is associated with a significant presence of semi-natural vegetation and high diversity of land cover (Paracchini *et al.*, 2008). In fact, European law (e.g. EU Habitats Directive) requires the protection of the biodiversity of species-rich grazed semi-natural grassland. The Directive protects ca. 200 so called 'habitat types' that are of prime importance to Europe. Looking at their dependencies on various agricultural land-use systems it can be shown that roughly 70 (35%) of the listed habitats relate to farming. Further analysis shows that all such habitat types refer to grassland management practices associated with, in general, low intensity livestock farming (Luick *et al.*, 2012). But the area covered by those 70 habitat types' amounts to much more than the 35% share of the total area covered by the protected habitat types might suggest.

There exists no coherent mapping of all European extensive grasslands or related HNV systems. Vague calculations can be drawn from the EU official data for designated NATURA 2000 sites, which comprise about 850,000 km^2 (18%) of the EU's land area (EU, 2012). Bearing in mind that ca. 50% of this area consists of agricultural land, and such figures may represent only 20-30% of all ecologically important areas, the actual area may well encompass more than 1 million km^2 and most of it correlates with grassland ecosystems which logically comprise livestock-(grazing)-keeping (EEA, 2012).

However, it is not just the scale that is important but also the ecological quality of such high nature value (HNV) farmland environments. Whereas in the more marginal areas of Europe, as in many of the southeastern countries and where millions of hectares of HNV farmland still have a high ecological value, the situation in the more central and western parts of Europe is frightening. For example, official assessments of the HNV distribution within Germany depict that only ca. 14% of all grassland areas have HNV properties and only ca. 7% can be judged has having good and high ecological values (Luick *et al*., 2012).

In 1999 the EU originally set the goal of stopping the loss of biodiversity by 2010, an aim that was not reached and which was postponed, and is now to be achieved by 2020 (COM, 2011). Therefore, agriculture is the most crucial locus of attention in the effort to stop the loss of biodiversity. The CAP, which at the moment is being developed for the period 2014 to 2020, is a key to a new policy to ensure and improve European HNV-farming, and thereby it is the most important instrument to protect the European biodiversity.

Materials and methods

From 2011, and ending in 2013, the authors have been conducting the research project ‘development of extensive grazings as a sustainable conservation tool’ which is supported by the German Environmental Foundation (DBU). The aim is to detect weaknesses of the current CAP which result in economic and structural problems for extensive grazing farming practices and to delineate, from the experience to date, more effective and beneficial measures within the new CAP. The research is based on farm surveys with extensive livestock-keepers using grazing systems of various systems (cattle and sheep, full-time and part-time, small-scale and large-scale). Furthermore, the existing agricultural policy framework and the ongoing process of outlining the CAP for the period form 2014 to 2020 had been scrutinized.

Results and discussion

Although the work focuses on practitioners in Germany, the situations to be examined and the problems to be solved are comparable to those of many other countries. The farm surveys clearly showed that the problems that face extensive grazing systems exist in both sectors of the CAP - Pillar 1 (direct area payments) and Pillar 2 (rural development measures). Key issues that have been examined and which need to be targeted within the debate for a new CAP are as follows:

- *Direct (first pillar) payments for extensive pastures*: there must be direct payments available for all areas used for grazing, which is not yet the case everywhere in Europe. This requires an adjusted definition of the types of grassland vegetation eligible for grant payment. The objective must be to allow spending of first pillar payments for all extensively used agricultural land including types such as heaths, semi-arid and arid grassland, wet grasslands with sedges, riparian zones, reedbeds, and traditional semi-open pastures with significant forest cover.
- *Definition and introduction of a specific land use code in the new CAP land use typology for sites of conservation interest which have agricultural uses:* So far, grazing lands rich in structural and biotic biodiversity but poor with respect to productivity are often excluded from

CAP grant money. This is the case for CAP first pillar payment and as well as in terms of eligibility for CAP pillar two programmes such as for agri-environment schemes. A targeted land use code (e.g. conservation areas used for agricultural purposes) and specified cross-compliance exemption for these areas would help to minimize administration and reduce risks from sanctions to farmers.

- *Making landscape features eligible for funding*: it should be possible to legally integrate up to 50% of landscape features such as hedges, stone walls or bare and rocky ground into the eligible area for CAP grant money on a lumped calculation. Currently, it is required to measure and map each of those elements with extreme precision and exclude the ground underneath them from the eligible grant payment area. At the same time, such structures must remain in a static condition as part of cross-compliance obligations; otherwise farmers are prone to sanctions. It is virtually impossible to measure such features, which often gradually appear or disappear over time in large-scale grazing environments, but maintaining these dynamic processes is a key factor to reach the ambitious EU targets for biodiversity. Furthermore, necessary management of such elements, for example reducing excessive scrub encroachment, must be permitted and not sanctioned. This could be handled and made legally correct if conservation-oriented site management plans were to exist and be applied.
- *Cutting red tape*: Mainstream animal husbandry provisions must be adjusted to the practical needs and manageability requirements of large-scale extensive grazing operations. Major issues to be addressed are the identification requirements for animals, veterinary medical surveillance, killing and slaughtering, and handling of carcasses. In all these areas, the volume of laws and threats of sanctions can be significantly reduced without lowering standards of food safety and veterinary hygiene.

Conclusions

Low-intensity grazing-oriented livestock farming represents modern, multifunctional and sustainable agriculture and also provides numerous public goods (ecosystem services). Necessary political changes within a new CAP comprise: (1) integration of extensive grazing into the first pillar of CAP, (2) advanced and more tailored agri-environment schemes in the second pillar of CAP, (3) general environmentally oriented extension services for farmers to inform them about conservation objectives and their integration into farming practice, and (4) simplification and other practical adjustments of regulations.

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Influencing effects of human activities on grassland biodiversity and degradation in south-west Hungary

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Abstract

Ecological investigations have been made in four succeeding years in grassland stands used for deer grazing with different management types in order to examine the effects of human activities on habitat degradation and biodiversity changes. In all stands, plant species were recorded and their cover was estimated in control and disturbed plots. The majority of human activities increased the cover of species that indicate disturbance, like weeds, adventive and invasive plants in different degrees. On the other hand, a decrease in the cover of species indicating naturalness was observable. Human activities like silage and manure deposits, or seeding, caused observable changes in humidity (Ellenberg moisture, F) of the affected plots.

Keywords: grassland, biodiversity, degradation, human activities, grassland management

Introduction

Ecosystem stability is directly related to high biodiversity (Tilman *et al.*, 2006). The effect of human disturbance on grassland is a permanent challenge between human impact and grassland biodiversity (Helm *et al.*, 2009). Human activities, like grazing, seeding and fertilizing, can cause a serious change in grassland biodiversity, aggravating the appearance of invasive weeds (Zimdahl, 2004). Our investigations were focused firstly on the appearance and spreading of common ragweed (*Ambrosia artemisiifolia* L.) in the designated plots. The conclusion was that all types of human activities have increased the ragweed cover in all affected plots (Hoffmann and Pál-Fám, 2012). In present work this investigation was expanded to all plant species indicating naturalness, with respect to disturbance, in order to get a more accurate image of the effect of human activities on grassland biodiversity change and degradation.

Materials and methods

The investigations were made in Bőszénfa (south-west Hungary) in natural and semi-natural grasslands, as well as in grass seeding sites used for deer pasturing. The aim was the comparison of the effects of different human activities on plant communities, with special regard to the appearance and changes in cover of species indicating naturalness, respectively disturbance. In each location, control and affected sampled plots were examined simultaneously over four successive years, with a summer and an autumn sampling per year. Plant cover values were compiled for the four years together for each individual plot. The surveyed plots were designated on the following sites:

(1) *Lolium perenne* grassland, control plot (LPC);
(2) Water pipeline construction plot on *Lolium* grassland (LPW);
(3) Intensive pasturing plot in *Lolium* grassland (LPP);
(4) Silage deposit plot in *Lolium* grassland (LPS);
(5) *Festuca pratensis-Lolium perenne* grassland control plot (FLC);
(6) Manure heap deposit plot in *Festuca-Lolium* grassland (FLM);
(7) *Lolium perenne* shrubby grassland, control plot (LSC);

(8) Deer-feeding plot in *Lolium* shrubby grassland (LSF);
(9) *Festuca arundinacea* grassland, control plot (FAC);
(10) Manure heap deposit plot in *Festuca* grassland (FAM);
(11) *Trifolium pratense-Lolium perenne* seeding made in 2007 (SE1);
(12) *Trifolium pratense-Lolium perenne* re-seeding in 2009 (SE2);
(13) *Trifolium pratense-Lolium perenne* seeding made in 2009 (SE3).

In the case of the three seedings the control plot was LPC. In all 2×2 m plots, the plant species list was recorded and cover percentages estimated for each species with the Braun-Blanquet method. The grouping in the different naturalness categories was made on the basis of Horváth *et al.* (1995). The Ellenberg (1992) moisture category (F) used was also adopted for the Hungarian Flora by Horváth *et al.* (1995). To establish the connection between the species composition of the different plots, classification was used (Jaccard index, Complete Linkage). The data analysis was made with NuCoSA software (Tóthmérész, 1993).

Results and discussion

Species numbers in almost all disturbed plots were higher than in the control plots, because of the appearance of the disturbance indicators (Table 1). This increase is well known in plant ecology, called 'Intermediate Disturbance Hypothesis' (Hobbs and Huennecke, 1992). The cover percentages of the disturbance indicators were also increasing in the disturbed plots, due to the appearance of new niches colonized by these species (Figure 1). Higher naturalness values were observed in the control plots (NI and DT), while the disturbance indicator values were higher in all plots affected by human activities. In the deer-feeding plot (LSF) the intensive trampling by deer decreased the species number, but the disturbance indicator cover was also increasing, similar to the other affected plots. The high naturalness indicator cover in the 2009 seeding is caused by *Trifolium pratense* L. and *Festuca rubra* L.

Table 1. Compilation of the basic data of the investigated plots.

	LPC	LPW	LPP	LPS	FLC	FLM	LSC	LSF	FAC	FAM	SE1	SE2	SE3
Species number	7	14	7	11	15	17	25	15	10	18	9	12	13
Naturalness indicator number	4	5	3	3	5	7	15	5	4	9	5	5	6
Disturbance indicator number	3	9	4	8	10	10	10	10	6	9	4	7	7
Average F-value	4.57	4.57	4.71	4.64	5.13	5.31	5.32	5.07	5.3	5.28	5.11	4.85	5.7

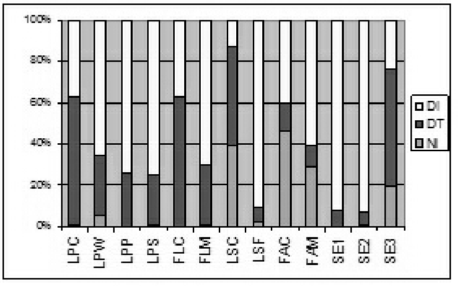

Figure 1. Distribution of the cover of species indicating naturalness (NI), disturbance-tolerance (DT) and degradation (DI) in the plots.

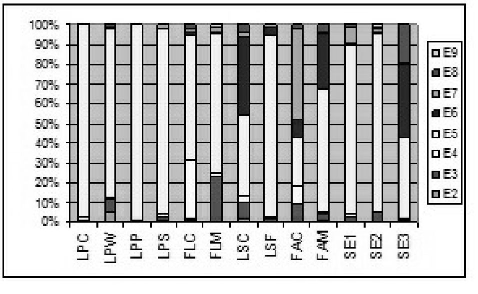

Figure 2. The Ellenberg F values distribution based on cover values in the plots.

The *Lolium* grassland is medium wet (E5), according to the control plot (LPC), based both on species and cover values (Table 1, Figure 2). The affected plots showed similar values, with the exception of the 2009 seeding (SE3). The *Festuca-Lolium* plot (FLC) is also medium wet, with higher moderately wet percentage. The manure-heap deposit does not change this feature (FLM), nor in terms of species composition and cover. The *Lolium perenne* shrubby plot

(LSC) is also medium wet, showing a significant change in cover values in the deer-feeding plot (LSF) resulting the cover increase of the E5 species, parallel with the strong decrease of E3, E4 and E7 species. A very similar tendency can be observed in *Festuca* plot (FAC), after the manure-heap deposit on this habitat (FAM). The first major group of the classification (Figure 3) contains the water pipeline construction plot (LPW), the silage-deposit plot (LPS) and the *Lolium* shrubby plots (LSC and LSF). The main similarity of this group seems to be the intensive trampling. One subgroup of the second group contains the three seedings (SE1, SE2, SE3); in the other are grouped all the other habitats. The *Lolium* control plot (LPC) and the intensive pasturing plot (LPP) are the most similar. The intensity of pasturing has a lower effect than the other disturbances on species composition change. The two manure-heap deposit plots (FLM, FAM) are also grouped together, close to their control plots (FLC, FAC). This means that manure deposit results in similar changes in different habitats, making the possibility of the appearance of nitrophil weeds.

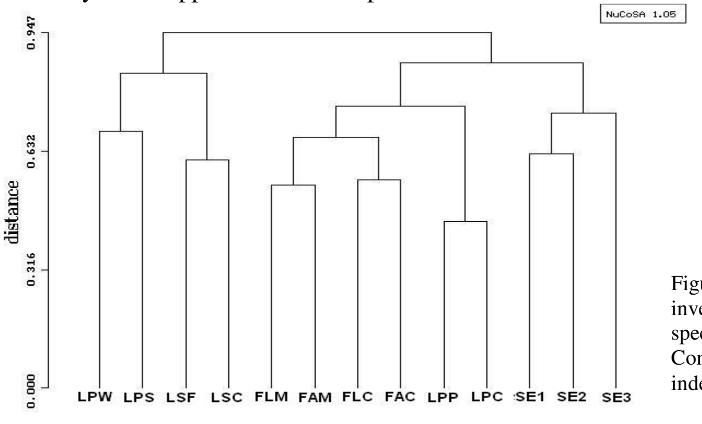

Figure 3. Classification of the investigated plots based on species composition, with Complete linkage and Jaccard-index.

Conclusion

The different human activities have a negative effect on grassland biodiversity, manifesting in species composition as well as in cover values. One of the most important changes is the appearance of the disturbance indicator species, with resulting species-number increase in the disturbed habitats, with parallel cover increase of these species in all investigated habitats. Changes in species composition are closely related to human activities causing trampling, but the effect of the manure-heap deposit is also very important. The negative effects of human disturbance on species cover are manifested in changes in Ellenberg F values of the species cover. Effect of seeding also causes changes in the original species composition and cover.

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Mixed effects of agricultural practices and agro-ecological structures on grassland plant diversity

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Abstract

For a long time, descriptive and experimental studies have shown that agricultural practices affect plant diversity in permanent grasslands. More recently, landscape organization and, more particularly, spatial organization of agroecological structures (AES) was identified as a major factor affecting plant diversity. AES are semi-natural elements, such as hedges, isolated trees, etc. The objective of this study was to evaluate the mixed effects of both agricultural practices and spatial organization of landscape on taxonomic and functional diversity in different French farming systems. We focused on twelve livestock farms distributed along two gradients: i) intensity of agricultural practices, and ii) density of AES on farm territory. In each farm, four types of permanent grasslands were selected in which variables of agricultural practices (date of mowing, livestock rate, fertilization), landscape (AES categories, AES density, AES connectivity) and floristic composition are sampled. Variance partitioning analysis showed that taxonomic diversity is mainly due to landscape variables, like density of hedges and diversity of AES included in a 300 m buffer zone around the floristic survey. The variance of functional indices depended both agricultural practices (livestock and mineral fertilization) and landscape variables such as density of hedges.

Keywords: taxonomic diversity, functional diversity, farming system, landscape, variance partitioning

Introduction

Since the 1970s, intensification of agriculture and simplification of landscapes have been observed. This dynamic in agricultural areas leads to a decrease of the area of permanent grasslands and hedgerows (Cousin, 2009). At the same time, biodiversity, and particularly plant diversity, decrease. Overexploitation of resources and destruction of habitats mainly due to intensification of agricultural practices have been the two main causes that explain this loss (Zechmeister *et al.,* 2003). The destruction of habitats is mainly due to an increase of artificial lands and a decrease of the grasslands area. This destruction of habitats causes changes in heterogeneity, connectivity and landscape fragmentation. These three landscape characteristics affect plant diversity (Burel and Baudry, 1999). In addition, some authors show that species richness increases near the edges of grasslands (Cousins and Aggemyr, 2008). However, no study shows the mixed effects of agricultural practices and landscape fragmentation on plant diversity (Gaujour *et al.,* 2012). In this study, we focus on this mixed effect in two farming systems (dairy cattle and meat cattle). We differentiate the effects on plant diversity in the centre and at the edge of permanent grasslands.

Materials and methods

The study site was in Normandy, eastern France, and included systems of dairy cattle and meat cattle. We selected 12 farms according to their intensity of agricultural practices combined with their density of agroecological structures (AES). The sample consists of four types of systems (BLh: dairy cattle with pasture; BLm: dairy cattle with corn; NEh: meat cattle with pasture; NEm: meat cattle with corn) with a repetition of three farms by type of system. Afterward, four types of permanent grassland were selected in each farms based on the intensity of agricultural practices and the density of AES. In order to describe the permanent grasslands, we focused on agricultural-practice variables (date of mowing, livestock rate...) and landscape variables (AES categories, AES density...). We also sampled floristic composition in two plots per grassland: grassland centre and grassland edge. Each plot consisted on six random quadrats (50×50 cm) in which we recorded the presence of species and their abundance.

We first characterized plant taxonomic diversity by classic indices: species richness, species diversity with Shannon Index. Then we characterized plant functional diversity by functional indices (i.e. functional richness, functional evenness and functional divergence) (Villeger *et al.,* 2008) calculated for strategy of reproduction, from the following four functional traits: seed mass, seed numbers, strategy of regeneration, and type of dispersion. Statistical analyses were performed using R software (R Development Core Team, 2012). Partitions of variance were carried out using the varpart function of package 'vegan' (Oksanen *et al.,* 2012). Canonical Redundancy Analysis (RDA) was performed with the rda function of package 'vegan' in order to determine the main variables involved in diversity. Their relative importance in diversity was obtained by forward selection with forward.sel function of package 'packfor' (Dray *et al.,* 2011) using 1000 permutations.

Results and discussion

Distribution of the Shannon index is asymmetric for the four types of farming system (Figure 1). The functional richness for reproductive strategy of BLh system seems to outweigh the other types of farming system (Figure 2).

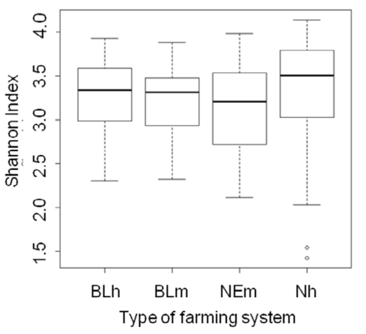

Figure 1. Shannon index in different farming systems (Abbreviations see text). Boxplots indicate interquartile ranges around median with maximum and minimum values.

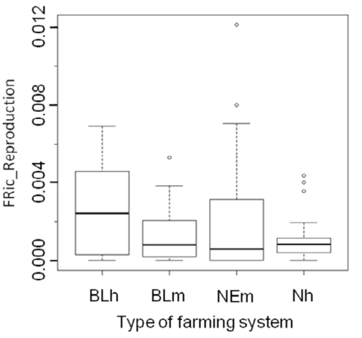

Figure 2. Functional richness (FRic) of the reproduction indexes in different farming systems (Abbr. see text). Boxplots indicate interquartile ranges around median with max. and min. values.

The striking result of our study shows that both landscape and agricultural practices can influence taxonomic diversity and reproductive strategy of permanent grasslands (Figure 3). At the centre of permanent grasslands a higher effect of the landscape (0.20) compared to agricultural practices (0.14) was observed. This effect was identical at the edge of permanent grasslands (Figure 3). Agricultural practices and landscape do not explain a lot of variation for reproductive strategy at the edge of grasslands. On the other hand, agricultural practices better explained this diversity (0.19) than the landscape (0.02), particularly in the centre of

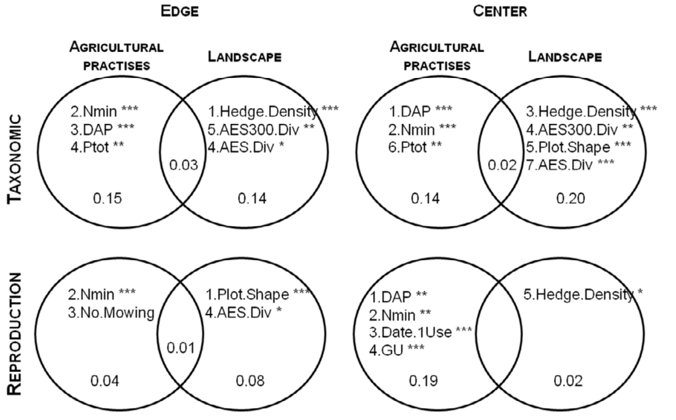

Figure 3. Partitions of variance of taxonomic diversity and of functional indexes of reproductive strategy, in the edge and in the center of permanent grassland. Variables that appear are the most influential (Nmin: Mineral nitrogen quantity; DAP: Day of attendance at pasture; Ptot: Total phosphorus; Hedge.Density : hedgerow density in 300m around grassland; AES300.Div: AES diversity in 300m around grassland; AES.Div: AES diversity of the technical plot; No.Mowing : number of mowing per year; Plot.Shape : form index of the technical plot; Date.1Use : date of first use; GU: grass use index). The star indicates significance level ('***': 0.001; '**': 0.01; '*': 0.05).

grasslands. The main variables are nitrogen fertilization, stocking rate, hedgerow density and AES diversity in 300 m around the plots. These results are consistent with previously reported trends (Gaujour *et al.,* 2012). The effect of agricultural practices on the diversity of reproduction forms is much greater in the centre of the permanent grassland than at the edge. Intensive management, with more fertilization at the centres of permanent grassland can explain this response by plant species. However, no study to date has reported on the effects of mowing and fertilization on functional diversity. Moreover, the presence of hedgerows influences the reproductive capacity of the plant species sampled. Future investigations on the dispersal vectors will be necessary to better understand the role of these agroecological structures. Indeed, the role of barrier-limiting of dispersal could be proposed.

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Vegetation monitoring of extensively cultivated floodplain grasslands in the lower Havel valley, north-east Germany

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Abstract

This paper deals with the effect of extensive management on grassland vegetation in a riverine floodplain area that was predominantly managed intensively before 1990. The analysis is based on vegetation surveys of 61 permanent plots in the floodplain of the Lower Havel River (German Federal State of Brandenburg). We used cluster analysis, regression analysis, correspondence analysis and ANOVA for the statistical evaluation of the data. The vegetation relevés were classified into 5 vegetation groups, which differed greatly in their site-specific characteristics. The average ecological Ellenberg values for nitrogen were negatively correlated to measured values of species diversity. After extensive grassland management extending over 20 years, the highest fodder values were mainly found in plant stands located on moderately moist to weakly wet sites. The historical land use before 1990 significantly affected the actual botanical diversity of the meadows. The findings underline the importance of unploughed and longstanding extensively managed permanent meadows as species pools for the promotion of biodiversity in grasslands that have, historically, been intensively managed.

Keywords: land use intensity, species diversity, extensification, vegetation development

Introduction

Species-rich floodplain grasslands in North Germany have been severely threatened by habitat loss and intensification over the past 50 years (Krause *et al.*, 2011). During this period, broad grasslands of the Lower Havel valley in north-east Germany have been drained and converted into intensive agricultural land with the consequence of species impoverishment (Haase, 1994/95). After 1990, the study area at the Lower Havel was included in programmes of nature conservation because of its great importance as a bird habitat. We installed permanent plots in that area and examined the state of the grassland vegetation based on vegetation surveys after 20 years of extensive use. The analysis allowed us to determine whether different levels of land-use intensity before 1990 influenced the species diversity of the actual grassland vegetation.

Materials and methods

The study area is located north-west of Berlin along the Lower Havel River in the German Federal State of Brandenburg and encompasses approximately 6100 ha, including the polder region Große Grabenniederung and parts of the neighbouring recent Havel River floodplains. This grassland area has been used for agriculture since 1992 on a low-input basis. As part of a monitoring programme, 61 permanent plots (size: 25 m^2) were installed in that area between 1993 and 1999. The historical land use before 1990 was determined through stakeholder interviews in 2000. We prepared vegetation relevés for half of the permanent plots in 2010 and for the other half in 2011. Species abundance was determined using the expanded Braun-Blanquet scale (Mühlenberg, 1989). Based on the vegetation data, the mean indicative Ellenberg values for moisture and nitrogen, and the mean abundance-weighted values for fodder quality according to Briemle *et al.* (2002) were calculated. The diversity measures were the species number and 'the number of species indicating extensive use' (method description in Kaiser *et al.*, 2010). For the statistical analysis, we used (i) cluster analysis, (ii) detrended correspondence analysis (DCA), (iii) regression analysis and (iv) ANOVA. The analyses were carried out with PC-ORD (McCune and Mefford, 1999) (i), CANOCO (Ter Braak and Šmilauer, 2002) (ii) and SPSS 12 (iii / iv).

Results and discussion

The cluster analysis resulted in 5 vegetation groups that represent a wide habitat range. To clarify the ecological differences, the relevès of the groups are presented in ordination diagrams with passive explanatory variables (Figure 1). The mean nitrogen values and species diversity measures are oriented in the opposite direction.

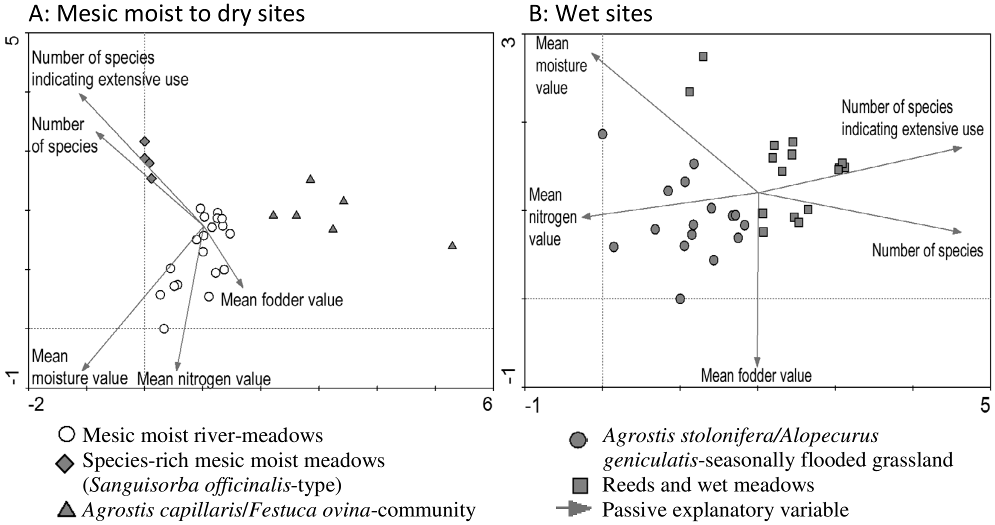

Figure 1. Ordination diagrams (DCA) of the vegetation relevés. The classification of the vegetation groups resulted from a cluster analysis.

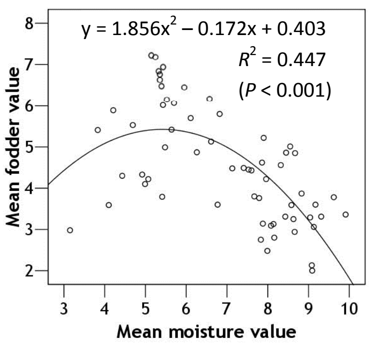

Figure 2. Mean fodder value in relation to the mean moisture value.

Grassland stands of moderately moist to weakly wet sites had the best fodder qualities (Figure 2) because the seeded forage grasses of the former intensive-use period were most abundant at this moisture level. The historical land use before 1990 significantly influenced the number of extensive-grassland species (Figure 3), even though all plots have been managed on a low-input basis for approximately 20 years. The long-standing extensively used meadows, which had not been ploughed historically, showed the highest species diversity by far.

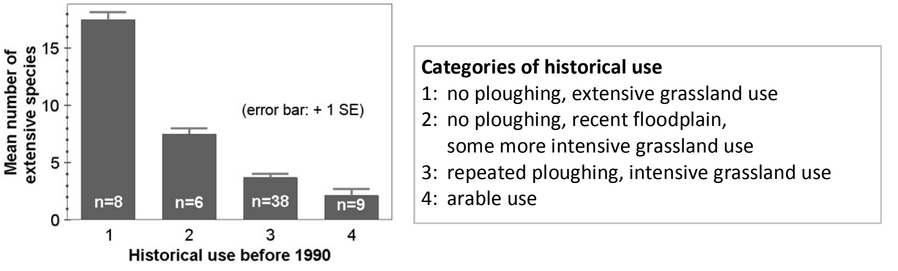

Figure 3. Mean number of extensive species subject to historical land use. The differences are significant (Tukey, $P<0.05$) except between categories 3 and 4.

Conclusions

The results underline the importance of unploughed and long-standing extensively managed permanent meadows for nature conservation. These meadows are species pools for the improvement of biodiversity in neighbouring grasslands that, historically, have been intensively cultivated.

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Management of extensive meadows influencing the distribution of rare plant species

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Abstract

Studies on a population of *Dactylorhiza majalis* in Grodzisk (Sokołow Podlaski district) were carried out in May and June 2007. Most generative specimens of *Dactylorhiza majalis* and numerous individuals of *Epipactis palustris* were recorded in a meadow used as a car park. The other 197 specimens were found in adjacent areas used for farming. The total number of 42 vascular plant species accompanying *D. majalis* was noted in the studied plot. The number of *D. majalis* individuals in the studied areas ranged from 2 to 13. No vegetative specimens were found in the investigated areas. Human activity seems to be the main threat to the studied population. The meadow park is a car park used by people arriving to a neighbouring wild watering place. Another danger can be a possibility of ploughing of the meadow by the owners of the adjacent fields. Expansion and overgrowing of the meadow by *Phragmites australis* is observed.

Keywords: orchids, botanical composition, meadow, management

Introduction

Dactylorhiza majalis (Reinchb.) Hunt et Summerh., like many orchids, is a species vulnerable to extinction because of its very specific requirements. The orchid grows in semi-natural plant communities. Incoming populations restrict the light access and create competition for orchid populations (Bateman *et al*., 2003). A large effect on the group characteristics of the population is the soil moisture, affecting the numbers and structure of the spatial arrangement (Herden *et al*., 2012). *Dactylorhiza majalis* is the most common orchid growing in Poland. However, the species is characterized by a fairly high risk due to changes in meadow management and its intensification (Sporek and Sporek, 2009). The main objective of the current study was to examine the individuals and populations of *D. majalis* and mass distribution of this species in the extensive meadow.

Materials and methods

The study was located on a wet meadow, used for recreational purposes, in Grodzisk village, Sokolow district. According to the physiographic division this area belongs to the province of Mid European Lowlands. The area was randomly divided into 6 differently used squares each of $1m^2$. Analyses of botanical composition were made from fresh material. Typical biometrics for all individuals of *D. majalis* was carried out. A deployment plan of *D. majalis* and *Epipactis palustris* individuals including the development phase was also made. Species lists of vascular plants associated with orchids on the tested surfaces were also compiled. Statistical analyses were performed.

Results

A total of 42 species of vascular plants associated with *D. majalis* were recorded on the study plots. The number of species on those surfaces ranged from 12 to 26. The species associated with the occurrence of *D. majalis* are listed in Table 1.

Table 1. Accompanying species of *Dactylorhiza majalis* (Reinchb.) Hunt et Surnmerh.

Species	Study site					
	1	2	3	4	5	6
Achillea millefolium	+					
Agropyron repens			+			
Agrostis vulgaris			+			
Alchemilla sp.			+	+	+	+
Anthoxanthum odoratum			+	+	+	+
Avenula pubescens				+		
Briza media		+		+	+	+
Carex nigra	+	+		+	+	
Carex hirta	+	+	+			+
Carex pallescens	+					
Carex echinata			+			
Centaurea jacea		+	+	+	+	+
Cerastium holosteoides			+			
Cichorium intybus				+		
Cirsium arvense	+	+	+	+		+
Cirsium rivulare			+			
Cynosurus cristatus				+		
Dactylis glomerata			+	+	+	+
Dactylorhiza mqjalis	+	+	+	+	+	+
Deschampsia caespitosa			+			
Epilobium hirsutum	+					
Epipactis palustris	+					+
Equisetum palustre	+	+	+	+		+
Festuca pratensis	+	+	+		+	+
Galium palustre	+					
Geranium pratense		+	+			
Juncus articulatus	+					+
Lysimachia nummularia			+			
Mentha arvensis		+				
Phleum pratense	+	+	+		+	+
Phragmites australis	+	+				
Plantago lanceolata		+	+	+	+	+
Plantago intermedia			+			+
Poa pratensis	+					+
Poa trivialis	+	+	+			
Potentilla anserina	+	+		+	+	+
Ranunculus acris	+	+	+	+	+	+
Ranunculus repens		+	+			
Rumex acetosa			+	+		
Scutellaria galerieulata	+					+
Stellaria media			+			
Taraxacum sp.			+			
Veronica chamaedrvs		+	+	+	+	
Number of species	18	17	26	16	12	18

Spatial structure (Figure 1) shows the concentrate distribution with the dominance of generative individuals. A similar distribution is presented by Herden *et al.* (2012). The meadow was harvested only one or two times a year. The extensive management of the meadow can protect the specimens of study orchids. Contribution of vegetative individuals in the population of *D. majalis* amounted 18%.

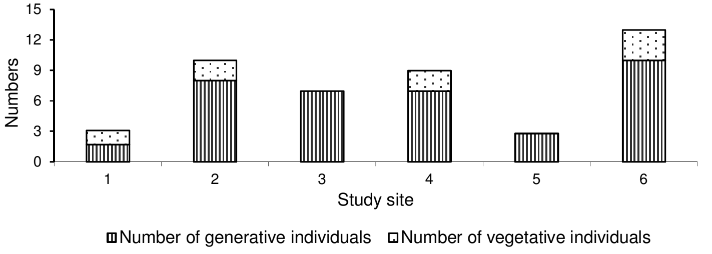

Figure 1. Number of generative and vegetative individuals at each study site.

A small proportion of juvenile individuals may indicate that the population is in regression. Non-flowering plants represent a development stage which precedes the generative stage. In the case of flowering plants, 'vegetative grown' (virginal) stage with three leaves dominated but juvenile stage (single leaf) was not reported. Generative individuals, which share 82%, were characterized by a typical biometrics.

Conclusion

Recreational use of the grassland area where the research was carried out created favourable conditions for the development and prevalence of *D. majalis*. The main threat for the *D. majalis* may be the intense use of this area by people using neighbouring area for swimming.

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Botanical composition and the quality of grazed sward in three groups of habitats

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Abstract

Studies were carried out in grazed grasslands in three groups of habitats: A – dry, B – post-bog and C – riparian. The sward in all habitats was dominated by grasses, or 72.2%, 68.4% and 56.9% in A, B and C, respectively; then by herbs and weeds, rush vegetation and legumes. *Poa pratensis* L. with *Lolium perenn*e L. dominated in habitat A, *Phalaris arundinacea* L. with *Alopecurus pratensis* L. in B, and *Agrostis stolonifera* L., *Glyceria maxima* (Hartm.) Holmb. and *Phalaris arundinacea* L. in C. *Poa pratensis* was present in all habitats and sites. Habitat moisture was determined with the phyto-indication method and qualified to optimum (A and B) and heavily moist or wet (C). Most valuable plant communities (for fodder) were found in habitats A and B, while definitely worse in C.

Keywords: habitats, sward, botanical composition, fodder value score

Introduction

Floristic diversity of plant communities on grasslands has become part of the Common Agricultural Policy since 2013, which highly rank the processes protecting biodiversity. There was reduced grassland utilization in the 2000s due to the abandonment of cattle breeding, especially on small family farms. The study aimed to assess the botanical composition and quality of grazed sward in grasslands in three different, typically Polish, groups of habitats.

Methods and habitats

Studies were carried out in lowland grasslands in the following types of habitats (Grzyb and Prończuk, 1994): A – dry (3 sites), B – post-bog (2 sites) and C – riparian (3 sites). Grasslands in habitats A and B are of anthropogenic origin (managed for at least 20 years). In the study period, grazing animal density was 1.5 to 3 LU ha^{-1}. Grasslands in habitats C are natural and their animal stock is hard to determine since the size of herds and of grazed area varied over time. Botanical composition was determined with the gravimetric method using fresh plant material. Sward fodder value scores (FVS) was calculated with the method by Filipek (1973) which, on a ten-point scale, shows very good value (8-10 points), good (6-7 points), mean (3-5 points) and poor value (below 3 points). Moisture conditions (moisture numbers Lw) in habitats were determined with the phyto-indication method based on indices worked out by Oświt (1992). Studied habitats were classified as follows: A-1, 2, 3, B-2 and C-2: fresh and moist (Lw from 5.2 to 5.3), B-1, C-1 and 3: heavily moist and wet (Lw from 7.2 to 7.5).

Results

Various phytocoenoses developed in studied grasslands, dominated by grasses mainly in habitats A and B (Figure 1). The proportion of grasses was found to decrease with increasing moisture of habitats. Legumes were found only in the sward of habitats A and B. Weed content of swards (with dicotyledons) was relatively large and reached 0.25 of the yield in habitats A and B and less in habitat C. There was a remarkable share of rush vegetation noted in the sward of the latter habitats. Species richness was moderate, being highest in habitat A (20-24 species) and lower in B (13-19 species) and C (13-22 species).

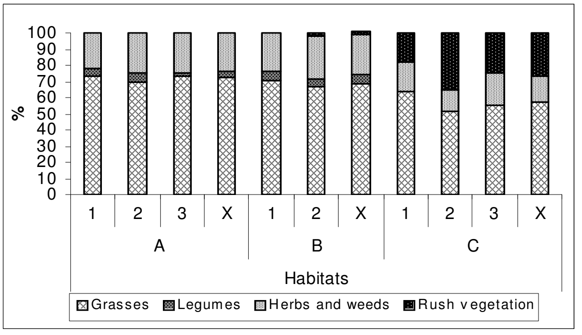

Figure 1. Percentage share of plant groups in the sward.

Poa pratensis L. was present in each habitat. Equally common were *Alopecurus pratensis* L., *Festuca rubra* L., *Festuca pratensis* L. and *Phalaris arundinacea* L. The order of percentage share in the sward was as follows: *P. pratensis*, *P. arundinacea*, *A. pratensis* and *F. rubra*. Dominating grass species (more than 5% contribution) were: In habitat A: *P. pratensis, Lolium perenne* L., *F. rubra* and *Dactylis glomerata* L.; in B: *P. arundinacea*, *A. pratensis* and *P. pratensis*; and in C: *Agrostis stolonifera* L., *Glyceria maxima* (Hartm.) Holmb., *P. pratensis* and *P. arundinacea*. Legumes were present in the sward of habitats A and B. This plant group was represented by *Trifolium repens* L. Most frequent and abundant in group of dicotyledon herbs and weeds were: *Ranunculus repens* L. s. s., *Potentilla anserina* L., *Taraxacum officinale* F. H. Wigg., *Achillea millefolium* L. and *Urtica dioica* L. The sward of habitat A was dominated by *T. officinale*, *A. millefolium* and *R. repens*, that of habitat B by *R. repens* and in C *P. anserina*. Rush vegetation was found only in habitat C apart from a small share of *Carex sp.* in site B-2. *Carex sp.* and *Acorus calamus* L. were noted in all sites of habitat C (Table 1).

Sward fodder value scores were differentiated. In sites of habitat A the sward fodder value score was 7.2 points (from 6.5 to 8.3), sites in habitat B the score was lower by 0.3 points and those in habitat C by as many as 3.1 points. The highest number of species of very good and good value was found in the sward of habitat A (11-12 species), habitat B had 6-9 such species and habitat C 4-5 species. In habitat A these species constituted 71% of yield on average (from 45 to 86%). In habitat B their contribution was 77% (from 73 to 82%) and in habitat C only 24% (from 23 to 25%).

Conclusions

Permanent grasslands are especially valuable fodder resources and sites of biodiversity. Unfortunately, large grassland areas are excluded from production and from any other use. Their maintenance is seen in grazing cattle. These animals eat various plant species and, due to their size and mass, largely affect the sward and entire habitat. Utility value of the sward indicated that most valuable (for fodder) plant communities occupied habitats A and B (good value) and less valuable were found in habitat C (of mean value).

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Table 1. Botanical composition of grazed sward (%).

Species	Sites A				Sites B			Sites C			
	1	2	3	mean	1	2	mean	1	2	3	mean
Poa pratensis L.	28.7	16.7	24.3	23.2	16.8	20.0	18.4	6.8	13.3	15.0	11.7
Festuca pratensis L.	4.0	7.0	0.3	3.8	-	1.7	0.9	-	1.7	-	0.6
Festuca rubra L.	0.7	2.3	37.7	13.6	0.6	+	0.3	-	-	-	-
Dactylis glomerata L.	8.3	6.0	1.3	5.2	-	2,3	1.2	-	-	-	-
Phleum pratense L.	0.7	1.7	0.7	1.0	-	+	+	-	-	-	-
*Agropyron repens (*L.) Beauv	5.0	3.7	1.3	3.4	-	-	-	-	-	-	-
Alopecurus pratensis L.	-	+	0.7	0.2	4.3	36.0	20.1	5.0	1.7	6.7	4.5
Alopecurus geniculatus L.	-	-	0.3	0.1	-	-	-	1.7	-	-	0.6
Lolium perenne L	21.7	32.0	0.3	18.0	-	-	-	-	-	-	-
Agrostis stolonifera L.	-	-	-	-	-	-	-	33.4	3.3	5.7	14.1
Phalaris arundinacea L.	1.7	-	-	0.6	48.4	6.7	27.5	13.4	3.3	-	5.6
Deschampsia caespitosa (L.) P. Beauv.	-	-	-	-	-	-	-	-	-	+	+
Arrhenatherum elatius (L) P. Beauv. Ex Presl et C. Presl.	-	-	3.0	1.0	-	-	-	-	-	-	-
Holcus lanatus L.	-	-	1.6	0.5	-	-	-	-	-	-	-
Bromus mollis L.	-	0.3	-	0.1	-	-	-	-	-	-	-
Festuca arundinacea L.	-	-	-	-	-	-	-	-	-	1.7	0.6
Agrostis gigantea L.	2.3	-	2.3	1.5	-	-	-	-	3.3	1.0	1.4
Glyceria maxima (Hartm.) Holmb	-	-	-	-	-	-	-	3.4	20.1	18.3	13.9
Glyceria fluitans (L.) R. Br.	-	-	-	-	-	-	-	-	5.0	6.7	3.9
Phragmites australis (Cav.)	-	-	-	-	-	-	-	-	+	-	+
Trifolium repens L.	5.0	5.3	1.7	4.0	6.0	5.0	5.5	-	-	-	-
Trifolium pratense L.	-	0.3		0.1	-	-	-	-	-	-	-
Trifolium hybridum L.	-	0.3		0.1	-	-	-	-	-	-	-
Taraxacum officinale F. H. Wigg.	2.3	8.3	5.3	5.3	3.3	+	1.6	-	-	+	+
Achillea millefolium L.	2.3	3.7	3.7	3.2	3.0	1.3	2.2	-	-	-	-
Plantago media L.	-	-	-	-	-	0.3	0.1	-	-	1.0	0.3
Rumex crispus L.	+	-	-	+	-	-	-	0.4	0.3	-	0.3
Ranunculus repens L. s. s.	6.7	3.0	5.0	4.9	11.0	17.7	14.4	-	1.7	1.7	1.1
Rumex obtusifolius L.	-	0.7	-	0.2	-	-	-	1.4	-	2.3	1.2
Potentilla anserina L.	-	+	0.3	0.1	-	0.7	0.3	10.4	5.0	7.7	7.7
Cirsium arvense (L.) Scop.	5.7	2.3	-	2.7	-	-	-	-	-	-	-
Lamium purpureum L.	-	0.7	-	0.2	-	-	-	-	-	-	-
Anthriscus sylvestris (L.) Hoffm.	2.3	3.0	-	1.8	-	-	-	-	-	-	-
Ranunculus acris L. s. s.	-	-	-	-	-	-	-	-	-	0.6	0.2
Heracleum sphondylium L.	+	0.7	-	0.2	-	0.3	0.1	-	-	-	-
Urtica dioica L.	0.7	1.0	-	0.6	0.7	1.7	1.2	-	-	3.3	1.1
Glechoma hederacea L.	1.3	0.7	-	0.7	0.3	-	0.1	-	-	-	-
Rumex acetosa L.	0.3	0.3	9.0	3.2	-	-	-	-	-	-	-
Cerastium holosteoides Fr. em. Hyl.	-	-	0.6	0.2	-	-	-	-	-	-	-
Veronica chamaedrys L.	0.3	-	-	0.1	-	-	-	-	-	-	-
Stelaria graminea L.	-	-	0.6	0.2	-	-	-	-	-	-	-
Capsella bursa-pastoris (L) Medik.	-	-	-	-	+	-	+	-	-	-	-
Cardamine pratensis L. s.s.	-	-	-	-	4.3	2.6	3.5	-	1.7	-	0.6
Leontodon autumnalis L.	-	-	-	-	1.3	2.0	1.2	-	1.7	1.7	1.1
Polygonum persicaria L.	-	-	-	-	-	+	+	+	-	-	+
Polygonum amphibium L.	-	-	-	-	-	-	-	5.7	2.3	1.7	3.3
Mentha aquatica L.	-	-	-	-	-	-	-	-	0.3	-	0.1
Epilobium palustre L.	-	-	-	-	-	-	-	-	-	+	+
Carex sp.	-	-	-	-	-	1.7	0.8	5.0	13.3	16.6	11.6
Juncus conglomeratus L. em. Leser	-	-	-	-	-	-	-	-	0.7	1.0	0.6
Acorus calamus L	-	-	-	-	-	-	-	13.4	9.3	5.6	9.4
Typha latifolia L.	-	-	-	-	-	-	-	-	12.0	1.7	4.5
Number of species	21	24	20	22	13	19	16	13	20	22	18
Total number of species		33				20			30		

Changes in floristic composition of the mountain pasture sward after the abandonment of sheep grazing

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Abstract

Grassland ecosystems play many important functions in the environment, e.g. in shaping landscape, which is particularly visible in the richness of species. Abandonment can lead to impoverished floristic diversity. The aim of our study was to compare floristic composition and species diversity in mountain pastures, both grazed and abandoned. The study was located in the Jaworzyna Krynicka Range, at 600 and 750 m a.s.l. It was divided into two parts, the first one was carried out in 2005 in cultivated pastures and it was a starting point for the comparison of such pastures after five years (in 2010), during which part was continuously utilized and some was abandoned, due to the decreased number of sheep. Five years after sheep grazing stopped the species richness of vascular plants had decreased, and dominance of a few expansive species was visible; e.g. *Deschampsia caespitosa* at 750 m a.s.l, and *Juncus effuses* and *Mentha longifolia* at some lower locations. Maintaining of utilization by grazing of such habitats has affected them positively, by sustaining a greater number of species.

Keywords: mountain pasture, floristic composition, pasture abandonment

Introduction

Meadows and pastures play numerous functions in the environment, including climatic, hydrological and shaping the landscape. Landscape values of grassland ecosystems largely depend on the biodiversity, which includes the range of plant species and also the diversity of plant associations. Grassland ecosystems are types of habitats where many different species often occur in small areas, and are therefore very valuable (Zarzycki and Wróbel, 2004). Maintaining this biodiversity depends on many factors, both natural and anthropogenic (Trąba, 1999). According to Kasperczyk and Szewczyk (1999), ceasing of pasturage in grassland ecosystems can lead to impoverishment of species composition, through the domination of one, or a few, expansive species and the loss of others. Abandonment also results in increasing erosion in such places. Sheep grazing may therefore be valuable for the environment, but nowadays it is limited because of the low profitability of small-scale livestock production (Musiał, 2004). However, the best form of active protection of areas covered by pastures, both in lowland and mountain areas, is maintaining the grazing by specific species of farm animals (Pławska-Olejniczak and Żywiczka, 2009).
The aim of the study was to compare the floristic composition of a mountain pasture sward, grazed by sheep, and after 5 years of abandonment.

Material and methods

The research was carried out in the Polish Carpathians on mountain pastures in the Jaworzyna Krynicka Range, at altitudes of 600 and 750 m a.s.l. There were 6 pastures included in the study, each about 1.5-2 hectares. Until 2005 all pastures were grazed by flocks of sheep. For the subsequent 5-year period, grazing was stopped on about 70% of this area due to a decrease in number of available animals. Analysis of the floristic composition was made by the Klapp Assessment Method (1962) and included 5 plots for each of 6 types of pastures, that were grazed (2005) and both grazed and abandoned (2010), at two altitudes above sea level. According to this method, the sward of pastures was divided into three quality groups: grasses (*Poaceae*), legumes (*Fabaceae*) and other vascular plant species, which included herbs and weeds. The percentage cover for those groups showed how the proportions changed at both altitudes, and depending on type of utilization.

Results and discussion

At the initial stage of the research, in 2005, analysis of the grazed pastures at both altitudes showed favourable species composition, which indicates a great diversity of plant species (Table 1). Some differences in number of species have been noted between the pastures at altitudes of 600 and 750 m a.s.l., regardless of the year of the study. At higher altitudes, there was a slightly smaller number of vascular plant species. In grazed pastures at 750 m, the mean value for 2005 and 2010 was 32 (pastures 2 and 4); on the other hand, in abandoned pastures the mean number was 20 (pasture 6). After 5 years of abandonment, the degradation of the mountain pasture sward became visible. This was manifested by the dominance of a particularly aggressive grass species, *Deschampsia caespitosa*, at both altitudes even though it was more dominant at the higher altitude. This led to replacement of other grasses but also legumes and other species including herbs and weeds. Floristic composition of abandoned pasture was reduced on average to 23 species at 600 m (Pasture 5). On the other hand, maintaining grazing resulted in greater plant diversity; the mean number of species was thus 33 in Pasture 3.

Table 1. The most frequent species in utilized and abandoned pastures (cover in %).

Year	2005		2010			
Type of use	Utilized				Abandoned	
Pasture	1	2	3	4	5	6
Altitude	600 m	750 m	600 m	750 m	600 m	750 m
Name of the species	Klapp Assessment Method					
Grasses (*Poaceae*)						
Festuca rubra	20	18	19	16	10	8
Cynosurus cristatus	15	12	15	10	2	+
Festuca pratensis	15	10	12	10	7	5
Lolium perenne	15	8	10	8	5	3
Poa pratensis	10	8	10	7	2	+
Poa trivialis	7	7	6	5	-	-
Dactylis glomerata	5	3	4	3	-	-
Agrostis vulgaris	1	1	1	+	-	-
Deschampsia caespitosa	1	1	+	2	45	50
Legumes (*Fabaceae*)						
Trifolium pratense	5	1	5	2	+	-
Trifolium repens	15	10	12	10	+	-
Herbs and weeds						
Alchemilla pastoralis	7	5	5	5	2	1
Ranunculus acris	3	1	2	+	+	-
Stellaria graminea	2	+	1	1	+	-
Cirsium arvense	1	+	+	+	5	15
Juncus effusus	1	-	1	+	15	2
Mentha longifolia	1	+	+	+	15	2
Plantago lanceolata	2	1	1	1	+	-
Potentilla anserina	1	+	1	1	+	-
No of species	35	32	33	32	23	20

Between the clusters of *D. caespitosa* there were visible bare soil surfaces, which may be unfavourable due to destructive action of water erosion. Moreover, at the altitude of about 600 m, after a 5-year period of abandonment, *Juncus effusus* and *Mentha longifolia* became quite dominant (Pasture 5). At the altitude of about 750 m, there was a large coverage of *Cirsium arvense* (Pasture 6). Comparisons of the utilized pastures showed that in 2005 and 2010, at both altitudes, there was visible similar floristic composition.

It is of interest that there was a greater coverage of the dicotyledons in abandoned pastures at altitude of 600 m, whereas lack of grazing at the higher altitudes led to the grasses dominating. Valuable legumes had insignificant coverage after ceasing of pasturage (Table 2). *Trifolium repens* was one of the species from this group that was more abundant in grazed pastures; similar findings to the studies done in mountainous region in Czech Republic (Hejcman *et al*., 2004). On the utilized pastures there occur not only a greater number of plant species, but also of botanical families, compared with abandoned pastures.

Table 2. The mean proportion of some quality groups in pastures: G - grasses, L – legumes, HW – herbs and weeds and number of families in different types of pastures in 2005 and 2010.

Altitude		600			750	
Groups of pasture sward	G	L	HW	G	L	HW
Utilized pasture						
Mean coverage (%)	75	10	15	80	8	12
No of families		13			11	
Abandoned pasture						
Mean coverage (%)	64	1	35	85	1	14
No of families		7			6	

Conclusions

Results of analysis show that abandonment can lead to a decrease in floristic diversity, through a reduction of the number of species and families at both 600 m and 750 m altitude. These negative changes are more visible in pastures at higher altitudes, where there is a smaller species composition, than in pastures situated at lower elevations. Maintaining utilization is particularly important in mountain areas, on a large acreage, since it determines the preservation of biodiversity and keeps its natural functions.

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Impact of abandonment on the floristic composition of permanent grassland and grassland created on former arable land

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Abstract

In 1995, permanent plots were established in the Babia Góra mountains (Polish Carpathians) in order to compare the rate and direction of plant composition change in cut and uncut meadows of different origin. One plot was situated on permanent grassland and the second on grassland created by spontaneous succession on former arable land. In both situations the lack of land management caused the establishment of trees and shrubs, which covered almost 50% of the abandoned plots after 17 years. On permanent grassland the differences in herbaceous plant composition among the cut and uncut plots were smaller than on former arable land. In 2011 the occurrence of herbaceous plant species was similar in both treatments, although there was a big difference in terms of their abundance. On grassland created on former arable land the difference in species composition in cut and uncut areas was observed from the first year of the experiment and persisted until 2011. The research findings indicated the impact of management and initial habitat conditions on the floristic composition of mountain meadows.

Keywords: mountain grassland, botanical composition, management, succession

Introduction

Meadow communities are greatly dependent on the direct and indirect effects of human activities. Changes in management practice can cause transformations in the floristic composition of these communities. The rate of reduction of meadow species and the appearance of species that are representative of subsequent phases of succession can vary, depending on habitat conditions. Knowing the rate and direction of changes in uncut grasslands is important for making decisions concerning the protection of multi-species plant communities (Pavlu *et al*., 2011), and their restoration potential. This is of particular significance in protected areas, where the grasslands have a role in conserving biological diversity and are not always associated with the use of the sward as a source of fodder. The objective of this study was to compare the rate and direction of changes in botanical composition of cut and uncut sward on permanent grasslands and on former arable land.

Materials and methods

Permanent experimental plots were established in 1995 on the Gubernasówka situated at 910 m a.s.l., within the Babia Góra Mountains National Park. The substrate is formed by leached brown soils, with acidity pH_{H2O} of 4.7, available phosphorus content (determined by Egner-Riehm method) of 15 mg kg^{-1} and potassium of 98 mg kg^{-1}. The long-term annual average amount of precipitation is 1190 mm per year, and the average temperature is 4.9°C (Obrębska-Starklowa, 1983). One plot was situated in a meadow that had emerged from self-seeding of a surface formerly used as arable land for several years. Its sward comprised mainly common bent (*Agrostis capillaris*) with minor proportions of other species. The second plot was under extensive cutting management (no fertilization), and had been used sporadically as a pasture. A community with a predominance of imperforate St. John's-wort (*Hypericum maculatum*), and species typical of poor habitats such as mat grass (*Nardus* *stricta*) and Lachenal's hawkweed (*Hieracium lachenalii*), occurred there. On both sites, two plots were delineated, each with an area of 100 m^2. One was cut each year, whilst the other was left uncut. The species composition of the sward was evaluated annually on the whole plots using the Braun-Blanquet scale. Changes in species composition over time were analysed with detrended correspondence *analysis (DCA*) using CANOCO software (ter Braak and Smilauer, 2002).

Results and discussion

Species composition changed in all plots, both cut and uncut (Figure 1). On the former arable land the course of change was strongly correlated with years of the experiment. A comparable impact of the duration of meadow management on the species composition has also been observed by Waesch and Becker (2009). As early as in the second year there was a differentiation of species composition between the cut and uncut plots, and these differences were maintained throughout the period of the study. After several years, some tree species were found on uncut plots: chiefly goat willow (*Salix caprea*) and common spruce (*Picea excelsa*), which covered over 40% of the plot area after 17 years. Among herbaceous species, the highest coverage was attained by wood softgrass (*Holcus mollis*), and also by certain tall-herb type species such as imperforate St. John's-wort and lady's mantle (*Alchemilla monticola*). The proportions of common bent, as well as some minor species such as cat's ear (*Hypochoeris radicata*) decreased markedly. On the cut plots, the abundance of species typical of low-fertile habitats, e.g. red fescue (*Festuca rubra*), increased, and even species typical of poor habitats appeared, such as mat grass.

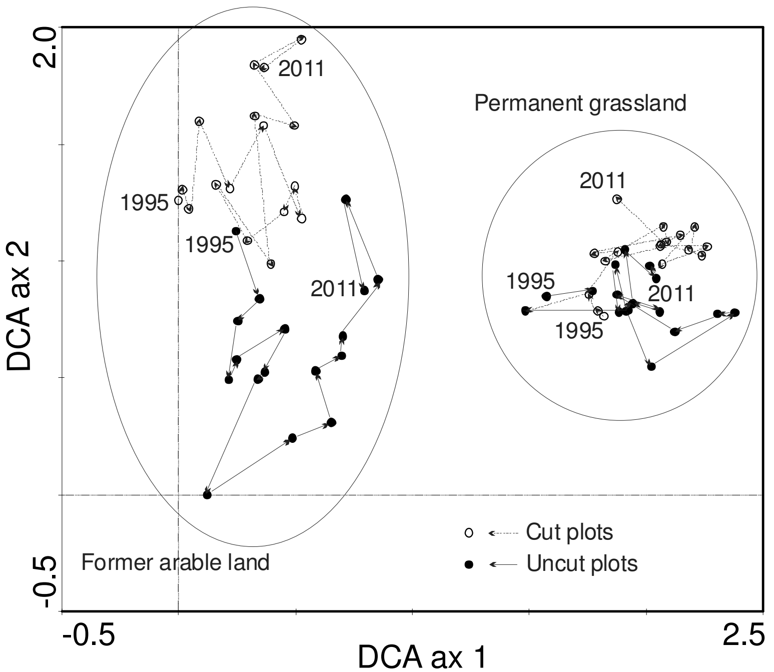

Figure 1. Changes in plant species composition of cut and uncut plots from 1995 until 201 as revealed by detrended correspondence *analysis (DCA).*

On the permanent grassland the changes in floristic composition in cut and uncut plots over the whole study period were markedly smaller than on the former arable land (Figure 1). The principal difference was in the intensive development of woody plants on uncut plots, such as spruce and rowan (*Sorbus aucuparia*), as well as red raspberry (*Rubus idaeus*). On this plot there was also a marked increase in the proportion of bilberry (*Vaccinium myrtillus*). Cutting resulted in increased proportions of species typical of poor meadows such as mat grass and tormentil (*Potentilla erecta*), and with decreases in the proportion of species vulnerable to cutting, e.g. imperforate St. John's-wort and blackberry. The species compositions on both plots were similar after 17 years of the experiment, although the majority of herbaceous species only covered small areas on the uncut plot. These results are in agreement with the results of a similar experiment carried out in the Czech Republic by Pavlu *et al.* (2011), but in their experiment there was no increase in the development of woody vegetation. In subsequent years of the experiment, certain fluctuations were observed in the species composition and proportions of particular species groups, irrespective of grassland management or its absence. The year 2003 was particularly atypical with regard to changes in meteorological conditions, when the development of most plants was inhibited and some grasses almost disappeared, which in turn enabled the development of woody plants on the uncut plots. Variability in the abundance of particular species and its dependence on weather conditions is often observed in meadow communities (Stampfli, 1992).

Conclusions

The rate and direction of changes in species composition is a product of many factors. The essential factor was the initial character of the community, as relatively stable meadow communities (under the same type of management for many years) were more resistant to succession-related changes after the management practice has been discontinued, than those emerging on former arable lands. Random factors, such as the course of weather conditions, could modify the course of succession to a significant degree.

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Changes in habitats and plant communities in reclaimed peatland meadows in relation to groundwater level

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Abstract

In the years 1991-2011 the groundwater level, mineralization of organic nitrogen, the content of nitrate- and ammonium-nitrogen, and changes in habitat moisture and meadow plant communities were studied in 9 permanent sites located on organic soils in central Poland. The aim of this study was to assess mineralization of organic nitrogen compounds and the changes taking place in organic soils and meadow communities due to the elevation of groundwater table. High groundwater level limited the actual mineralization of organic nitrogen compounds and the content of N-NO_3. The re-colonization by communities of the class *Scheuchzerio-Caricetea nigrae* and *Phragmitetea* was observed.

Keywords: groundwater level, organic matter mineralization, habitats, communities

Introduction

Reclaimed bog areas in central Poland were managed and used in the 1960s. Meadow communities of the class *Molinio-Arrhenatheretea* developed there. They were utilized as meadows and pastures. The abandonment of the maintenance of reclamation facilities in the beginning of the 1990s led to uncontrolled water outflow from river valleys. The degradation of organic soils intensified the expansion of shrubs or *Phagmites australis* (in wetlands). In the spring, stretches of rivers fed water from adjacent areas and the overgrown ditches could not channel the excess water. The groundwater level elevated and finally flooded the meadows. The elevation of groundwater level in the spring sections of streams caused secondary bogging of reclaimed bogs and the recovery of species typical of wet and bog habitats (Kozłowska *et al*., 2012). Long-term actions such as limitation of uncontrolled water outflow from the river valley, increasing groundwater level and mitigation of its fluctuations are needed. Such actions should increase water retention, protect soil organic matter, improve biomass production in the river valley and accelerate the recovery of bog vegetation to their former habitats. The aim of this study was to assess mineralization of organic nitrogen compounds, and the changes taking place in organic soils and meadow communities due to the elevation of groundwater table, finally to flood of the meadows.

Study area and methods

Studies were carried out in central Poland in the years 1991-2011 on reclaimed peatland meadows. Kind of soil, groundwater level (in two terms: the beginning of June and the turn of July and August), bulk density and organic matter content in the 0-20 cm soil layer were determined in 9 representative sites in the years 1995 and 2010. Mineralization of organic N compounds according to the ETH method modified by Frąckowiak (1980), was estimated in 1995 and the content of nitrate- and ammonium-nitrogen in soil extract (colorimetric method with the use of flow-through analyser after extraction of fresh soil samples with 1% K_2SO_4) in 2011. Botanical composition was determined with Klapp's method.

Results and discussion

Studied grasslands were situated on organic soils (Table 1) which differed in the type of peat (alder, moss, rush), the degree of decomposition, depth, silting, organic matter content, thickness and the degree of transformation of muck layer (Kozłowska and Frąckowiak, 2011). Different soil conditions were further differentiated by soil moisture determined by the depth of groundwater table. The depth varied seasonally and among study years (Figure 1). In the beginning of this study, differentiation of the groundwater table depth was smaller. Reclamation facilities were maintained and grasslands were mown or grazed. In sites situated in the spring (5, 4, 8) and in the flooded (7) part of the valley, groundwater table increased in the 1990s. The elevation of groundwater table extended, though to a lesser degree, over sites 6, 9, 2 and 3 in the 2000s with marked variability of groundwater depths. In 2009 and 2011 groundwater table declined due to sporadic maintenance of basic reclamation ditches.

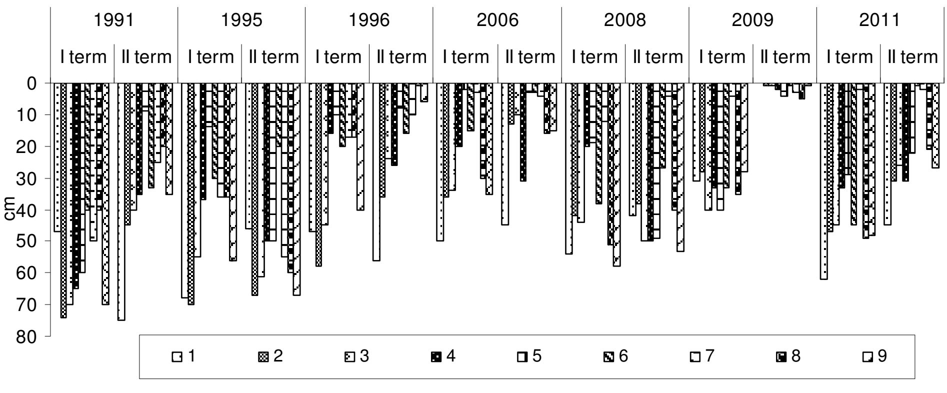

Figure 1. Groundwater level in the study years (cm) determined in two terms: the beginning of June and the turn of July and August.

Table 1. Characteristic and selected properties of soils in nine reclaimed grassland sites.

Site	Soil type	Bulk density (kg dm^{-3})	Organic matter content in 0–20 cm soil layer (%)		Mineralization of organic N (kg ha^{-1} yr^{-1})	
			1995	2010	Po[1)]	Rz[2)]
1	Mt_nIIc_ne (Ol)	0.78	21.4	16.2	385	124
2	MtIIc2 (Ol)	0.25	63.7	54.4	296	119
3	MtIac (Me)	0.21	79.5	73.5	225	90
4	Mibc (Me)	0.17	83.4	77.6	431	92
5	MtIa1 (Me)	0.18	86.7	86.5	258	40
6	MtIIcc (Szu)	0.28	53.6	52.5	352	69
7	MtIIcc (Ol)	0.25	62.6	52.4	464	148
8	Mr11 (Me)	0.24	55.1	43.0	218	71
9	Mr11	0.71	20.1	15.0	443	124

[1)]Po - potential – under optimum conditions, [2)]Rz – actual – under natural conditions

Groundwater table depth determines mineralization of organic N compounds and the release of mineral forms of nitrogen (Frąckowiak, 1980). Actual mineralization of organic N (under natural conditions) was highest in flooded site (no 7; 148 kg N ha^{-1} yr^{-1}) with large variations of groundwater level in the growing season and in sites with the lowest levels of groundwater (no 1, 2, 9). Moistening and drying of the soil accelerates mineralization of organic N (Frąckowiak, 1980). In other sites with higher groundwater table, mineralization was smaller (71-92 kg N ha^{-1} yr^{-1}). The smallest mineralization (40 kg N ha^{-1} yr^{-1}) was on the site with highest groundwater table. Mineralization rate did not depend on the content of organic matter in soil. Potential mineralization (under optimum conditions) was high in all sites; c. 5-6 times higher than the actual in sites of the highest groundwater level (no 4, 5, 6) and 2-3 times higher in sites of lower groundwater table and/or at its large variability. These results indicate large possibilities of N release under optimum conditions (Frąckowiak, 1980).

The content of $N\text{-}NO_3$ and $N\text{-}NH_4$ in soil extract in the autumn 2011 was differentiated and always higher in 0-10 cm soil layer (Table 2). In sites with the lowest ground water level the concentration of $N\text{-}NO_3$ was high and varied from 10 to 37 mg dm^{-3} in both soil layers (sites 1 and 2), in soils formed from moss peat, at similar groundwater level, these concentrations slightly exceeded 10 in the upper and 6 mg $N\text{-}NO_3$ dm^{-3} in the lower soil layer (sites 3 and 4). Moss peatlands are characterized by stable hydrological conditions which inhibits the mineralization of organic matter. In other sites with higher ground water levels the concentrations were low (0.86-3.14 mg $N\text{-}NO_3$ dm^{-3}). Concentrations of $N\text{-}NH_4$ were low and less differentiated (0.08-4.53 mg $N\text{-}NH_4$ dm^{-3}) being usually higher in sites with higher ground water level. In site 5 with the highest and less variable ground water levels, the actual mineralization of organic N, the content of $N\text{-}NO_3$ and the decrease of organic matter content (in 1995-2010) were smallest. The content of $N\text{-}NH_4$ in this site was higher than in sites with low ground water level. Ammonification takes place in aerobic conditions and less intensively in anaerobic. In this site the high groundwater level protected the soil from loss of organic matter. The greatest decline of organic matter content was found in sites with a high variability of groundwater level in the beginning of this study (site 7), and that had a thin organic layer (site 8).

Table 2. The content of nitrate- and ammonium-nitrogen in soil.

Site	Content [mg dm^{-3}]			
	$N\text{-}NO_3$		$N\text{-}NH_4$	
	Layer [cm]			
	0–10	10–20	0–10	10–20
1	14.83	10.64	0.96	0.50
2	37.21	20.56	1.14	0.65
3	10.00	6.66	3.57	2.16
4	13.55	6.44	1.85	0.64
5	1.07	0.86	1.16	0.87
6	1.21	1.31	1.04	0.49
7	1.23	1.07	3.57	2.63
8	2.52	1.24	4.53	0.32
9	3.14	1.54	0.13	0.08

Communities of *Molinio-Arrhenatheretea* were present in all sites in the beginning of the study. At five sites they became dominated by *Deschampsia caespitosa* with a gradually increasing contribution of sedges. In other sites the elevation of groundwater level resulted in the transformation of meadow communities that were then attributed to class *Scheuchzerio-Caricetea nigrae* (site 5) or *Phragmitetea* (sites 6, 7, 8) (Kozłowska *et al*., 2012). Protected species *Saxifraga granulata* and *Ostericum palustre* appeared in the communities. Most sites were not used but there was no expansion was observed of shrubs or *Phagmites australis*.

Conclusion

High groundwater level limited the actual mineralization of organic nitrogen compounds, the content of $N\text{-}NO_3$ and the loss of organic matter, despite a great susceptibility of organic soils to these processes as evidenced by a high potential mineralization. At high soil moisture, former bog habitats were re-colonized by communities of the class *Scheuchzerio-Caricetea nigrae* and *Phragmitetea*. Protected species were also noted. Species, habitat and landscape diversity increased which is beneficial for both economic and biological reasons.

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The Icelandic highland communal grazing areas

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Abstract

The interior highland communal grazing areas in Iceland extend to about one third of Iceland. with limited erosion problems to nearly barren desert areas characterized by severe erosion. Deserts and severe erosion is common within the volcanic zone. The extent of land with extreme erosion severity is comparable to the driest deserts on Earth, in spite of Iceland's cold and humid climate. There is a need for protection from grazing of commons that are in poor condition.

Keywords: land use, erosion, deserts, volcanism

Introduction

Grazing is one of the fundamental uses of Icelandic natural resources. Cattle and most of the horse population are grazed within confined lowland grazing pastures. The Icelandic highlands, however, are grazed by sheep, together with various species of wild birds, predominantly geese and swans. There are currently about 475,000 winter-fed sheep in Iceland, including 374,000 ewes, producing, on average, about 25 kg lamb meat ewe^{-1} yr^{-1} (Icelandic Farmers Association, www.bondi.is). The sheep roam freely in both highland and lowland private and communal grazing areas, from about late June to early September. In this review we outline the characteristics of the central highland communal grazing areas based on recently established national surveys and databases on soil erosion and vegetation cover.

Materials and methods

We surveyed 45 highland communal areas, covering about 31,200 km^2 (nearly one-third of Iceland) all commons lying within the central (interior) highlands. The Westfjord and Snaefellsnes peninsulas and communal areas south of the Vatnajökull and Mýrdalsjökull glaciers were excluded from this survey as these commons are not used for, or have been protected from, grazing. Data on soil erosion was extracted from the 'Soil Erosion in Iceland' (SEI) database maintained by the Agricultural University of Iceland (AUI) (Arnalds *et al.*, 2001). The AUI Farmland Database (Nytjaland) was used to obtain additional information on erosion and vegetation cover for selected communal areas.

Results and discussion

The highlands have a sub-Arctic climate characterized by cool summers with temperatures (in June, July and August) ranging from 6 to 10°C (Einarsson, 1984) and with annual precipitation of <500 mm to >1500 mm for North and South Iceland, respectively (Crochet *et al.*, 2007). The interior highlands are intersected by the North Atlantic Rift Zone, with frequent volcanic eruptions. Soils of the highlands can be divided into four main categories: wetland Andosols (Gleyic Andosols), dryland Andosols (Brown Andosols) of vegetated areas, Cambic Andosols and Sandy Andosols of desert areas (Arnalds and Óskarsson, 2009). The highland areas are subjected to intense cryoturbation due to frequent freeze-thaw cycles (Orradottir *et al.*, 2008), while permafrost is only sporadic.

The size of the communal areas ranged from 75 km^2 to >1500 km^2 with an average of 693 km^2. The largest commons were in Northeast Iceland and the smallest ones in West Iceland. On average, about half of the areas were barren or with poorly vegetated mountains (Table 1). Erosion was most severe within the volcanic zone in the South and Northeast, whilst severe erosion was reported in <10% of the commons in the West and Northwest, outside of the volcanic zone. Table 2 shows soil erosion and vegetation cover from ten selected highland commons. The vegetation cover of individual commons varied from <10% to >90%. The lack of vegetation cover in many of the communal areas is striking. Areas of severe erosion (classes 4 to 5 on a scale of 0 to 5) within the commons range from being almost absent to a cover of over half of the land. This level of extent of land with extreme erosion severity is globally rare, except within the driest deserts. Consequently, Iceland ranks among the most active dust sources on Earth (Arnalds, 2010). The commons with limited vegetation cover and severe erosion have been deemed non-suitable for grazing (Arnalds *et al.,* 2001). The commons outside the volcanic rift zone generally had continuous vegetation cover and wetland ecosystems were also common due to denser bedrock. Other parts were dominated by heathland vegetation, which is further divided into rich and poor heathland based on their species composition. The rich heathlands had higher cover of palatable species, but the poor heathlands, on the other hand, were dominated by species such as *Vaccinium* spp., *Empetrum nigrum, Calluna vulgaris, Betula nana* and non-vascular plants (Thorsteinsson *et al.,* 1971). Areas with low cover of vascular plants (classified as partly barren and barren) often have biological soil crusts if they are lightly grazed or non-grazed.

Table 1. Average area and erosion characteristics of 45 grazed highland communal areas divided after highland sections.

Section of highlands	N	Average area km^2	Little erosion %	Considerable erosion %	Severe erosion %	Barren/ mountains %
South	15	718	28	35	37	65
West	4	281	59	33	9	40
Northwest	7	363	83	15	1	17
North	5	674	16	54	30	77
Northeast	7	1220	23	44	33	66
East	7	693	52	23	25	43
Average		693	40	33	25	52

Table 2. Soil erosion and vegetation cover of selected highland communal areas, ordered by vegetation cover. Sources: 'Soil Erosion in Iceland' and 'Nytjaland' databases (AUI).

Communal area (section)	Size km^2	Desert/ Mountains %	Severe erosion %	Vege-tated %	Barren$ %	Heath-land %	Wet-land %
Þverárrétt (W)	412	3	0	93	7	48	17
Afr. Midfjarðar (NW)	369	0	0	91	5	>51	28
Fljótsdalsheidi (E)	671	26	2	68	31	54	11
Audkúluheiði (NW)	742	28	3	55	36	43	11
Grímstunguheidi (NW)	394	55	41	51	48	>45	<1
Álftaversafréttur (S)	208	38	59	42	58	>14	<1
Gnúpverjaafréttur (S)	617	51	31	39	55	>23	10
Rangárvalla.afr. (S)	789	76	39	31	69	na	na
Landmannaafréttur (S)	953	83	66	16	80	na	na
Austurafr. Bárddæla (N)	949	92	57	4	95	na	na

$: classes barren and half vegetated in the AUI Nytjaland database, na: not available

Ecosystem degradation of Icelandic highland areas during the 1100 years of settlement has been catastrophic. This degradation has been most severe within and in proximity to the volcanic rift zone, where soils are most susceptible to erosion and the systems are exposed to periodic impacts of volcanic eruptions. Commons that are already in poor state continue to be grazed, even though scientists have urged for protection of these areas from grazing for several decades. Furthermore, grazing is heavily subsidized by the government (50% of the sheep farmers' income, Arnalds and Barkarson, 2003). The animal production does not have to rely on these degraded rangelands as there are ample amounts of good grazing lands in Iceland, sufficient to maintain the present levels of sheep numbers (Table 1).

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Landscape changes in two agricultural communities in coastal West Norway

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Abstract

The impact of agricultural change in transforming Norwegian coastal landscapes was analysed in two areas in Western Norway; one (18 km^2) in an urban, and another (12 km^2) in a rural district. Both areas were dominated by small holdings and agricultural abandonment was evident. In the urban district the trend was stable throughout the period while in the rural area the frequency of abandonment increased in the 1989-1999 period, mainly due to changes in socio-economic conditions. To study landscape changes, land use/land cover maps were created based on aerial photographs for 1969 and 2008, and a number of landscape metrics were calculated. The observed landscape changes were linked to a number of farm level and socio-economic drivers, most of which significantly affected the landscape change. Improved understanding of the process of agricultural change could enhance the role of agricultural policy as a tool for integrated landscape management and regional planning.

Keywords: land use change, land cover change, socio-economic drivers

Introduction

Agricultural landscapes are mosaics of patches, varying in size, shape, and complexity (Ihse, 1995; Fjellstad and Dramstad, 1999). Land use and land cover (LULC) changes have been studied in agricultural communities in several countries (Ihse, 1995; Zimmermann *et al*., 2010), and found to be one of the main ways whereby humans influence biodiversity (Ojima *et al*., 1994; Vitousek *et al*., 1997). Farmers in coastal areas of Norway maintain some of the oldest landscapes in the country. Traditionally, livestock farming based on extensive grazing has been the basis for coastal agriculture, and abandonment may affect several aspects of the landscape. Norwegian agriculture is highly subsidized and the markets are protected from competition. However, the political willingness to support agriculture has changed through the last decades, and several subsidy schemes have changed. The objective of the study was to explore the LULC changes in the coastal parts of Western Norway, and investigate farm-level and socio-economic driving forces affecting agriculture and landscapes in two contrasting (in terms of location and population development) coastal agricultural areas.

Materials and methods

Study area A is an urban area located within a growing municipality, close to the regional centre and a town. During the study-period from 1969 to 2010, population increased from 7800 to 14200. Study area B is a rural area located far from the regional centre. Population in B was at a peak, with 5700 inhabitants, in 1969 and decreased gradually to 3800 in 2010. Farming information was obtained for every 10^{th} year between 1969 and 2010 from the agricultural census (Statistics Norway). At the end of the study period the number of active farms was reduced by 80% in both areas. The annual decrease was 4.3±1.8 farms. The reduction was almost stable throughout the successive decades in A, but increased in the last two decades in B. Total livestock number decreased by 37% in A and by 59% in B.

LULC data were derived from interpretation of black and white panchromatic aerial photos (1:15000) from 1967 for A and from 1969 for B. The historical photos were digitized and rectified to a universal mercator projection. Information of present LULC was interpreted from RGB orthophotos from 2008. The LULC polygons were drawn in ArcGIS 9 (1999-2008 ESRI) with minimum polygon size set to 300 m^2, and minimum polygon width to 5 m. LULC types were classified into 24 polygon and 11 linear classes. The maps from each study area were intersected and LULC transitions from one land class to another were collected at one random point for each 0.35 ha, the number of points determined from the mean of median patch size. Landscape indexes were calculated using Patch Analyst 4.2.13 (Ontario Ministry of Natural Resources). Driving forces at the farm level or socioeconomic scale were investigated using Binary Logistic Regression (BLR) (Minitab Inc.) with maintenance of husbandry, coded as a dummy, yes (1) or no (0), as dependent variable. Socio-economic information was collected from different local sources and from a record of the major subsidy payments obtained by the Norwegian Agricultural Economics Research Institute (NILF).

Results and discussion

Only 29.8% (A) and 53.9% (B) of the arable land at the beginning of the study period was still arable in 2010 (Table 1). Transition frequency to abandoned land is highest in both areas or 0.280 (A) and 0.209 (B). Interestingly, there is a tendency to higher transition frequency, of arable to built-up, in the rural area (B). The hayfield, pasture and heathland classes also displayed a major frequency of change. The transitions of the home-near pastures and the outlying pastures (coastal heathlands) were somewhat different in the two areas. In area A, succession towards a mixed forest has been most important in both categories due to cessation of grazing. In B, the transition of home pastures has been to heather-dominated coastal heathland or to abandoned land. There is a notably lower frequency of transition of coastal heathlands to other categories in B (0.741 unchanged) than in A (0.371 unchanged).

Table 1. Frequency of transitions between different LULC categories in study area A (1967-2010) and B (1969-2010), calculated from transitions in randomly selected points.

A 1967	Arable	Pasture	Abandoned	Deciduous	Conifer plantation	Mixed forest	Coastal heathland	Built-up
Arable	0.298	0.257	0.280	0.028	0.014	0.083	0.000	0.073
Hayfield	0.059	0.157	0.118	0.059	0.039	0.294	0.020	0.216
Pasture	0.040	0.169	0.164	0.025	0.179	0.358	0.169	0.060
Heathland	0.002	0.029	0.003	0.002	0.302	0.484	0.371	0.018
B 1969								
Arable	0.534	0.084	0.209	0.017	0.002	0.020	0.027	0.123
Hayfield	0.118	0.196	0.363	0.088	0.039	0.147	0.078	0.078
Pasture	0.034	0.299	0.126	0.069	0.017	0.109	0.374	0.011
Heathland	0.002	0.088	0.017	0.013	0.065	0.102	0.741	0.012

For maintaining biodiversity at the landscape level, the decline of the hayfields, pasture and coastal heathland classes are most important. Hayfields are believed to be hotspots for biodiversity in both landscapes. The term is used to classify area of uncultivated, sparsely fertilized grasslands, harvested once and grazed during spring and autumn. During the period of the study, hayfields have been abandoned and transformed to several different classes.

In 2010 only one hayfield patch was left (in B), sustained within a national conservation scheme. Changes in the use of the landscape have increased landscape diversity (LD) in both areas (Table 2). Moreover, mean patch size (MPS) has decreased, which together with increased edge density (ED) indicates fragmentation of habitats.

Table 2. Values of selected landscape ecological indexes.

	Study area A		Study area B	
	1967	2010	1969	2010
Landscape diversity, (LD-index)	1.59	2.04	1.74	2.00
Mean Patch Size (ha)	4.3 (±26.3)	1.8 (±13.2)	1.3 (±12.7)	0.8 (±4.0)
Edge density (m/ha)	254	420	435	582

Two farm-level drivers were positively correlated with continued activity (Table 3). The off-farm income has the highest odds-ratio values, 15.6 (A) and 35.3 (B) and is considered most beneficial for continued farm activity. Farm size is also beneficial but less so. Age of farmer, changed farm-ownership and decreased subsidy payments were insignificant. Of the socio-economic drivers, improved local communication negatively affected farming activity in both areas; however, significant only in A. Local job availability, increasing in A and decreasing in B, affected farming negatively in both areas. When farming has been given up, reduction in job availability does not bring activity back to the farms. In B, where farming traditionally has been supplemented with off-farm income, reduction in availability of industrial jobs might have led to increased emigration. The effect of increased subsidy payments on the dependent variable was ambiguous: in A it was positive but insignificant, in B it was negative and significant. Only major changes in subsidy payments have been considered; the effect of several minor changes, which can sum up to a large effect, has not been included.

Table 3. Parameter estimates for the significant variables in the Binary Regression Model.

		Study area A			Study area B		
Class variable	Independent variables	BLR-coefficient	*P*-value	Odds ratio	BLR-coefficient	*P*-value	Odds ratio
Farm related	Farm size	0.14	$P<0.01$	1.15	0.27	$P<0.01$	1.3
	Off-farm income	2.8	$P<0.05$	15.6	3.6	$P<0.01$	35.3
Socio-economic	Improved communications	-6.3	$P<0.05$	0.00	-4.6	n.s.	0.01
	Increased local job availability	-4.8	$P<0.10$	0.01	Not relevant		
	Decreased local job availability	Not relevant			-3.6	$P<0.05$	0.03
	Increased subsidy payments	1.1	n.s.	2.88	-2.7	$P<0.05$	0.06

Conclusions

Changes in LULC in coastal Western Norway have been substantial in both the rural and more urban area, and overall they resulted in more diverse landscapes. However, the effect on biodiversity is likely to be negative due to loss of habitat and fragmentation. Among the variables tested, the opportunity for off-farm income has been the most important driver to maintain farm activity in both study areas. Changes in subsidy payments seem to be ambiguous and further studies are needed to clarify its influence on farming decisions.

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Influence of maternal age and pasture use on lamb growth on islands in Northern Norway

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Abstract

While coastal heathland on islands traditionally are grazed by the Old Norwegian Sheep breed, we know little about how the more-common Norwegian White Sheep utilize and produce on island pastures. We used GPS-collars to track Norwegian White Sheep grazing semi-natural pastures on two different islands during the summer where lambs were weighed frequently. Lambs' growth rates were comparable to previous studies in mountainous pastures. Yearling mothers spent less time in predefined pasture areas, and had lighter lambs than two-year-old mothers. However, the direct link between habitat choice and lamb growth was less clear. This study confirms that island grazing is a good alternative to mountainous areas for Norwegian White sheep

Keywords: body mass, maternal effect, body mass growth, habitat selection

Introduction

Grazing in Norway is based on the utilization of the huge variety in nature, which is reflected in different management systems and local adaptations. A recent White Paper from the Norwegian Government (St.prop.Nr. 9, 2011-12) encourages increased grazing in all regions of the country. Traditionally, sheep have grazed rangeland pastures in the mountainous areas but increased interests to utilize pastures on islands have developed in recent years. The coast of Nordland County (ranging from 65°N 12°E to 69°N 15°E) is scattered with islands of all sizes, from small islets of approximately 1 ha to inhabited islands up to 500 km^2. Many of the smaller islands, previously inhabited, are now abandoned and traditional farming with meadow harvesting and livestock grazing is disappearing. The terrain of islands is mainly flat (rising up to 40-50 m a.s.l.) and fresh water is a limiting factor. The Norwegian Agricultural policy is offering special subsidies and encouraging farmers to farm abandoned islands to keep the landscape open. Stocking rate on grazed islands is defined by experience over years by the farmers, but this gives us little information about the potential for using non-grazed islands due to lack of information on stocking rate, growth rate and production (carcass weight and classification). In a newly initiated project, ewes with lambs were followed using GPS-collars during summer grazing on island pastures in 2012. The aim was to investigate ewe's choice of habitat and link this information to the lambs' growth rate.

Materials and methods

The experiment was carried out at two randomly selected, but typical islands in Nordland County, Norway during the summer 2012. Buoya is located at 66.6°N 12.9°E and the size of the island is about 36 ha, while Sandvaer (66.3°N 12.7°E) is approximately 52 ha. Fifteen one- and two-year-old ewes with twin lambs grazed Buoya between 24 May and 3 September. At Sandvaer, six one- and two-year-old ewes with twin lambs grazed between 3 June and 8 September. All lambs were weighed at birth, when sent to the island, twice during the summer (22 June and 17 July), and when collected from the island. All the ewes (21) were equipped with a GPS (Global Positioning System) collar (Telespor©) logging locations at six-hour intervals during the grazing period. The vegetation at Sandvaer is species rich with a grass-dominated coastal heath being the dominant vegetation type. This type of vegetation is typical for islands where the rock type is rich in lime. In contrast, the rock types on Buoya are poor in lime and also contain species-poor heather (Calluna vulgaris) vegetation. In addition, the island contains areas of salt marsh and abandoned hayfields. By using aerial photos we were able to distinguish between types of habitat on the islands. On both islands a 'best pasture area' was predefined based on the domination of grass. The predefined best-pasture area at Sandvaer is about 15 ha, while at Buoya it is about 4 ha.

The influence of maternal age and habitat use on lamb growth in the period 24 May to 7 September was estimated using linear mixed models. Island and maternal ID was included as a random intercept in the models. The identity of each lamb was included both as random intercept and random slope in the models to control for repeated measurements (library lme4) and to allow for individual growth rates (Pinheiro and Bates, 2001). Pasture use was defined as the proportion of recorded GPS locations within the predefined pasture areas. For easier interpretation of interaction effects, weighing date was centred to the median date in the period, July 14 (Julian date 196). The predictors included in the models was selected using Akaikes Information Criterion adjusted for small samples sizes (AICc) (Burnham and Anderson, 2002), and their influence was assumed significant when 95% confidence intervals (95% CI, estimated by 1000 Monte Carlo Markov chain resampling or for random slopes models approximated by using 2×SE) did not include zero. All statistical analyses were done in R, version 2.14.1 (R Development Core Team, 2008).

Results

The lambs showed a linear growth rate of 0.31 kg per day (95% CI [0.29, 0.32] during the grazing period; however, the rate decreased during the period (Table 1). Lambs of 2-year-old females were, on average, 1.56 kg heavier than lambs of yearling mothers (Table 1). The proportion of pasture use ranged from 0.30 to 0.71 among the females. Pasture use was influenced by age of females (ΔAICc = 6.6), 2-year-old females having 11% more of the locations within pastures than yearlings. Use of pastures positively influenced body mass of lambs (i.e. included in the model of lamb growth ΔAICc = 29.39); however, this influence was not significant and decreased throughout the season (Table 1).

Discussion

Lamb growth rate during the summer averaged 310 g per day for twin lambs. This growth rate will allow the lambs to reach a live weight minimum of 42 kg in the autumn, which is the preferred weight prior to slaughter. This corresponds well with the findings in 2004, in which the average lamb growth rate was 353 g per day in June and July (Lind and Eilertsen, 2004). Quality of the pasture decreases throughout the grazing season and can thus influence lambs' growth rates. Hence this study may indicate that quantity and/or quality of pastures satisfied nutritional needs for lamb growth. Lambs of yearling mothers grew less than lambs of older mothers. Although this may be caused by differences in reproductive allocation, our study indicates a link to feeding strategies. Two-year-old mothers spent more time in the defined pasture areas. This habitat selection tended to influence weight of the lambs. However, due to the covariance with age, it is not possible to separate the effect of habitat selection from additional age effect.

Table 1. Parameter estimates for the mixed models of lamb body mass and pasture use of mothers. Date was centred to July 14. For all models random effects are given as standard deviation units. For mothers age yearlings is set as reference level.

Response	Predictor	Estimate	SE	95% CI
Lamb body weight (kg)	Intercept	27.47	1.81	(23.16, 30.4)
(n = 161)	Date	0.31	0.01	(0.29, 0.32)
	$Date^2$	-1.08×10^{-3}	0.11×10^{-3}	$(-1.3, -0.86) \times 10^{-3}$
	Mothers age	1.56	0.77	(0.02, 3.1)
	σ_{LambID}	10.83		
	$\sigma_{Date|LambID}$	0.001		
	$\sigma_{MotherID}$	1.35		
	$\sigma_{Location}$	8.59		
	$\sigma_{Residual}$	2.51		
Pasture use (proportion)	Intercept	0.41	0.10	(0.33, 0.54)
(n = 18)	Mothers age	0.11	0.02	(0.07, 0.17)
	$\sigma_{Location}$	0.14		
	$\sigma_{Residual}$	0.04		
Lamb body weight (kg)	Intercept	26.20	3.52	(16.22, 29.26)
(n = 145)	Date	0.31	0.01	(0.29, 0.32)
	$Date^2$	-1.12×10^{-3}	0.11	$(0.5, 2.1) \times 10^{-3}$
	Pasture use	11.89	6.14	(-0.39, 24.17)
	Pasture use x $Date^2$	-4.70×10^{-3}	0.75×10^{-3}	$(-6.20, -3.20) \times 10^{-3}$
	σ_{LambID}	3.22		
	$\sigma_{Date|LambID}$	0.04		
	$\sigma_{MotherID}$	1.56		
	$\sigma_{Location}$	1.97		
	$\sigma_{Residual}$	1.26		

Conclusion

This study shows that using islands as summer pastures is a good alternative to mountainous pastures. This can be of particular importance in areas where other factors, for instance predation, may limit the use of the latter. However, it also shows that habitat selection within the islands is dependent on individual characteristics like age, which may hence influence somatic growth of females or their allocation into growth of offspring. The details of these interactions will, however, need more data before the effects can be disentangled.

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Grazing intensity and ecosystem services in subalpine pastures

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Abstract

Subalpine pastures have a great value for society by providing a number of ecosystem services. These are linked to natural factors as well as to the intensity of livestock grazing. We assessed the relationship of grazing intensity and of environmental factors to (i) plant species richness as an ecological service, (ii) fodder production as an economic service, and (iii) carbon sequestration as an climatic service. Grazing intensity was measured by GPS tracking. Thus, we were able to separate its effect on services from the influence of topographic and pedologic factors. Simple correlations showed a positive relationship of grazing intensity to biomass production, a negative relationship to plant diversity and no relationship to soil organic carbon (SOC). Including environmental factors, there was an increasing complexity of the resulting mixed models from plant species richness over fodder production to SOC content. Plant species richness was mainly explained by grazing intensity and terrain slope, whereas fodder production and SOC content were also influenced by interactions between environmental variables and grazing intensity.

Keywords: fodder production, plant diversity, carbon sequestration, topography

Introduction

Subalpine pastures provide important services to society: they are habitats for numerous plant species, deliver fodder for animals and they sequester carbon. While plant diversity is of increasing interest as a regulator of other ecosystem functions (e.g. Diaz *et al.*, 2007), fodder production has a direct economic value to farmers. Soil organic carbon (SOC) plays a role in regulating climate and soil fertility. Since pasture ecosystems have been formed by grazing animals, the provision of ecosystem services is likely governed by the intensity of livestock grazing. At the same time, grazing intensity is the primary system variable influenced by herders. However, in the highly heterogeneous subalpine environments, the assessment of grazing intensity remains a challenge. Using average stocking rate as the intensity measure, for example, does not cope with the small-scale patterns of grazing on large subalpine pastures (Homburger *et al.*, 2012).

In order to understand the role of grazing intensity for the provision of ecosystem services, we need to know how it is interacting with other environmental factors. For this purpose, grazing intensity has to be quantified independently of environmental characteristics (soil and vegetation), in order to discern between grazing and environmental effects on ecosystem services in statistical analysis. Therefore, in this study, we measured grazing intensity by GPS tracking of cows, so that we were able 1) to quantify small-scale differences in intensity and 2) to separate effects of grazing intensity and of other environmental variables on ecosystem services.

Materials and methods

Plant species richness, fodder production and SOC content were assessed on 55 sites on two summer farms in Obwalden (north-central Swiss Alps) and on three summer farms in Engiadina Bassa (south-eastern Alps). The farms, all with dairy cows, were chosen in order to present different management systems. Two farms had a well-organized pasture management with many rather small paddocks. Two of the farms had very few and large paddocks and one farm was additionally grazed by suckler cows. On each farm, we established eleven study plots along the inclination gradient at varying distances from the primary farm building. A suitably homogenous sampling area of 25 m^2 was used for determination of plant species richness. Fodder production was measured as biomass dry matter production using 1 m^2 grazing exclusion cages. SOC content (% dry weight) of the upper 5 cm soil layer was quantified from 16 pooled soil cores per plot. In addition, the variables terrain slope, distance to the farm building, altitude and soil phosphorus content in the upper 5 cm soil layer were quantified for each study plot. The method of GPS-tracking to determine grazing intensity is described in Homburger *et al.* (2012). We used generalized linear mixed models for statistical analysis in order to account for differences between alpine farms as random effect. For each ecosystem service we started with the same set of variables in the full model (Table 1). We reduced the models by using sequential likelihood-ratio-tests.

Results and discussion

Simple linear correlations between grazing intensity and each ecosystem service showed a significant negative relationship between grazing intensity and plant species richness, a significant positive one between grazing intensity and fodder production and none between grazing intensity and SOC content (Figure 1).

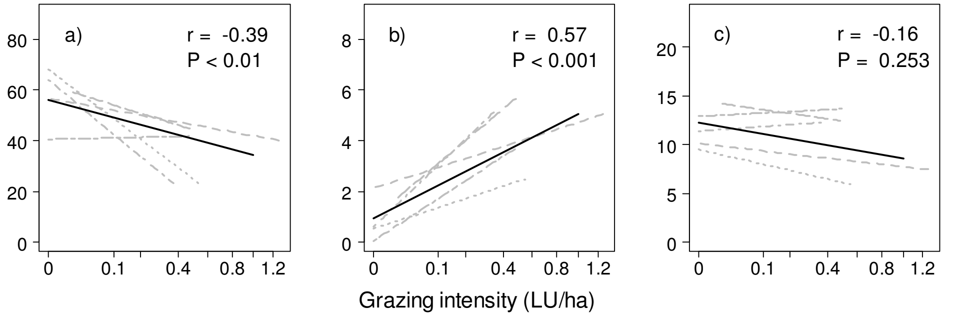

Figure 1. Relationships between grazing intensity (livestock units ha^{-1}) and ecosystem services on five alpine farms: a) Plant species richness (counts), b) fodder production (t ha^{-1}) c) soil organic carbon content (%). Spearman's correlation coefficient is provided for the relationship over all five alpine farms (black line). Grey lines show relationships for each farm separately.

Linear mixed models including environmental variables showed that grazing intensity had a negative effect, and terrain slope a positive effect on plant species richness (Table 1). Fodder production was negatively related to terrain slope and distance to the farm building and positively to grazing intensity. Additionally, we found a strong negative interaction between terrain slope and grazing intensity influencing fodder production. This is because the negative effect of terrain slope on fodder production is especially present at high grazing intensity. The final reduced model for SOC showed the highest complexity. Only soil phosphorus content explained a part of the variation in SOC content as a single variable. Terrain slope, altitude and grazing intensity had an effect only in interaction with one another. The negative effect of terrain slope on SOC content was diminished by increasing altitude and it was reinforced by grazing intensity.

Table 1. Linear mixed models explaining plant species richness, fodder production and soil organic carbon (SOC) content by grazing intensity (A), environmental variables (B) and the interactions between the two (C). Data from five alpine farms in the Swiss Alps (+ positive effect, - negative effect, *** P<0.001, ** P<0.01, * P<0.05, ° P<0.1, ns non-significant).

		Species richness	Fodder production	SOC content
A	Grazing intensity	- **	+ ***	- °
B	Terrain slope	+ ***	- **	ns
	Distance to farm building	ns	- *	ns
	Altitude	ns	ns	ns
	Topsoil phosphorus content	ns	ns	+ ***
C	Terrain slope × Altitude	ns	ns	- *
	Slope × Grazing Intensity	ns	- ***	+ *

The analysis highlights terrain slope and grazing intensity as primary influencing factors for the three ecosystem services (Table 1). Hence, these two variables are especially important in shaping the subalpine pasture ecosystem. For plant species richness, they even were the only variables with significant explanatory power. The fact that they act together means that terrain slope not only affects plant species richness by reduced grazing, but, in addition, also by lower nutrient and water availability with increasing terrain slope (terrain slope × soil phosphorus: r = -0.50, P<0.001). This causes lower productivity on steep slopes, which is related to higher plant species richness (Schneider *et al.* 2011).
We did not expect plant species richness to sho that rather clear pattern, as there are complex underlying processes, such as seed dispersal and competition. In the case of fodder production, terrain slope also acts as a factor which integrates other environmental influences. Therefore, soil phosphorus does not appear to determine fodder production in our model, although representing a major plant nutrient.
As reported by Klumpp *et al.* (2009), grazing can have a negative influence on SOC content through altered plant physiology and disturbance of the soil leading to carbon losses. In our study, environmental influences on SOC in the top 5 cm soil layer are much stronger than grazing. Grazing intensity slightly alters environmental effects but has no effect alone.

Conclusions

In order to make decisions in target-oriented pasture management, we need to dissect the effects of grazing and of other environmental variables on ecosystem services. Doing so, we find that different ecosystem services are not controlled by grazing intensity in the same way. The effects of grazing intensity on fodder production and on plant species richness are opposite and, therefore, the adjustment of grazing intensity requires consideration of the aims on a grazed site. The same is true with SOC content, which shows a very complex response to grazing intensity in interaction with environmental factors.

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Influence of different storage conditions on quality aspects of harvested seed material from an *Arrhenatherion* meadow

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Abstract

No guidelines are available for quality criteria and storage conditions of harvested seed material coming from semi-natural grassland. The aim of this work was to find some methods which are practicable and recommendable to assess such quality aspects. The seed material from an *Arrhenatherion* meadow was harvested via on-site threshing in 2009. After the determination of purity and thousand seed weight, the germination capacity was tested in a greenhouse. The harvested seed material was stored under different conditions up to three years and tested once a year. A specific volume of seed material was sown on organic growing media. The results showed that storage under different conditions and the length of storage influenced the germination capacity significantly. Storage under cool and dry conditions revealed better results. There is a strong positive relationship (correlation) between the proportion of mature seed and germination percentage.

Keywords: on-site threshing, germination capacity, greenhouse, storage, *Arrhenatherion* meadow

Introduction

Until now there has been no prescribed method for determination of the germination capacity of seed mixtures harvested from *Arrhenatherion* meadows. Therefore, a method was developed to gain sufficiently valid information about the seed potential of a harvested donor site within a defined period of time, with as limited technical and personnel expenditure as possible. Contractors are interested in obtaining sufficient information about the quality of sowing material, especially in terms of seed proportion and germination capacity (GC). The seed production of plants and the biomass of a meadow stock are dependent on the course of precipitation and temperature during the year, and these differ from year to year (Scotton *et al.*, 2012). An especially high content of diverse seeds in the seed material is necessary for successful restoration. The share of mature seeds with high germination capacity in the harvested material is decisive for the transfer rate on the newly sown area. The main questions of this research work are: i) is there any influence on the seed material by storing it under different conditions and ii) is there a change of germination capacity over the course of time?

Materials and methods

The experimental site is an *Arrhenatherion* meadow and situated in the north-western part of Austria, the so called 'Welser Heide' (48°18'27" N, 14°03'98" E; 310 m a.s.l). During the year of harvest the mean annual air temperature was 9.6°C and annual precipitation was 1017 mm. The pH-value of the soil is neutral to alkaline (6.3-7.4). The seed material was harvested on 1 July 2009, using an on-site threshing (OST) with a plot combine thresher (Wintersteiger) that had a cutting width of 150 cm. After harvesting, the material was air dried, cleaned using a 6 mm sieve, and divided into three fractions. The material was stored over three years (2009-2012) under different conditions: i) room temperature (15-20°C) with 7-15 g m^{-3} of absolute humidity ('room'); ii) cooling chamber (2-5°C) with 3-4 g m^{-3} absolute humidity ('cool'); and iii) freezer (at -18°C) ('freeze'). A germination capacity (GC) test in the greenhouse was done every year. The experimental design for the greenhouse trials were designed after determination of the purity, the thousand seed weight (TSW) and following the results of pre-tests in the phytotron presented in Haslgruebler *et al.* (2011). For determination of the TSW a 100 g sample was taken and divided in subsamples and 8×100 randomly available full seeds were counted and weighed. For the purity assessments the samples were divided into chaff and full seeds. The TSW, purity and the GC is important to define the sowing density of the harvested material. Before the germination trial started, a 4×1.2 g sample was taken and divided into monocotyledons, dicotyledons and chaff. The seeds of every sample were counted and weighed. Afterwards, the samples were mixed again and sown in bulb trays (40 cm × 60 cm × 0.6 cm) on growing organic media (OGM) according to the International Seed Testing Association (ISTA, 2011). The duration of the trial was 4 weeks and the samples were counted once a week and divided into monocotyledon and dicotyledon seedlings. The statistical analyses were done with the statistics program R 2.15.1 (R Core Team, 2012). A Shapiro Wilk Test was done, testing the sample originating form a normally distribution of the data. Afterwards a two-way ANOVA was used to test for differences between the length of storage and the storage under different conditions. Finally, the Bonferroni post hoc test was used to test for significant differences between variants where necessary.

Results and discussion

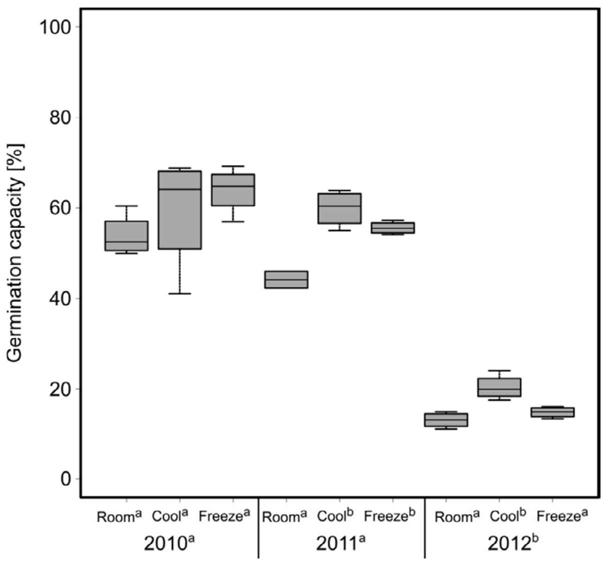
The TSW of the harvested OST material was 1.057 g with a purity of 63.05% pure seeds. The length of storage (P<0.001, Eta Sq = 0.95) and the different storage conditions (P<0.001, Eta Sq=0.46) showed a highly significant impact on the germination capacity of the tested OST material but there was no significant interaction between duration and storage method (see Table 1).

Table 1. Results of the two-way ANOVA of the greenhouse germination trial from seed material harvested with on-site threshing, stored under different conditions over three years.

	Df	Sum Sq	Mean Sq	F-value	*P*-value	Partial Eta Sq
Year	2	13038.3	6519.2	237.99	<0.001***	0.95
Storage	2	618.7	309.4	112.94	<0.001***	0.46
Year × Storage	4	232.1	58.0	21.18	0.106	0.24
Residuals	27	739.6	27.4			

R^2: 0.95: Adjusted R^2: 0.93
Significance level: P (*P<0.05, **P<0.01, ***P<0.001)

A minimum germination capacity threshold at 50% is shown in Figure 1, and this is the level that should be shown by the harvested material if it is to guarantee sufficient quality for restoration success (Krautzer and Hacker, 2006). After one year of storage, the germination capacity (Figure 1) of the OST material partly reached 70%. In the second year the samples stored under 'room' temperature declined to 40%, but the samples of the 'cool' and 'freeze' treatments still obtained a high result, ranging between 55 and 65%. After three years of storage all three treatments (room, cool and freeze) showed a significant reduction of the germination capacity to below 20%. In general, samples stored under cool and dry conditions revealed higher germination rates than the samples stored under room temperature. Variance of all treatments strongly declined over the years. This could be explained by the fact that seeds of single species have different germination strategies and, after storage over the years, some species loose vitality earlier than others (Grime *et al.*, 1981; Haslgruebler *et al.*, 2011).



a, b – different letters indicate significant differences

Figure 1. Germination capacity of stripped seeds from an *Arrhenatherion* meadow stored under different temperature and humidity conditions over three years (2010, 2011, 2012).

Conclusion

To obtain satisfactory quality results, seed material should be stored under cool and dry conditions. To avoid rapid decrease of germination capacity, the storage of seed material should not exceed two years. The presented method for testing seed material harvested from an *Arrhenatherion* meadow can be recommended to practitioners because it is easy to apply in practice and it gives sufficient information about the quality of seed material.

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Quality characteristics of seed material from selected species of a nutrient poor *Arrhenatherion* community

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Abstract

Arrhenatherion meadows are rich in biodiversity and the most important type of semi-natural grassland in central Europe. A problem is that the seed quality of selected wild flowers from local provenance is broadly undetermined. Therefore seven species were tested according to the rules of the International Seed Testing Association (ISTA, 2011) to determine the quality of wild flower seeds. An Arrhenatherion meadow was harvested in July 2009 with a plot combine thresher. After the material was dried and roughly cleaned, it was split and stored under different conditions. Three years after harvesting the thousand seed weight and the germination capacity were tested for seven of the main characteristic species: *Arrhenatherum elatius, Bromus erectus, Dactylis glomerata, Festuca pratensis, Poa pratensis, Trisetum flavescens* and *Dianthus carthusianorum*. The results showed that the germination capacity of the seed material stored in the cooling chamber is higher than at room temperature, except for *Dactylis glomerata*. The germination capacity of the assessed species still remained between 60% and 90% under both storage conditions.

Keywords: germination capacity, local provenance, Jacobsen germination apparatus, ISTA

Introduction

Before the marketing of cultivars of forage species in Central Europe was made possible by plant breeding, the most commonly used material for sowing grassland was the seed of semi-natural meadows, which was obtained from hay lofts (Scotton *et al*., 2009). The importance of seed propagation of native ecotypes has now increased, to provide seed for restoration and re-establishment of species-rich grassland (Kirmer *et al*., 2012). The quality parameters of a single species of regional provenance, propagated for seed mixtures, should as far as possible be aligned with the methods of the International Seed Testing Association (ISTA). The primary purpose of the Association is to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for evaluation of seeds moving in international trade (ISTA, 2011). Seed of some native ecotypes (e.g. most grasses) can sprout almost immediately, but many seeds require a period of dormancy before they are able to germinate. This paper addresses the following research questions: (i) are there any differences in the germination capacity of seven dominant species of an *Arrhenatherion* community; (ii) is it influenced by different storage conditions; and (iii) how is the germination capacity of the assessed species affected after three years of storage?

Materials and methods

The study site (nutrient-poor *Arrhenatherion* community) is located at 48°18'27" N, 14°03'98" E; 310 m a.s.l., in the province of Upper Austria near the capital Linz. The mean annual air temperature in 2009 (year of harvest) was 9.6°C and the annual precipitation was 1017 mm. A combine plot harvester with a cutting width of 1.5 m was used for harvesting the meadow on 1 July 2009. The harvested material was air-dried, roughly cleaned and afterwards the seed material was split in two fractions and stored under different conditions (i) room 15-20°C with 7-15 g m^{-3} absolute humidity, and (ii) cooling chamber 2-5°C with 3-4 g m^{-3} absolute humidity. The germination capacity of the seven most characteristic species was tested after three years (2012) of storage under different conditions. The tested species were *Arrhenatherum elatius, Bromus erectus, Dactylis glomerata, Festuca pratensis, Poa pratensis, Trisetum flavescens* and *Dianthus carthusianorum.* Before testing the germination capacity, the thousand seed weight (TSW) was analysed for both storage conditions (Table 1). From every species 4×100 resp. 4×50 pure seeds were randomly separated from the samples and tested on a Jacobsen germinations apparatus. The germination capacity indicates the proportion of seeds that have produced seedlings (ISTA, 2011). The seeds were uniformly spread on moist (distilled water) filter paper. Two Jacobsen apparatuses were needed because of different night and day temperatures (20/30°C, 15/25°C) as well as different dormancy breaking treatments: (i) pre-chilling for a week; (ii) addition of KNO_3 and (iii) light (Table 1) The duration of the germination trial is described in Table 1. The first count was after 5 or 7 days depending on the species, and afterwards at a 4-day interval up to the last counting date. The statistical analysis was done with the statistical language R (R Core Team, 2012). A one-way ANOVA was created to obtain significant differences between the different storage conditions.

Results and discussion

The results clearly showed that seeds stored under room temperature revealed a lower TSW than seeds stored in the cooling chamber (Table 1). The lower TSW measured under room temperature can be explained by higher respiration losses caused by higher temperatures and higher humidity (Sherman, 1921).

Table 1. Prescriptions for testing the germination capacity of selected species in the Jacobsen germination apparatus according to ISTA (2011). The results of the thousand seed weight (TSW) for both storage conditions.

Species	Night/day temperature, °C	Duration days	Pre-chilling	KNO_3	Light	TSW-room g	TSW-cool g
Arrhenatherum elatius	20-30	14	yes	-	yes	3.18	3.47
Bromus erectus	15-25	14	yes	yes	yes	4.37	4.49
Dactylis glomerata	15-25	21	yes	yes	yes	0.72	0.79
Festuca pratensis	15-25	14	yes	yes	yes	1.98	2.04
Poa pratensis	15-25	28	yes	yes	yes	0.18	0.19
Trisetum flavescens	20-30	21	yes	yes	yes	0.21	0.23
Dianthus carthusianorum	20-30	14	yes	-	yes	0.63	0.70

The moisture content of the seed mixture was on average 9.5%. The results show that *Trisetum flavescence* (P>0.001, R^2=0.94), *Bromus erectus* (P>0.01, R^2=0.76), *Arrhenaterum elatius* (P=0.1, R^2=0.39) and *Festuca pratensis* (P=0.08, R^2=0.43) revealed a significantly higher germination capacity when seeds were stored in the cooling chamber compared to room temperature. There were no significant differences in the germination capacity for seeds of *Dactylis glomerata, Poa pratensis* and *Dianthus carthusianorum* concerning the storage conditions. Seeds of *Bromus erectus, Festuca praensis* and *Poa pratensis* reached a germination capacity of 90% and higher, when stored in the cooling chamber, and were even exceeding the ISTA (2011) threshold. Grime *et al.* (1981) assessed that, for most species, germination capacity increased during dry storage conditions, but our experiments showed opposite results. All species except *Dactylis glomerata* reached higher germination capacities when the seeds were stored under cool and dry conditions. Thompson and Ooi (2010) reported in their paper that temperature can both break dormancy and stimulate or reduce germination capacity, sometimes even at the same time, which underlines our results.

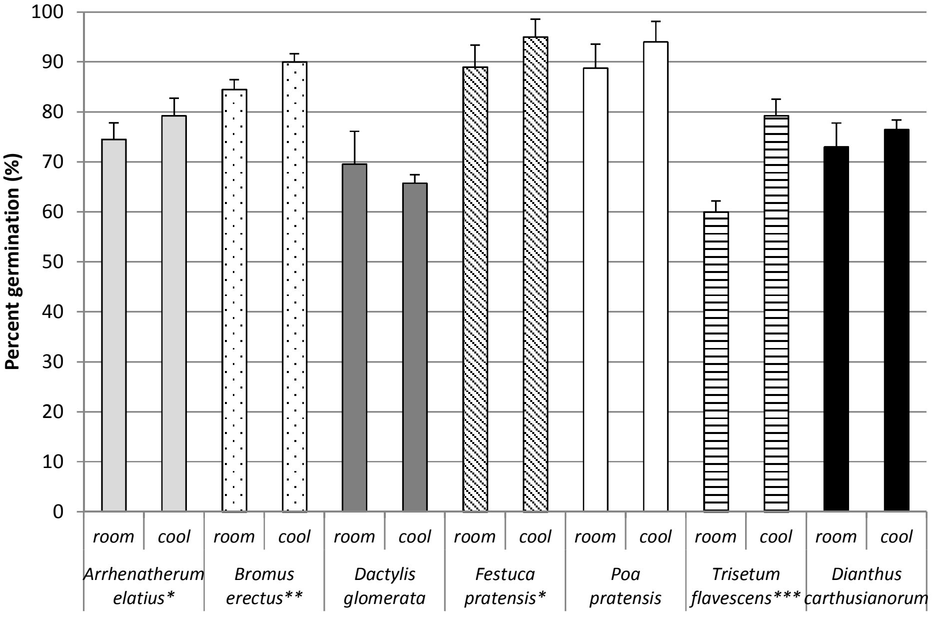

Figure 1. Results of the germination capacity after three years of storage under different conditions (i) room 15-20°C with 7-15 g m^{-3} absolute humidity and (ii) cooling chamber 2-5°C with 3-4 g m^{-3} absolute humidity of seven dominant species *Arrhenatherum elatius, Bromus erectus, Dactylis glomerata, Festuca pratensis, Poa pratensis, Trisetum flavescens* and *Dianthus carthusianorum* of an *Arrhenatherion* community (significance level: *P<0.05, **P<0.01, ***P<0.001).

Conclusion

Wild flower seeds harvested with on-site threshing can be tested with the same methods as commercial seeds according to the rules of the International Seed Testing Association (ISTA 2011). The germination capacity of the selected main characteristic species is generally high and seed dormancy strategies could not be assessed. Harvested seeds of wild flowers can be easily stored at least for three years under dry and cool conditions and will still hold sufficient germination capacity.

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Effects of fertilization on flower size in species-rich grasslands

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Abstract

Reduction of soil fertility is a key factor for restoration of species-rich grasslands. However, when soil fertility is already low, a further reduction may reduce flower size and weight. Low fertilization rates could then enhance grass production and enlarge the size of the flowers of grassland herbs and legumes. To test this hypothesis we collected inflorescences of *Leucanthemum vulgare* and *Trifolium pratense* on two species-rich grasslands with three different treatments: a) control: no fertilization; b) application of cattle slurry; c) application of P and K fertilizer. The length of the flowering stem and the diameter and weight of the flowers of 50 inflorescences were measured per treatment. As expected, with fertilization, grassland productivity increased as well as the length of the flowering stem for both species. A limited application of cattle slurry or P and K did not affect species-richness of the grasslands. For *L. vulgare* the diameter and weight of the flowers were significantly larger with slurry application, but not with P and K fertilization. For *T. pratense,* only the diameter of the flowers was increased by slurry application. We conclude that at nutrient-poor conditions the flower size of the *L. vulgare* and *T. pratense* could be increased by limited application rates of slurry or P and K fertilizer, without affecting the overall species-richness of the swards.

Keywords: flower size, re-introduction, restoration management, species-rich grasslands

Introduction

Most grassland on farms is nowadays heavily fertilized, intensively managed and frequently reseeded. It generally contains only about ten plant species (Korevaar and Geerts, 2007); some sown grass species and common weeds. Reduction in soil fertility is a key factor for restoring these intensively used grasslands into species-rich grasslands. At high soil fertility, tall grass species dominate the sward and suppress herbs. Therefore, a reduction in soil fertility generally is a first step in restoration management (Isselstein *et al*., 2005; Pierik *et al*., 2011; Pywell *et al*., 2012). Agri-environmental schemes stimulate farmers to adapt their farm management to more environmentally friendly production systems. Reduction of the fertilization level is one of the options; it favours the development of species-rich grasslands. However, when soil fertility is already low, a further reduction of soil fertility may also decrease sward productivity and decrease flower size and weight. To test the hypothesis that under nutrient-poor conditions flower size is smaller than under more fertile conditions, we collected inflorescences of *Leucanthemum vulgare* (oxeye daisy) and *Trifolium pratense* (red clover) on two fields with different fertilization treatments and measured their sizes.

Materials and methods

On two farms on sandy soils in the eastern part of the Netherlands, near the municipality of Winterswijk, two fields of 1.3 and 2.0 ha, respectively, were ploughed and sown with a species-rich seed mixture from an *Arrhenatheretum elatioris* community in the first week of October 2002. Seeds were sown at a quantity of 20 kg ha^{-1}. Of the total seed weight, 75% originated from nine grass species: *Cynosurus cristatus, Lolium perenne, Festuca rubra, F. pratensis, Dactylis glomerata, Phleum pratense, Poa pratensis, Arrhenatherum elatius* and

Anthoxanthum odoratum. The other 25% was harvested on a species-rich grassland in the neighbourhood with, among others, seeds from *Achillea millefolium, Bellis perennis, Centaurea jacea, Crepis biennis, Holcus lanatus, Hypochaeris radicata, Leucanthemum vulgare, Lotus corniculatus, Plantago lanceolata, Prunella vulgaris, Ranunculus acris, R. bulbosus, R. repens, Rumex acetosa, Stellaria graminea, Trifolium dubium, T. pratense* and *T. repens.* In 2003 the new swards received no fertilization. In 2004 the fields were divided into three equal parts. Each part received one of the following three treatments: a) control: no fertilization, b) application of cattle slurry (15 tonnes ha^{-1}), and c) fertilization with 11 kg ha^{-1} phosphorus (P) and 83 kg ha^{-1} potassium (K). These treatments were introduced to study the impact of low fertilizer application rates on species-rich grasslands. The background is the on-going discussion in the Netherlands between farmers and nature conservationists on 'maintenance' fertilization. Farmers argue that without any fertilization the nutrient status could become too low, with even a decline in species diversity. In practice 'maintenance' fertilization generally is performed through the application of cattle slurry. For herbs, notably legumes, P and K are essential. N stimulates the growth of tall grass species, resulting in the shading of herbs and legumes. In treatment (c) we therefore applied only P and K. Fertilization was applied annually after the first cut at the end of June. Total number of plant species, dry matter (DM) production and feeding value of the grass for ruminants were measured during 2005-2008. After 2008 the fertilization treatments were continued, but production was no longer recorded.

In June 2010, fifty inflorescences of *Leucanthemum vulgare* and *Trifolium pratense* were collected randomly from each treatment at the two sites. The length of the flowering stem, diameter of the flower and weight of the flower without stem were measured for each inflorescence collected.

Results and discussion

The effects of the fertilization treatments on species numbers, DM production and feeding value of the grass are presented in Table 1.

The data demonstrate that a limited application of cattle slurry or P and K did not affect species-richness of the grasslands. As expected, grassland productivity increased with fertilization. Feeding value, presented as the net energy content in the DM, was not affected by the treatments. Similar results were observed in another field experiment in which two different species-rich grasslands were compared with a *Lolium perenne* sward (Korevaar and Geerts, 2012).

Table 1. Number of plant species, DM production and feeding value of grass on two farms. The figures are average values of two sites and four years (2005-2008).

Treatment	Fertilization (kg ha^{-1} yr^{-1}) N	P	K	Total number of species (per 100 m^2)	DM production (kg ha^{-1} yr^{-1})	Net energy (MJ kg^{-1} DM)
Control	0	0	0	27.4 a	5653 a	5.02 a
P and K	0	11	83	27.9 a	7260 b	4.97 a
Cattle slurry	35	9	85	26.6 a	7896 b	4.99 a
LSD (*P*≤0.05)				1.9	1337	0.14

Different letters: parameters are different *P* ≤ 0.05

The flowering stems of both species were longer on treatments with fertilization (Table 2). For oxeye daisy, flower diameter and weight were significantly greater on the treatment with slurry application than on the control, P and K fertilization had no significant effect. For red clover, the diameter of the flowers was larger with slurry application, but the weight did not differ from the control. The impact of P and K on diameter and weight of the flowers was limited and not significantly different from the control.

Table 2. Length of flower stem, flower diameter and flower weight of *Leucanthemum vulgare* and *Trifolium pratense*.

Treatment	*Leucanthemum vulgare*			*Trifolium pratense*		
	Length stem (cm)	Flower diameter (cm)	Flower weight (g)	Length stem (cm)	Flower diameter (cm)	Flower weight (g)
Control	38.4 a	4.0 a	0.52 a	18.3 a	2.4 a	0.28 a
P and K	48.1 b	4.2 ab	0.59 a	25.1 b	2.6 ab	0.36 a
Cattle slurry	53.8 c	4.4 b	0.69 b	30.6 c	2.7 b	0.40 a
LSD ($P \leq 0.05$)	3.2	0.3	0.09	3.7	0.3	0.18

Different letters: parameters are different $P \leq 0.05$

In a three-year experiment growing different combinations of cereals (barley or rye), peas and associated plants (arable weeds), Stilma (2008) concluded that in a mixture with cereals and associated plants, peas did not affect the number of associated plants, but stimulated individual plant biomass resulting in larger associated plants. The positive effect of pea was due to the accumulated nitrogen stocked in the soil by the pea in previous years.

Galen (1999) hypothesized that smaller flowers require less investment of essential resources from the plant than large, showy flowers. Reduced flower size will be advantageous under resource-poor conditions, but it could be a disadvantage for pollinators like bumblebees, because they prefer large flowers (Galen, 1999).

Conclusion

We conclude that there is evidence that under nutrient-poor conditions the flower size of *Leucanthemum vulgare* and *Trifolium pratense* can be increased by limited application rates of slurry or P and K fertilizer, without affecting the overall species-richness of the swards.

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Effect of ammonium nitrate concentration on the germination of *Pulsatilla pratensis*

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Abstract

The most critical phases in the life of many plant species are seed germination and seedling recruitment. Both these phases are claimed to be negatively affected by high nitrogen availability in the soil. In our experiment, we investigated the effects of different concentrations of ammonium nitrate (NH_4NO_3) in water solution on the germination of *Pulsatilla pratensis* subsp. *bohemica*, an endangered species of dry grasslands in the Czech Republic. We tested the germination response of seeds collected at two localities to eight concentrations of ammonium nitrate: 0, 6.8, 34, 170, 509, 848, 2543 and 4239 mg N L^{-1}. Increased concentrations of ammonium nitrate had a negligible effect on seed germination up to the N concentration of 848 mg L^{-1}. Total germination over 31 days was 40-50%. In the 2543 and 4239 mg N L^{-1} treatments, germination was only 1 to 2%. In conclusion, common nitrogen concentrations in rainwater, which range from 10 to 13 mg N L^{-1}, have no effect on the germination rate of *P. pratensis*.

Keywords: seed germination, atmospheric N deposition, *Ranunculaceae,* pasqueflower, recruitment

Introduction

Pulsatilla pratensis is an endangered species of European dry grasslands. A significant part of the species' area of distribution lies in Central Bohemia. Many populations are even found within the capital city of Prague. Most populations have declined rapidly or have become extinct during the last decades. It is assumed that the main reasons for its decline are the cessation of management such as grazing or mowing (Mayerová *et al.*, 2010) and high deposition of atmospheric nitrogen (Bobbink *et al.*, 1998).

Deposition of N compounds (NH_4^+ and NO_3^- in particular) can affect populations of *P. pratensis* indirectly through an increase in biomass production resulting in stronger competition for light from other species (Wilson and Tilman, 1993). As previously recorded for several other species, low concentrations of NH_4^+ and NO_3^- ions in solution can stimulate germination whereas high concentrations can inhibit it. Nitrogen concentrations in rainwater in Prague range from 10 to 13 mg N L^{-1} (Fišák *et al.*, 2002). In the soil solution, however, concentrations of N can be at least ten times higher, especially because of the use of nitrogenous fertilizers (Hejcman *et al.*, 2007). Germination of *Galium tricornutum*, for example, was stimulated by 70 mg N L^{-1} and inhibited by higher concentrations (**Chauhan** *et al.*, 2006). Germination of *Thlaspi villosa, Medicago arabica, Amaranthus powellii, Atriplex sagittata* was stimulated by 10 to 500 mg N L^{-1} and inhibited under concentrations higher than 1000 mg N L^{-1}. Similar results have been obtained for *Dactylis glomerata* and *Anthemis arvensis* (e.g. Brainard *et al.*, 2006; Pérez-Fernández *et al.*, 2006; Mandák and Pyšek, 2011).

The aim of this study was to ascertain the range of N (NH_4^+ and NO^-_3) concentrations in solution that enable germination of *P. pratensis* seeds. We particularly asked whether common concentrations of N in rainwater in areas with higher N deposition can decrease or stimulate germination of *P. pratensis* seeds.

Materials and methods

Seeds of *P. pratensis* were collected in June 2011 at two localities SW of Prague. The first was Křešín (49°48'N, 13°57'E) and the second was Pání hora (49°57'N, 14°9'E). The altitude of both localities is 400 m a.s.l. The germination experiment was carried out in a laboratory at the Czech University of Life Sciences in Prague. The ambient temperature was 22°C, and the seeds were subjected to a light/dark regime of 16/8 hours, respectively. We used Petri dishes 10 cm in diameter with three layers of filter paper sterilized in a Labo Autoclave (Sanyo). We used only fully developed and healthy seeds, 30 per Petri dish. Altogether we used 40 dishes and 1,200 seeds for each locality. To simulate atmospheric N deposition, we used ammonium nitrate (NH_4NO_3, 35% N) diluted with distilled water to obtain required N concentrations for eight solution treatments (Table 1), each replicated five times. Then we added 5 ml of the solution to each Petri dish. N solutions were added only once during the course of the experiment. Dishes were watered only with distilled water.

Seed germination was recorded after 12, 15, 19, 25 and 31 days from the beginning of the experiment. Seeds with visible sprouts were considered as germinated. The obtained germination rate data were evaluated by a repeated measures ANOVA (effects of time, treatment and their interaction) and by a one-way ANOVA followed by Tukey's post-hoc test (effect of treatment on germination on the 31st day).

Table 1. Concentrations of nitrogen solutions used for the germination experiment.

Treatment	1(Control)	2	3	4	5	6	7	8
mg N L^{-1}	0	6.8	34	170	509	848	2543	4239
mg NH_4NO_3	0	19	97	484	1453	2422	7267	12111

Results

Germination of seeds from both localities was significantly affected by the treatment, time and by the treatment × time interaction (Figure 1). Germination started on day eight of the experiment. Total germination over all treatments was 35% for Pání hora and 50% for Křešín, and differences between localities were significant (P=0.002). The cumulative germination rates in all treatments for both localities on the 31st day of the experiment are presented in Table 2. For both localities, treatments 1-5 significantly differed from treatments 7 and 8.

Table 2. Mean values, SE and results of Tukey's *post-hoc* test for cumulative germination on the 31st day of the experiment for each locality. Numbers marked with the same letters were not significantly different (P=0.05).

Treatment	1(Control)	2	3	4	5	6	7	8
Křešín	49 ± 5.4a	43 ± 2.7a	39 ± 3.1a	38 ± 7.1a	43 ± 3.5a	32 ± 2.9ab	11 ± 1.2b	14 ± 8.4b
Pání hora	36 ± 4.2a	36 ± 5.4a	38 ± 5.7a	36 ± 4.2a	30 ± 4.7a	21 ± 3.1ab	5 ± 2.2b	3 ± 1.2b

Discussion

We found that *Pulsatilla pratensis* seeds are able to germinate under a wide range N concentrations up to 848 mg N L^{-1}. Concentrations above this level strongly reduced the rate of germination. These concentrations are considerably higher than concentrations of N commonly recorded in rainwater, which range approximately from 10 to 13 mg N L^{-1}. In addition, as shown by Kellner (1993) for *P. vernalis*, a single dose of N fertilizer can improve bud formation. Concentrations above 2500 mg N L^{-1} almost blocked the germination of *P. pratensis* seeds. The highly negative effect of N concentrations above 1000 mg N L^{-1} on the germination of *P. pratensis* are in accordance with results obtained for several other species (e.g. Brainard *et al.*, 2006; Pérez-Fernández *et al.*, 2006; Mandák and Pyšek, 2011). We also found a difference in total germination and in the germination response between localities, which can be caused by genetic or environmental factors.

It seems that common N concentration in city rain do not have a negative effect on seed germination of *P. pratensis* even though this species usually grows in nutrient-poor, dry grasslands. Rainwater is nevertheless only one of several sources of nitrogen, which is only one of many factors present in the soil solution, so further research is needed to pinpoint the reasons for the ongoing decline of *P. pratensis* populations.

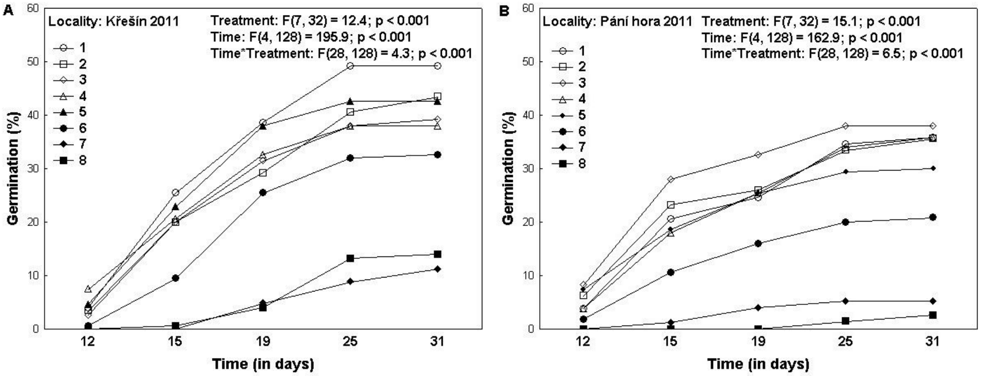

Figure 1. Seed germination at the localities Křešín and Pání hora in eight different nitrogen solutions (treatments) in Petri dishes. Treatment abbreviations are given in Table 1. We used 150 seeds of *P. pratensis* for each treatment. The *F* and *P* values were obtained from repeated measures ANOVA.

Acknowledgements

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Seasonal evolution of herbaceous components inside and outside a plane afforested wood

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Abstract

During the last decades, the European Union Common Agricultural Policy has promoted the creation of hedges and woods, especially in intensively cultivated areas where significant environmental impacts are most expected. A study was conducted in an afforested wood and neighbouring grasslands established in 1992. In 2007, the neighbouring grasslands were compared to an afforested wood, in order to study species richness, herbage dry matter (DM) and herbage quality during the four seasons. During each season, number of species and herbaceous cover were significantly higher in the grasslands than in the woods. High differences in herbage DM production between grasslands and afforested woods were observed in spring (0.37 *vs.* 0.79 t ha^{-1}) and summer (0.68 *vs.* 0.14 t ha^{-1}). Higher concentrations of crude protein (CP) were found in the grasslands than in woods, while crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) were lower in the grasslands. Our results demonstrate that grassland areas in proximity to afforested woods can increase plant biodiversity and provide high quality herbage for wild herbivorous animals.

Keywords: species richness, dry matter yield, herbage quality

Introduction

For many years the Common Agricultural Policy (CAP) has promoted policies that lead to environmental restoration. Where there are highly intensive agricultural systems, as, for example, in most areas of the Po Valley, special attention to environmental impacts is needed. The main actions have consisted of planting of hedges and woods, because these arboreal coenoses can play several important landscape and environmental functions. If they are enriched with grassland areas, these restoration interventions offer an increase in species richness and shelter for wild herbivorous animals, together with an improvement of herbage quality (Susan *et al.*, 2010). The present research focused on seasonal differences in the number of species, herbage dry matter (DM), and herbage nutrient contents of the herbaceous layer between afforested woods and their neighbouring grasslands.

Materials and methods

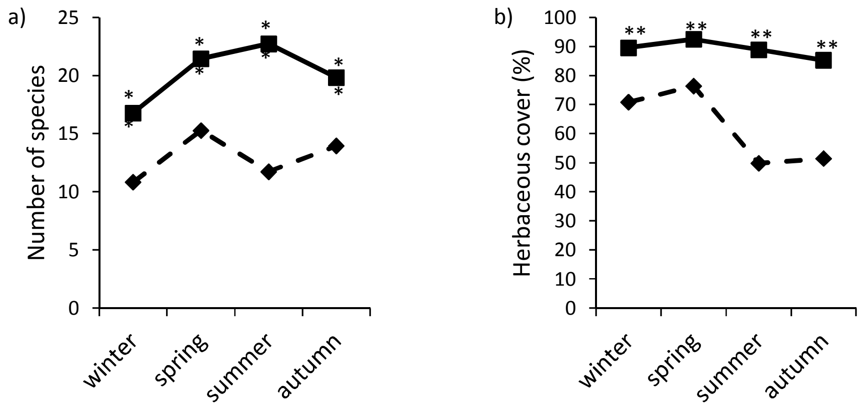
The research was conducted at the Vallevecchia agricultural farm in Caorle (north-east Italy, altitude 0 m a.s.l.), which is an area characterized by a mean annual temperature of 12.6°C and a mean annual rainfall of 854 mm, well distributed throughout the year. In 1992, three woods were planted using deciduous native species, on a 14 ha surface. Each area of wood was surrounded by a grassland area having the same age of woods. The grasslands were composed of spontaneous herbaceous species and were subjected to three cuts per year in order to avoid tree regeneration.

In each of the experimental areas, three plots each measuring 100 m^2 (Pirola, 1960) were selected within the afforested wood and the neighbouring grassland; thus, a total of 18 plots

(9+9). In 2007, surveys were conducted in each plot during the four seasons and consisted of measurements of: number of species, visual estimation of herbaceous cover (%), and herbage DM production (from a test area of 0.5 m^2). Herbage samples were then analysed on a DM basis to determine: crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) (all by Van Soest method), ash (dry-ashing procedure), crude protein (CP; Kjeldahl method), and ether extract (EE; Soxhlet method).
Number of species, herbage DM (t ha^{-1}), and herbage nutrient contents were subjected to analysis of variance using R 2.15.1 (R Core Team, 2012) to determine the effect of afforested wood, season, and their interaction.

Results and discussion

The number of species and coverage of the herbaceous layer (Figure 1a, 1b) were significantly higher outside the afforested woods than in the grasslands in all seasons. However, the difference was more evident in summer and autumn, leading to a significant interaction between wood and season (P<0.001). This was probably due to higher competition for light from arboreal plants during summer and autumn.
A significant interaction between wood and season was detected also for herbage DM (P<0.001), which was significantly higher outside the wood in spring, summer and autumn (Figure 1c).The maximum difference in herbage DM was observed in summer, while there was no difference in winter.



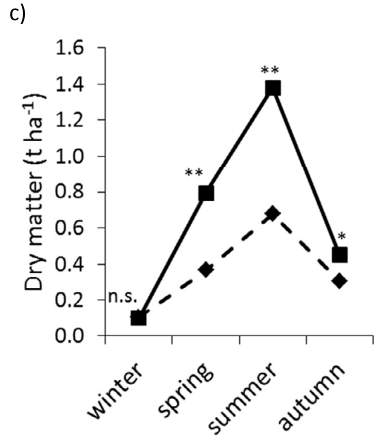


Figure 1. a) Number of herbaceous species, b) herbaceous cover, and c) herbage dry matter of coenoses in Po Valley.
Dotted line: afforested wood; solid line: neighbouring grasslands.

In each season, CP and NDF were significantly higher inside the woods than in grasslands (Table 1). Similarly, ADF and ADL were significantly higher inside than outside the woods, except for ADF in winter and ADL in autumn (Table 1). No significant difference was observed for Ash content throughout the year. Moreover, higher contents of CP and EE were observed outside afforested woods than inside in all seasons. These results clearly revealed that the grassland herbage had higher quality then the herbage inside the woods, year-round.

Table 1. Herbage nutrient contents [crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude protein (CP), ash and ether extract (EE)] as affected by season inside afforested woods (W) and neighbouring grasslands (G).

Nutrient content (% of DM)	Winter			Spring			Summer			Autumn		
	Mean value		Sig.	Mean value		Sig.	Mean value		Sig.	Mean value		Sig.
	W	G		W	G		W	G		W	G	
CF	23.5	20.0	*	33.4	27.0	**	34.3	29.2	**	29.4	22.8	**
NDF	58.6	51.7	*	66.2	57.2	**	68.6	54.9	**	57.7	51.2	*
ADF	31.3	29.4	ns	40.2	34.7	**	41.0	33.2	**	33.6	28.1	**
ADL	5.7	5.2	*	7.2	5.9	**	7.3	5.9	**	6.5	6.0	ns
CP	17.5	20.4	**	9.4	12.8	**	8.6	12.9	**	13.2	16.5	**
EE	2.9	3.3	*	2.5	3.5	**	2.9	3.5	**	2.5	3.0	*
ash	9.7	10.5	ns	10.7	10.5	ns	9.8	9.5	ns	11.2	11.0	ns

Significance: ** (P<0.001), * (P<0.05), ns not significant

Conclusions

The results of this study demonstrate that herbage DM production and herbage quality are consistently lower inside the 15-year-old afforested woods than in the neighbouring grasslands of the same age. Therefore, when hedges and woods are used as tools for environmental restoration, it may be useful to provide grasslands near these coenoses. As such, along with the improvement in species richness, there would be also an increase of food availability for wild herbivorous animals.

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The role of fertilization and cutting in the productivity of an abandoned meadow

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Abstract

The changes occurring in the socio-economic situation of mountain areas during the second half of the twentieth century resulted in significant changes in land use. Several meadows disappeared due to the cessation of the standard cultivation. Most of them, and especially the most productive ones, have been readily invaded by pioneer tree species. Currently, the most accepted opinion is that the re-establishment of meadow management is essential for the biological conservation and mountain land protection. However, the possibility to maintain meadows in mountain areas depends on their productivity. A 2-year study was conducted on an abandoned sub-alpine meadow in order to verify the role of fertilization and cutting on its productivity. Four treatments were compared in a randomized complete block design with four replicates: (1) untreated meadow; (2) meadow cut two times per year; (3) fertilized meadow (120 N, 80 P_2O_5, 80 K_2O kg ha^{-1} yr^{-1}); and (4) meadow cut as treatment 2 and fertilized as treatment 3. The results confirm that productivity of meadow grassland depends on cutting, which reduces yield, while fertilization greatly increases the latter. Moreover, when fertilization is combined with the cutting, it compensates, thereby reducing the negative effect of cutting.

Keywords: yield, herbage components, sub-alpine meadow

Introduction

The socio-economic reforms that occurred in the Alps during the second half of the twentieth century resulted, among other things, in the abandonment of vast surfaces that were previously used for meadow or pasture. As a result of the abandonment, many such meadows and pastures, and especially those located in a natural forestry environment, were invaded by woody vegetation. However, while the relatively more fertile areas have been quickly reclaimed from the forest (Da Ronch *et al.*, 2010), the remaining areas maintained the characteristics of oligotrophic grasslands. With the aim to understand the effect of fertilization and cutting on the recovery of productivity of abandoned alpine meadows, a field study was conducted for two years on an abandoned sub-alpine meadow in north-eastern Italy.

Materials and methods

A field trial was carried out for two years (2006-2007) on a meadow in the southern side of the Pre-Alps of the Veneto region (45°57'N, 12°05'E) which had been abandoned for 25 years. The meadow had the following characteristics: 415 m elevation, 102° exposition, 66.83% slope, and calcareous soil (Redzicleptosols category), and the vegetation referable to the alliance *Bromion erecti* Koch 1926 (syn. *Mesobromion erecti* (Br.-Bl. et Moor 38) Oberdorfer 1949) (Da Ronch *et al.*, 2007). The mean annual temperature and precipitation over 25 years (1982-2007) were 12.5°C and 1502 mm respectively.
Four treatments were compared: (1) untreated meadow (control); (2) meadow cut according to the local management procedures: first cut at the flowering of the dominant species, second

cut at the maximum summer regrow of the Poaceae species; (3) meadow fertilized (120 N, 80 P_2O_5, 80 K_2O kg ha^{-1} yr^{-1}) and uncut; and (4) a meadow cut as treatment 2 and fertilized as treatment 3. The experimental design was a randomized block with 4 replicates. The plot size was 10.0 m^2 (2.0×5.0 m) with a test area of 4.0 m^2 (0.8×5.0 m). Fertilization was applied on 15 April 2006 and 5 April 2007. Plots were cut on 28 June and 29 September 2006 and on 18 July and 2 October 2007. The fresh weight of herbage was measured in the field and a subsample of 0.5 kg was collected at each cut and dried for 36 h in a drying oven at 105°C to determine dry matter (DM). Dry matter yield was determined at each cut for treatments 2 and 4, while for treatment 1 and 3 it was determined only at the end of the experiment, namely at the second cut of treatments 2 and 4 (2 October). On 27 September 2006 and on 30 September 2007, three forage samples of 25×40 cm were collected from each plot of treatments 1 and 3. The total biomass of each sample was then separated into green and dead biomass. The two components were dried in a drying oven at 105°C to determine the DM. In addition, in June 2006 and 2007 floristic surveys were performed in each plot using the transect method (Daget and Poissonet, 1971).The number of species, the total DM yield and the DM of single biomass fractions were subjected to analysis of variance, and Tukey's honestly significant difference test was used at the *P* level of 0.05 to identify significant differences among means.

Results and discussion

The analysis of variance for the number of species revealed that the effects of treatment and year, and their interaction, were all not significant. The average number of species over the four treatments and the two years was 31.5, and the most abundant species were *Brachypodium rupestre* (Host) R. et S., *Bromus erectus* Hudson, *Festuca nigrescens* Lam. non Gaudin; *Galium mollugo* L., *Salvia pratensis* L., and *Thymus praecox* Opiz.

A significant year × cut × treatment interaction (P<0.001) was detected for DM yield. In both years of investigation, treatment 4 had higher DM yield than treatment 2; however, the difference was higher in 2007, and especially at the second cut (Table 1). Significant differences in total DM yield among treatments were also found. Total DM yield was highest for the fertilized treatments (3 and 4), followed by the control, then by the treatment not receiving fertilization (Table 1). These results clearly point out the positive effect of fertilization on DM yield. The results of analysis of variance for DM of single biomass components revealed a significant interaction between treatment and year (P<0.001) for the green and the total biomass (green + dead). The amount of green biomass was higher in treatment 3 than 2, and the difference increased in 2007. Moreover, the total biomass obtained at the end of each growing season for treatments 1 and 3 is in line with the total DM yield detected for treatments 2 and 4, respectively (Tables 1 and 2). From these results it is evident that fertilization rapidly enhances the productivity of the meadow by promoting new growth plants.

Table 1. Dry matter yield (t ha^{-1}) of a sub-alpine meadow in 2006 and 2007.

Treatment	2006			2007			total
	Cut		total	Cut		total	
	1	2		1	2		
1 (control)							3.32 b
2 (cut meadow)	1.03	0.38	1.41	0.57	0.70	1.27	2.68 c
3 (fertilized meadow)							5.69 a
4 (cut and fertilized meadow)	1.59	0.56	2.15	1.30	1.79	3.09	5.24 a
Significance	**	**	**	**	**	**	**

** Significant at the 0.01 probability level

Means in the column followed by the same letter are not significantly different (P<0.05)

Table 2. Dry matter (t ha^{-1}) of above-ground biomass components at the end of growing seasons in 2006 and 2007.

Year	Treatment	Biomass		
		Green	Dead	Total
2006	1 (control)	0.46	1.27	1.73
	3 (fertilized meadow)	0.89	1.25	2.14
	Significance	**	ns	**
2007	1 (control)	1.00	1.84	2.84
	3 (fertilized meadow)	3.58	1.62	5.20
	Significance	**	ns	**

** Significant at the 0.01 probability level

Conclusion

Two years of experimentation were not sufficient to detect any substantial change on species richness of the studied meadow in consequence of cutting or fertilization. However, results demonstrate differences from cutting and fertilization on the recovery of productivity. While the former depresses the productivity of the meadow, the fertilization, apart from cancelling the negative effect of cutting, significantly increases yield favouring the neo-formation of biomass.

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Effects of abandonment on some characteristics of mountain pastures

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Abstract

In the second half of the 20^{th} century many pastures in the Alps were abandoned. In order to study the effect of abandonment on some characteristics of mountain pastures, a study was carried out at the bottom of the valleys of Val Menera (86 ha) and Pian Cornesega (15 ha), in the Cansiglio Forest (north-eastern Italy). Some of these pastures are still used for cattle grazing, while others have been abandoned for more than 30 years. A botanical survey was conducted on 100 m^2 plots established in grazed pastures and in abandoned ones. Herbage yield, forage value and herbage digestibility were also determined for each plot. The results demonstrate that vegetation of all pastures belongs to the association *Festuco commutatae-Cynosuretum*, although the cover of *Deschampsia caespitosa* (L.) Beauv was higher in the abandoned pastures than in the grazed ones. Both the number of species and the forage value decreased with increased *D. caespitosa* cover. The herbage digestibility was higher in the grazed than in the abandoned pastures, whilst no significant differences in herbage dry matter were observed between the two types of pastures.

Keywords: *Deschampsia caespitosa*, number of species, herbage yield, herbage digestibility

Introduction

In the European Alps, during the second half of the last century, many small villages on the mountain slopes became largely abandoned, and people migrated to more urban types of settlements along the floors of the main valley (Conti and Fagarazzi, 2004). As a consequence of this, pastoral activities in these areas, and especially summer pasturing, no longer guaranteed sufficient earnings from dairy farming. In the Italian Alps and Pre-alps these two facts caused a progressive underutilization of large grasslands previously used as pastures. This led, in many cases, to total abandonment of these areas, which has caused changes in the vegetation such as establishment of woody species (Finegan, 1984) or herbaceous species low in forage quality (Peter, 2007). In order to identify and quantify some consequences of this phenomenon, the characteristics of an extensive area of pastureland in the Cansiglio Forest were studied. Part of this area is still regularly utilized and a part has been abandoned for more than 30 years. We report here the effects of abandonment on productivity, forage value, herbage digestibility and species richness of these pasturelands.

Materials and methods

Cansiglio Forest (6000 ha) grows on a plateau, bearing the same name, which occupies the south-western-most area of the Carnic Pre-alps, partly in the Veneto Region and partly in the Friuli-Venezia Giulia Region. The Cansiglio plateau (46°06'N, 12°40'E) is a karstic formation composed essentially of a central depression (800-1000 m a.s.l.).
The climate of the plateau is characterized by an annual average temperature of 4.9°C and annual rainfall of 1872 mm. In this area there are several forest vegetation types including the mountain spruce (Picea excelsa (Lam.)) forest (Del Favero, 2000), which surrounds the pastures at the bottom of the valley of Val Menera and Pian Cornesega. There are 86 ha of

grassland in Val Menera of which 61 ha are divided into seven enclosures where cattle still graze in rotation at a stocking rate of 0.74 LSU ha^{-1}. The remaining 25 ha, as well as the 15 ha of adjacent pasture in Pian Cornesega, have not been used for more than 30 years. During the summer 2008, the vegetation of 74 plots, each of 100 m^2 (Pirola, 1960), randomly established in the 86 ha of Val Menera and 15 ha of Pian Cornesega grasslands, was studied following the Braun-Blanquet method. To summarize vegetation data, a cluster analysis was performed using the statistical package MULVA 5. The forage value of each plot, based on botanical surveys, was calculated according to the method proposed by Horroks and Vallentine (1999).
In 2008 and 2009, nine cages of 1 m^2 were set up in both grazed and abandoned pastures before livestock were introduced. At the end of the growing season (late August-early September) the herbage of each cage was harvested to determine herbage dry matter (DM). Herbage in a 1 m^2 plot in proximity to each cage was also harvested to determine the grazed herbage DM. All herbage samples were then analysed to determine *in vitro* dry matter digestibility (Tilley and Terry, 1963).

Results and discussion

Cluster analysis revealed two macro clusters of 38 and 36 plots, the first included only plots of the grazed pastures, while the second included all the plots of the abandoned pastures and some of the grazed pastures. The plots of each macro cluster were grouped into 2 and 3 sub-clusters (Table 1).
According to Mucina *et al.* (1993), the first macro cluster included plots with numerous characteristics and differentiating species of the class *Molinio-Arrhenatheretea*, of the order *Arrhenatheretalia* and the alliance *Cynosurion*. All those species are considered characteristic and differentiating of the association *Festuco commutatae-Cynosuretum*. Therefore, these surveys referred to characteristic vegetation types of fertile pastures at an elevation above 800 m a.s.l. and extensively managed. The 22 plots of the first sub-cluster belonged to the more fertile pastures, while the 16 plots of the second sub-cluster belonged to more thermo-xerophilic pastures.
Plots included in the second macro cluster were composed of most of the characteristic and differentiating species of the association *Festuco commutatae-Cynosuretum*, despite the fact that these species were present a much lower frequency and average percent cover than that observed in the first macro cluster. *Deschampsia caespitosa* (L.) Beauv. was one of the species that were exceptions to this trend, presenting an average percentage cover of 2.85 in the first and of 36.96 in the second macro cluster (Table 1). In addition, the second macro cluster includes four species of woodland edges, and which can also generally be found in forest communities. The 3 sub-clusters were grouped according to different average cover of *D. caespitosa* (19.69, 36.40 and 57.22 in the first, second, and third sub-clusters, respectively). Moreover, the characteristic species of forest plant communities or woodland edges are only present in the third sub-cluster.

Table 1. Cluster analysis result of 74 surveys conducted in grazed and abandoned mountain pastures, with mean *Deschampsia caespitosa* (L.) Beauv cover (%), number of species and forage value of macro and sub clusters.

	Number of surveys	Cover % of *D. caespitosa*	Number of species	Forage value
Cluster 1	22	2.25 ± 1.56	46 ± 7	1.9 ± 0.2
Cluster 2	16	3.67 ± 2.90	53 ± 4	1.7 ± 0.1
Macro cluster 1	38	2.85 ± 2.30	49 ± 7	1.8 ± 0.2
Cluster 1	10	19.69 ± 4.68	37 ± 10	1.3 ± 0.3
Cluster 2	17	36.40 ± 5.31	33 ± 5	1.0 ± 0.1
Cluster 3	9	57.22 ± 8.82	24 ± 8	0.4 ± 0.2
Macro cluster 2	36	36.96 ± 15.07	32 ± 9	0.9 ± 0.4

Hypothetically, the sub-clusters that formed the second macro cluster, represented three stages in the evolution of the *Festuco commutatae-Cynosuretum* identified in the first macro cluster, following its abandonment. In fact, *D. caespitosa* cover was significantly correlated to number of species, corresponded to a decrease of 4.7 species for each 10% increase of *D. caespitosa* cover (Figure 1a). Forage value was negatively correlated with *D. caespitosa* cover and positively correlated with number of species (Figure 1b and 1c).

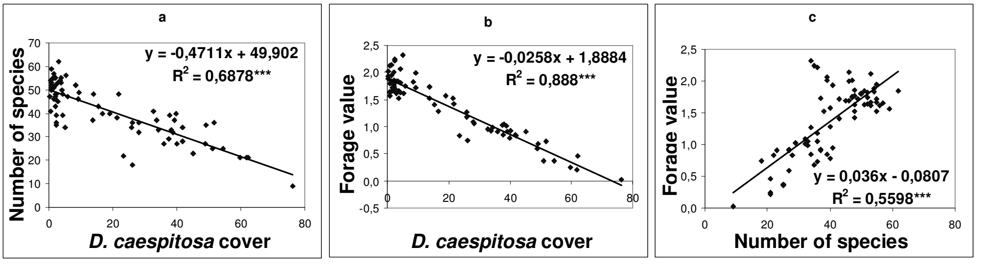

Figure 1. Correlations between a) *Deschampsia caespitosa* (L.) Beauv cover and number of species; b) *Deschampsia caespitosa* (L.) Beauv cover and forage value; c) number of species and forage value of 74 plots in grazed and abandoned mountain pastures.

The herbage dry matter yield of grazed pastures was 5.5±0.8 t ha^{-1} of which 2.5±0.8 was grazed by livestock. In the abandoned pastures herbage dry matter yield was 5.2±0.5 t ha^{-1}, of which 1.2±0.3 was presumably eaten only by wild animals such as deer. In fact, as demonstrated by Zweifel-Schielly *et al.* (2011) the Alpine red deer diet is mainly represented by graminoids that are more abundant in pastures than forests. Furthermore, the herbage digestibility of the grazed pastures was clearly higher than that of the abandoned ones.

Conclusions

In the studied area, as a consequence of the abandonment of the pasture ascribable to *Festuco commutatae-Cynosuretum*, there was a consistent increase of *D. caespitosa* presence and a decline of both the number of species and forage value. For this reason, while the annual herbage yield did not vary substantially, its digestibility was reduced significantly on the abandoned pastures.

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Short-term effects of prescribed fires on soil microbial properties of shrublands in the Western Pyrenees

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Abstract

In less favoured areas such as the mountainous valleys of the Western Pyrenees, shrubland expansion is a consequence of rural depopulation and the reduction in livestock activities. As a consequence, prescribed fires to control shrub encroachment are frequent practices. In March 2012, we initiated research to assess the effects of prescribed fires in interaction with grazing, on the soils of three shrubland areas that received the following treatments: burned and grazed, burned and non-grazed, non-burned and grazed, and non-burned and non-grazed. We describe the short-term changes observed in the chemical and microbiological properties of the soils, sampled twice: ten days and then four months after burning. N mineral contents increased in the burned plots, with a time lapse between the pulses of NH_4^+ and NO_3^-. Furthermore, soil microbial biomass and activity declined in burned areas after 4 months, which suggests that indirect effects of burnings on soil microbial populations are more important than immediate effects caused by a potential thermal shock.

Keywords: CLPP, Biolog, soil enzyme activity, shrub encroachment, prescribed burning

Introduction

Shrub encroachment in less favoured areas of the Western Pyrenees is a consequence of rural depopulation and reduction of livestock activities in rangelands. Traditionally, prescribed fires have been used as a tool to control biomass accumulation and shrub encroachment in order to improve grassland. These fires are made in winter time, when dry vegetation and wet soils increase their efficiency and avoid a rapid and hazardous spreading of flames. In Mediterranean areas, where wildfires are frequent and intense, prescribed fires have been used as a prevention tool to control fuel loads, and have been exhaustively studied (Eugenio and Lloret, 2006). But few studies have assessed the effects of prescribed fires in the temperate ecosystems of Southern Europe (Marcos *et al.,* 2009). Studies of this type are in urgent demand for environmental managers, who need solid criteria and tools to decide about the convenience of maintaining this deeply-rooted practice. The aim of this research is to evaluate the short-term effects of prescribed fires on the chemical and biological properties of the soil in highland communities, particularly focusing on the effects on the C and N cycles.

Materials and methods

The study site was located in the Aezkoa Valley, in the Spanish Western Pyrenees. In March 2012, three areas of 60×15 m were selected at different slopes around 1000 m a.s.l. Each area was divided in 4 plots of 15×15 m with the following treatments: burned and grazed, burned and non-grazed, non-burned and non-grazed, and non-burned and grazed. Burnings, made by the traditional method of shrub-to-shrub, attained low soil temperatures (the highest temperature recorded at 2 cm depth was 71°C). The soils were sampled from the first 10 cm under burned and unburned shrubs 10 days and 4 months after burning, and analysed for N mineral pools, microbial biomass C (chloroform fumigation and direct extraction), enzyme activities (acid phosphatase and β-glucosidase) and metabolic characteristics of the microbial community (31 C substrates on Biolog-EcoPlate^{TM}, Garland, 1997). Community level physiological profile (CLPP) was assessed by calculating average well colour development (AWCD), richness (S) and Shannon–Wiener index (H´) (Nair and Ngouajio, 2012). Data were transformed when necessary and analysed using a split-split-plot design with fire as the main plot factor, grazing as the sub-plot factor and date after fire as the sub-subplot factor.

Results and discussion

Because of the short-term of the samplings presented here, we did not find significant effects due to grazing, and we will focus on the effects of the fire. Prescribed burnings increased mineral N pools of burned soils. The NH_4^+ pulse was immediate and maintained over time whereas the NO_3^- pulse was delayed (Figure 1). These results were consistent with previous literature describing an increase of NH_4^+ pools after a burning, followed by an increment of NO_3^- pools anywhere from 30 days to 1 year (DeLuca and Zouhar, 2000). Plant combustion causes a pyromineralization of organic N, and the incorporation of ashes into the soil increases the NH_4^+ soil content. This ammonium may be partly nitrified and NO_3^- become available in the soil solution due to a decrease of plant uptake.

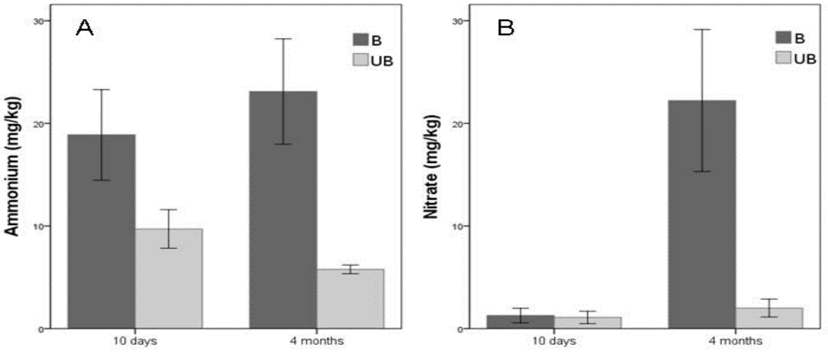

Figure 1. Prescribed fire effects on soil nitrogen mineral pools. B burned soils, UB unburned soils. Error bars, 1 standard error.

Size and activity of soil microbial populations were significantly affected by the fires, despite the fact that the microbial richness and diversity (Shannon–Wiener index) remained unchanged. Microbial biomass C, AWCD, acid phosphatase and β-glucosidase activities were not immediately affected by burnings but declined 4 months afterwards (Figure 2). This suggested that microbial populations were not affected by the thermal shock, but by indirect effect of a lack of vegetative cover after the fire. In burned areas, soil water content was lower and temperature oscillations higher because of a lack of a protective canopy. In addition, the partial elimination of an active rhizosphere in burned soils and the resulting decrease of root exudates inputs to the soil may also have led to a reduction of soil microbial activity.

Conclusion

Prescribed fires caused an increase of soil mineral N pools, which was immediate for NH_4^+ and delayed for NO_3^-. Depressing effects on soil microbial populations were reported 4 months later, which suggests that the indirect effects of fire (such as canopy elimination, reduction of root exudation,..), were more important than the direct effects caused by the thermal shock (<100°C at 2 cm depth). Long-term effects will be evaluated in order to provide new tools to decide on the convenience of maintenance of these practices.

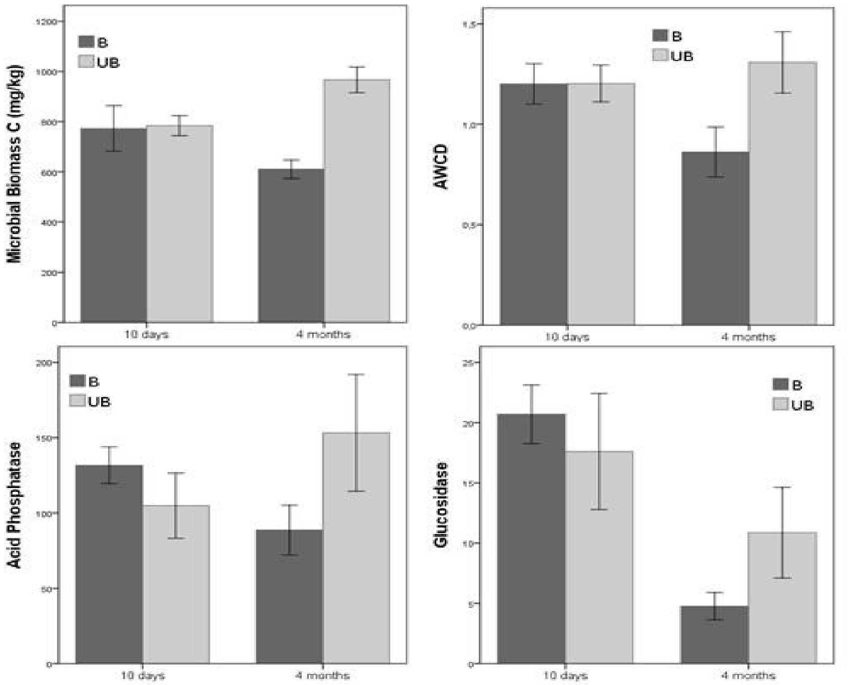

Figure 2. Prescribed effects on size and activity of soil microbial populations. B burned soils, UB unburned soils. Error bars, 1 standard error.

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Impact of different grassland management regimes on population biology and phytochemistry of *Colchicum autumnale*

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Abstract

In Austria, Colchicum autumnale reaches high population densities in grasslands under low management intensity. Farmers face problems using this fodder as the main toxic substance of the plant, colchicine, persists in hay and silage and is highly toxic to livestock. We tested four different management regimes at seven sites with the objective of reducing the abundance of *C. autumnale*. In 1m^2 plots, all *C. autumnale* individuals were recorded and assigned to one of six life stages every year (2008-2011). We analysed data with matrix population models, calculated population growth rates (λ), and used life-table response experiments (LTRE) to determine the contribution of demographic processes to Δλ between treatments and control. Colchicine concentrations were measured by high-performance liquid chromatography at biweekly intervals. The spring cut treatments resulted in the lowest λ in all years, and the lowest overall λ of 0.6. The LTRE showed that negative growth and positive regression caused the largest proportion of Δλ in the spring cut treatments. Generally, colchicine content was highest in flowers, capsules and young leaves. Different cutting regimes had no significant effect on the alkaloid content in the leaves. Cutting in early May, when colchicine content is very high, reduced *C. autumnale* populations most effectively.

Keywords: colchicine, *Colchicum autumnale*, low input-grassland, matrix population models, life-table response experiments (LTRE), phytochemistry

Introduction

Colchicum autumnale (autumn crocus) is a perennial geophyte, which is native to Europe. This toxic grassland species mainly occurs in grasslands of low management intensity (Winter *et al.*, 2011), which are often also species-rich habitats. High population densities are hindering fodder utilization, causing particularly negative impacts on hay sale, because toxic colchicine persists in high concentrations in hay and silage. Although livestock usually avoid *C. autumnale* under free-range grazing conditions, intoxications have been reported if livestock were fed with hay (e.g. Chizzola and Janda, 2002). High population densities of *C. autumnale* might lead to management intensification to eradicate this toxic plant or to complete abandonment; both changes will significantly reduce biodiversity. In this paper, we analysed the effects of different management regimes on the population biology and the colchicine content of *C. autumnale* to identify the optimum management date for a reduction of its abundance and colchicine concentration.

Materials and methods

Our investigation sites can roughly be characterized as mesophilous lowland hay meadows (Molinio-Arrhenatheretea) of low to medium management intensity. We applied four different management regimes on 20 permanent plots (1 m^2) at each of 7 sites in Lower Austria: a control treatment with the first cut in June (C), a flower treatment with the first cut in June and repeated flower-removal in 10-day intervals in autumn (F), a late spring treatment with the first cut in mid-May (LS), and an early spring treatment with the first cut in early May (ES). Each individual of *C. autumnale* was recorded and assigned to one of six life stages: seedling, vegetative plant with one, two and three or more leaves, generative plant with capsules, and dormant plant which does not appear above ground for one or two years (Jung *et al.*, 2011). We constructed transition matrices and calculated population growth rates (λ) for each treatment, site and transition period. To identify significant differences between treatments, we generated a 95% confidence interval for λ by bootstrapping the data (10,000 iterations). The contribution of different demographic processes to the difference in λ between the treatments F, LS and ES and the control treatment was analysed with a life-table response experiment (LTRE; Caswell, 2001). For the phytochemical investigations, *C. autumnale* samples consisting of 30 plants were collected and dried at biweekly intervals from April to October. The colchicine content in leaves, capsules and flowers was assessed by HPLC after ultrasonic assisted extraction with 30% methanol.

Results and discussion

The early (ES) and late spring (LS) treatments resulted in a reduced λ (Figure 1a), whilst the flower treatment (F) fluctuated slightly below or above 1, indicating population equilibrium. The population growth rate of the control treatment (C) was in all years, except for the last, greater than 1. The early spring (ES) treatment had the lowest overall λ of 0.62 in 2010/2011.

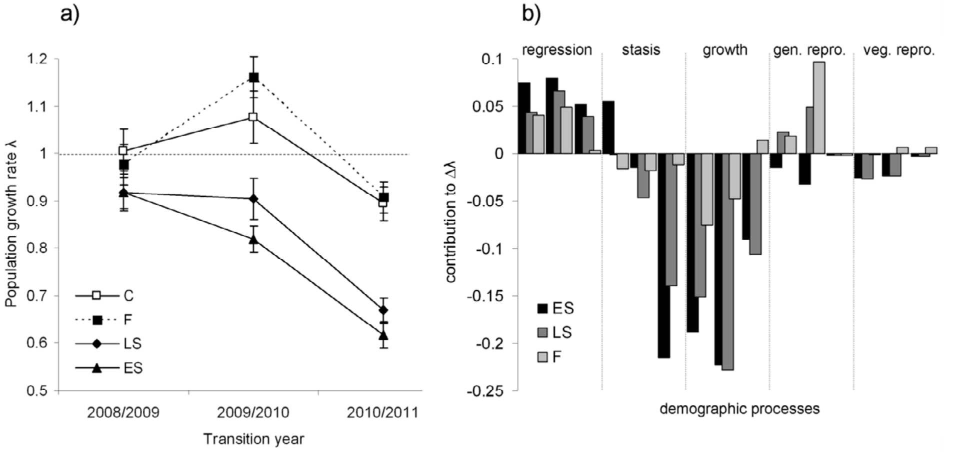

Figure 1. (a) Mean population growth rates (λ) with confidence intervals of the different treatments from 2008/2009 until 2010/2011. The broken horizontal line indicates a stable population (λ=1). (b) Contribution of demographic processes to Δλ between treatments and control (LTRE). The bar-triplets show the transitions 2008/2009, 2009/2010 and 2010/2011.

Life-table response experiments analysis revealed that differences in population growth rates between treatments and control were mainly caused by decreasing growth and increasing regression (Figure 1b). Stasis was negative in all transition periods (except for the early spring treatment, ES in 2008/09). The spring treatments (ES and LS) resulted in the lowest values of growth and in the highest of regression; vegetative reproduction was negative in all years.

The content of colchicine differed from site to site; it was highest in flowers, followed by capsules and young leaves. Colchicine concentration in the leaves decreased during the sampling period (Figure 2a), while in the capsules it remained nearly constant from May to early June, dropping rapidly afterwards (data not shown). The colchicine concentration in the flowers (7.5-10.1 mg g^{-1}) did not decrease until late September (data not shown). The treatments had little influence on the colchicine content in leaves and capsules (Figure 2b). In most of the seven monitored populations, slightly higher colchicine concentrations were observed in the control compared to the late spring (LS) treatment.

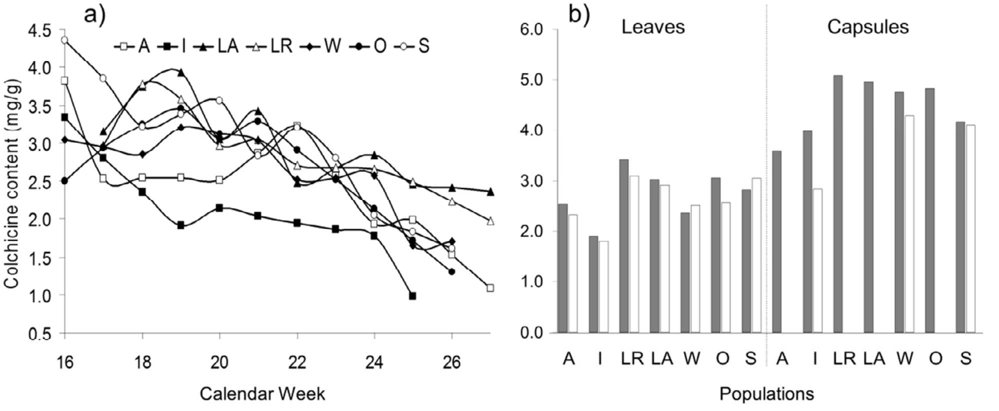

Figure 2. (a) Colchicine content in the leaves of seven populations from mid-April (16) to the end of June (26) 2010. (b) Colchicine content in leaves and capsules of the control treatment (grey) and the late spring treatment (white) of seven populations in 2010.

The negative effects of the spring treatments on population growth rate can be explained by the life cycle of the species: the corm renews itself each year and leaves emerge in March, whilst flowers appear in autumn. The plant starts to store nutrients for the next year in late April/early May when the old corm has depleted its storage reserves (Jung *et al.*, 2012). Consequently, an early leaf removal in spring reduces the nutrient reserves of the corm more than a later cut does, when the plant has likely already stored nutrients in the corm. At that time, however, colchicine concentration is very high and this might cause problems for fodder utilization.

Acknowledgements

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Hybrid of *Rumex patientia* × *Rumex tianschanicus* (*Rumex* OK-2) as a potentially new invasive weed in Central Europe

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Abstract

Since 2001 a hybrid of docks *Rumex patientia* × *Rumex tianschanicus* (*Rumex* OK-2) has been grown as a new energy crop in the Czech Republic. It was originally bred as a forage crop plant in Ukraine and then introduced for the same reason to the Czech Republic. In the past five years a successive spreading in the surroundings of the fields of the original plantation was observed. This paper evaluates the results of the two monitoring years (2011 and 2012) at the eastern edge of Prague where *Rumex* OK-2 was established on arable land. In 2011 each plant of the *Rumex* OK-2 was located by geodetic GPS equipment in the study area. In 2012, the presence of *Rumex* OK-2 plants was verified and some newly discovered plants were recorded. By comparison of the two successive years, we have shown successive spreading of *Rumex* OK-2, mainly in man-made habitats. It seems that *Rumex* OK-2 could have an invasive potential. Further detailed study of its biology and ecology is needed.

Keywords: energy crop, weed, spreading, *Rumex* OK-2

Introduction

Many broad-leaved *Rumex* species are considered to be the most difficult weeds in grasslands and arable land worldwide (Zaller, 2004). A new forage and energy crop hybrid *R. patienta* × *R. tianschanicus* registered as cv. *Rumex* OK-2 (hereafter referred as *Rumex* OK-2) also known as Uteush (after the breeder Prof. Uteush from Ukraine) was introduced to the Czech Republic about ten years ago (Usťak, 2007). This taxon has, moreover, recently been introduced into other European Countries (Bulgaria, Germany, Norway). It can potentially become a new invasive weed species, because the escape of *Rumex* OK-2 plants from cultivation into surrounding grassland has been recorded (Hujerová, 2010).

Rumex OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness, tolerance to salt stress and increased humidity (Kosakivska *et al*., 2008). Although *Rumex* OK-2 has been planted for ten years in the Czech Republic, its detailed ecological requirements, spatial distribution, possibility to hybridize with native species, and its potential to become a new weedy species has never been investigated.

In this study we present preliminary results of *Rumex* OK-2 spreading within two monitoring years in the vicinity of the former field where *Rumex* OK-2 was grown experimentally. In particular, we consider whether the taxon is able to spontaneously spread in the countryside.

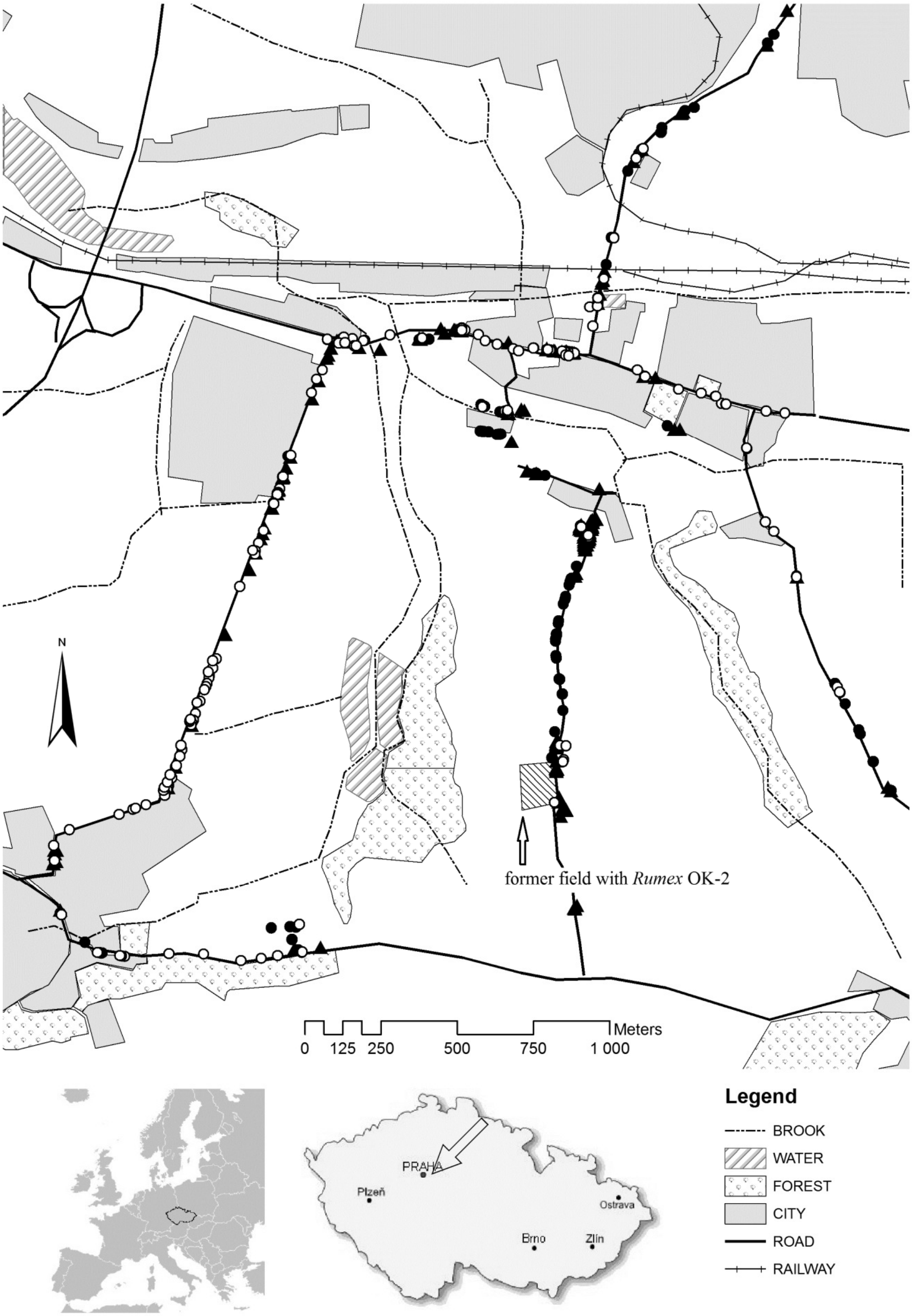

Figure 1. Map showing the distribution of *Rumex* OK-2 in the vicinity of Prague where a experimental field of the *Rumex* OK-2 was established about ten years ago. ▲ plants recorded in 2011 and 2012, ● plants recorded only in 2011, ○ plants recorded only in 2012.

Materials and methods

Monitoring was conducted over the years 2011 and 2012 in the eastern margin of Prague where *Rumex* OK-2 was experimentally grown about ten years ago. The ditches along both sides of the roads (± 3 m) were monitored, in total length about 20 km (Figure 1). Each plant of *Rumex* OK-2 was located (± 3 cm) using geodetic GPS equipment ProMark 200 and its distribution was recorded in a special map.

Results and discussion

In the first monitoring year (2011) 375 plants were found in the study area. In 2012, the second monitoring year, 264 plants (70.4%) were verified again. Furthermore, 275 additional plants were discovered. By comparing the two successive years we have shown successive spreading of *Rumex* OK-2 in the study area (Figure 1). The majority of recorded plants were present on the edges of fields and in grasslands along the roads. Although Usťak (2007) characterized *Rumex* OK-2 as a competitively weak plant that disappears from grassland vegetation after 2-3 years, our previous monitoring (Hujerová, 2010) showed that it is able to persist in grassland for a much longer time.

Conclusion

The preliminary results of two monitoring years (2011-2012) showed the expansive spreading of the *Rumex* OK-2 from the former field especially along roadside ditches. It seems that *Rumex* OK-2 could have an invasive potential and that further detailed study of its biology, ecology and distribution strategy in the landscape is needed.

Acknowledgments

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Rumex alpinus removal by integrated management methods

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Abstract

Results of different methods of *Rumex alpinus* management in mountain pastures are represented. *Rumex alpinus* is considered as a noxious weed on grasslands and can spread on large areas of mountain and alpine pastures. These pastures are often part of Natura 2000 areas or other areas under nature protection so the use of herbicides is not preferred. Therefore, we tested different integrated methods: controlled cattle grazing, pig rooting, mowing, heat treatment, covering with black foil, and manual digging. We used 4×4 m plots, with 4 four replicates. After applying the treatment the plots were sown with commercial grassland mixtures. The highest reduction of *Rumex* biomass after the first year was on the manual digging and foil treatment. Pig digging also proved to be successful, while other methods just reduced shoot number, and the cover and biomass of vegetation remained high. Further monitoring will be needed, at least for some seasons, but also the transfer of these methods on to large-scale treatment surfaces has to be tested.

Keywords: mowing, alpine pastures, heat treatment, Natura 2000, foil, cattle grazing, pig digging

Introduction

Rumex alpinus L. grows in most parts of the Alps, and other alpine areas in the Balkans, Asia Minor and the Caucasus, and in the subalpine region in the altitudinal range of 1500-2500 m a.s.l. (Hegi, 1957). The species generally occurs in nutrient-rich natural, anthropogenic, and transitional types of habitats that are well supplied with water (Dihoru and Dihoru, 1985; Ellenberg, 1996; Šťastná *et al.*, 2010). While the plant is regarded in some areas as one of the most unwanted weed (Šťastná *et al.*, 2010) it is also historically known as a valuable plant (López-Muñoz *et al.*, 2006).

R. alpinus is a perennial species consisting of a horizontal rhizome, above-ground vegetative shoots with three to five big leaves, and fertile stalks bearing smaller leaves and up to several thousands of flowers and fruits (Šťasna *et al.*, 2010). The rhizomes grow usually at a depth of up to 5 cm (Klimeš, 1992), less frequently at 10-12 cm (Kliment and Jarolímek, 1995). If the apical meristem of a rhizome is damaged, one or several resting lateral buds, usually the youngest ones situated closely to the former apical meristem, are activated (Klimeš, 1992). Because *R. alpinus* is a strong competitor forming species-poor stands, and the cover of *R. alpinus* is often close to 100% (Kliment and Jarolímek, 1995), integrated-removal management methods are seldom sufficiently effective, or there implementation needs to be long term. In this context the results of different integrated methods of *R. alpinus* removal management on Korošica planina are presented.

Materials and methods

The removal experiment was set on Korošica planina (1500-1700 m a.s.l.) in June 2012. Different management practices for *R. alpinus* removal were applied (heat treatment with

open flame and mowing every 14 days starting on 14 June, manual excavating, cattle grazing with 6 cattle for 2 h a day through the whole season, pig rooting with 2 pigs for 4 weeks, black foil as permanent cover, control). For each management we installed plots (4×4 m) with four replicates, except that for grazing and pig rooting we established one large plot (10×15 m) due to logistical constraints (we were unable to set more large plots and the number of animals was limited). In each plot we sampled at 14-day intervals the height of plants, number of shoots and visually estimated (van der Maarel, 2005) the cover of *R. alpinus*. Within each plot these characteristics were sampled in two 1m^2 plots, except in the large plots we sampled four small plots. Plants of *R. alpinus* were cut in autumn 2012 in 0.5×0.5 m plots (two replicates per sample plot) for biomass estimation. Biomass was air-dried and then dried in an oven (105°C for 24 h) and weighed.
Univariate statistics was made in Statistica 8 (Anova and Tukey HSD post-hoc test).

Results and discussion

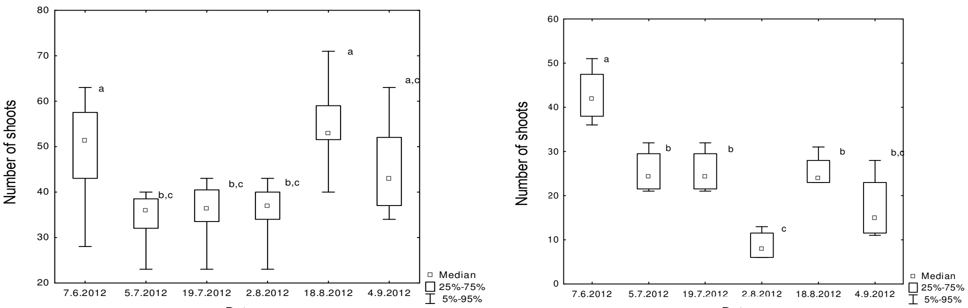

Figure 1. No. of shoots of *R. alpinus* in mowing (left) and pigs (right) treatment during first vegetation period.

Number of shoots shows there was an evident decline in the beginning of the vegetation season regardless of management (Figure 1). In mown plots there was a decline in the middle of the vegetation period and a peak in August, though the decline was not significant. Pig grazing reduced the number of shoots significantly at the end of monitoring.

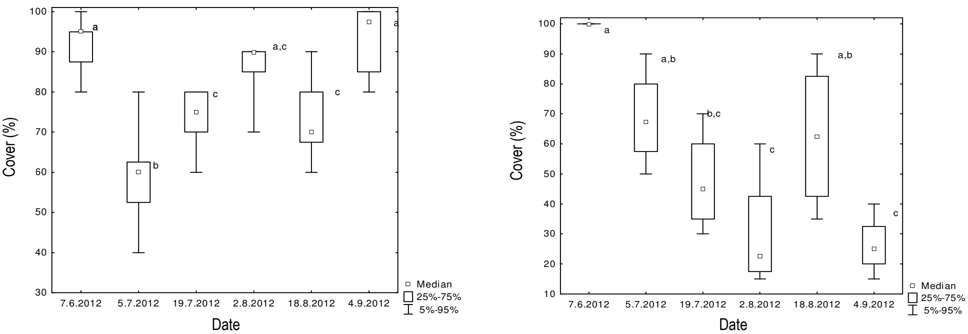

Figure 2. Cover of *R. alpinus* in mowing (left) and pigs (right) treatment along first vegetation period.

Changes of cover of *R. alpinus* show distinct changes during the vegetation season (Figure 2). Mowing reduced cover after the intervention, but the cover was quickly restored. At the end of the season the cover was the same as at the beginning. The pig-grazing treatment was more successful and cover at the end was significantly lower than at the start.

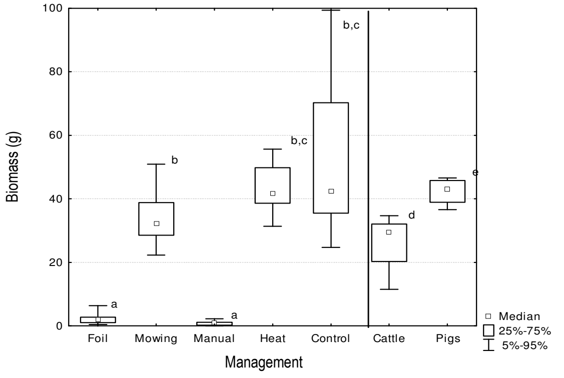

Figure 3. Biomass of *R. alpinus* in all treatments. Results are presented on the same graph although two sampling designs were used.

Similar results were obtained for above-ground biomass (Figure 3). Differences between groups were significant (ANOVA, F = 23.96, $P<0.001$). Manual excavating and use of foil are very successful in biomass removal and are significantly different from other treatments (Tukey HSD test). Cattle and pig grazing differ significantly in their effect on biomass removal (Mann-Whitney test, Z = -3.36, $P<0.001$).

Conclusion

Results of the first project year opened several solutions and questions, and more definite answers will be provided in further monitoring years. Different parameters used in monitoring give biased data on the success of *R. alpinus* removal as different treatments have different influence on plant parameters.

Nevertheless, manual and foil treatments proved to be the best solutions for biomass removal as a first step for successful *R. alpinus* management. But these treatments are applicable only on smaller surfaces. Use of animals shows intermediate success. Mowing is known to be a successful method but it requires a long term effort.

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Effects of low-input management on the biodiversity of a *Festuca valesiaca* L. grassland

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Abstract

Grassland biodiversity is affected by multiple factors including natural or anthropogenic actions. The alteration of natural agricultural ecosystems through modern agriculture as well as climate changes and environmental pressures has led to a series of changes in grassland biodiversity. The interest in immediate economic benefits of intensive, high-input agricultural systems, lacking any consideration for long-term environmental negative effects, raises the question of an alternative solution of low-input management of grasslands with reduced environmental impact. This paper aims to indicate the effect of reduced-input management on phyto-diversity of a *Festuca valesiaca* L.-dominated meadow from the Moldavian sylvosteppe. We used PC-ORD software with the MRPP method and Multidimensional Scaling NMS Autopilot for the interpretation of the floristic data. The greatest differences between treatments were registered between the plot fertilized with 10 t ha^{-1} cattle manure annually combined with mineral N_{50+50} P_{72} kg ha^{-1} (V3) compared with the plot fertilized with 40 t ha^{-1} manure every three years combined with mineral + N_{50+50} P_{72} kg ha^{-1} (V9), P<0.05. The botanical composition of these two plots was heterogeneous (A = 0.4843). The obtained results have shown that the positive effects of mineral and organic inputs have a positive effect on maintaining and improving grassland biodiversity.

Keywords: grassland management, biodiversity, low-input, floristic composition

Introduction

In the recent past, the main objective of grassland management in many European countries was to intensify the grassland system for the improvement of soil fertility and for increasing of forage yields per hectare (Maruşca *et al*., 2010). These actions also had negative effects on grassland ecosystems by reducing biodiversity, increasing groundwater pollution, and general landscape changes. Maintaining biodiversity through durable management of grassland ecosystems is a necessity and a major preoccupation of researchers in this field (Jankowski *et al.*, 2003). Among sources that threaten biodiversity and need to be considered are anthropogenic activities, aggressive pressure over natural resources, fragmentation, alteration and distruction of habitats, excessive use of pesticides, and chemical fertilizers (Vîntu *et al.*, 2007). The objective is to study the effect of low technological inputs, applied in small quantities and at different time intervals, on the biodiversity of the green canopy.

Materials and methods

Experimentation took place on a *Festuca valesiaca* L. meadow from the Moldavian sylvosteppe, situated on moderately sloping land, with a north-east exposition, and cambic chernozem weakly leached soil, silt clay texture; humus content was 4.2-4.8%, and with moderate mobile phosphorus (30-37 ppm) and highly supplied with mobile potassium (235-320 ppm). Soil pH is 6.5-6.9 in the 0-20 cm soil layer. The experiment was organized by the method of randomized blocks in three replicates and the harvesting method was cutting. We used the following experimental treatments:

V_1 - unfertilized control
V_2 - 10 t ha^{-1} cattle manure annually + P_{36} N_{50} kg ha^{-1}
V_3 - 10 t ha^{-1} cattle manure annually + N_{50+50} P_{72} kg ha^{-1}
V_4 - 20 t ha^{-1} cattle manure applied every 2 years + N_{50} P_{36} kg ha^{-1}
V_5 - 20 t ha^{-1} cattle manure applied every 3 years + N_{50+50} P_{72} kg ha^{-1}
V_6 - 30 t ha^{-1} cattle manure applied every 3 years + N_{50} P_{36} kg ha^{-1}
V_7 - 30 t ha^{-1} cattle manure applied every 3 years + N_{50+50} P_{72} kg ha^{-1}
V_8 - 40 t ha^{-1} cattle manure applied every 3 years + N_{50} P_{36} kg ha^{-1}
V_9 - 40 t ha^{-1} cattle manure applied every 3 years + N_{50+50} P_{72} kg ha^{-1}.

The floristic composition was studied with the Braun-Blanquet method. We used the PC-ORD software (McCune and Grace, 2002) for the interpretation of the floristic data. From the abundance of co-ordinates offered by this program, we selected the method MRPP (Multi Response Permutation Procedure) and multidimensional scaling NMS Autopilot.

Results and discussion

When experimental plots were overlapped, with the help of NMS Autopilot, we showed that technological inputs can bring major changes to green canopy biodiversity (Figure 1). As shown in the charts, phytocenosis do not overlay exactly, which demonstrates that there is not a high similarity between the floristic composition of treatments. The lack of high similarity between all 27 floristic surveys shows a high biodiversity with a high number of species, which varies from one fertilization treatment to another. In the case of treatments V_2 and V_8, the floristic composition overlays show a high similarity of the phytocenoses. We observed that some of the treatments (V_7, V_9) have distances which indicate the positive effect of the mineral and organic fertilization on maintaining and improving grassland biodiversity.

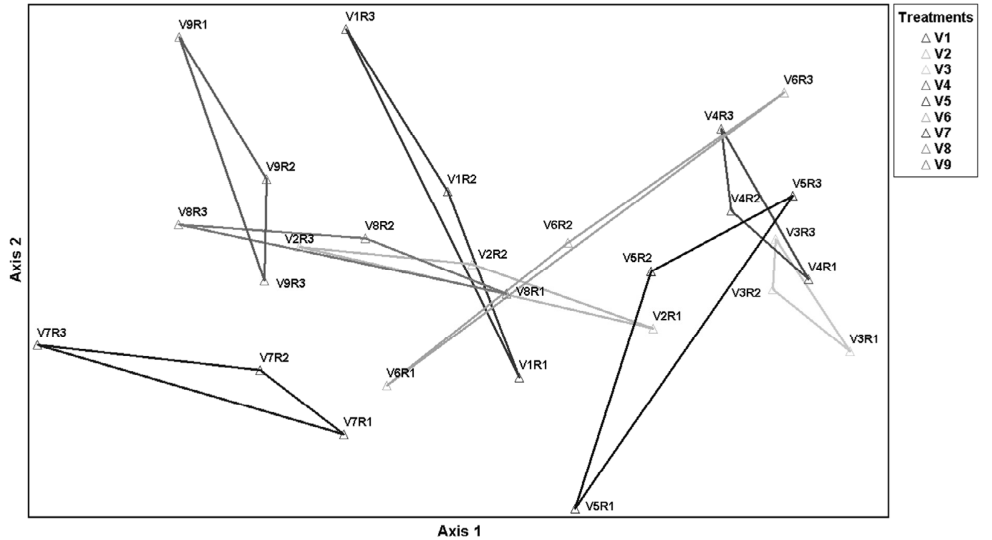

Figure 1. Ordering floristic composition according to combined organic and mineral treatments (*R1*, *R2*, *R3* – replicates).

With the help of MRPP 36, comparisons were performed between the nine experimental treatments. Table 1 shows only the comparisons between treatments that are statistically different (P<0.05). The greatest difference occurred between the treatment V_3, fertilized with

10 t ha^{-1} cattle manure annually + N_{50+50} P_{72} kg ha^{-1}, against the treatment V_9, fertilized with 40 t ha^{-1} cattle manure applied at 3 years + N_{50+50} P_{72} kg ha^{-1}; the floristic composition of these phytocenoses being heterogeneous (*A*=0.4843). As in the case of NMS Autopilot, the results obtained with the method MRPP underline the fact that mineral and organic inputs applied in moderate rates help the development of grassland biodiversity. The statistical method MRPP uses the t test, which describes the separation between groups. The higher that t is negative the separation is stronger. In our case, t had values between -1.735 (V6 vs. V7) and -2.905 (V3 vs. V9).

Table 1. Comparing floristic composition experimental treatments with MRPP (*t* - t test; *A* - homogeneous group).

Compared	t	A
1 vs. 3	-2.775	0.375
1 vs. 4	-2.698	0.338
1 vs. 5	-2.203	0.173
1 vs. 7	-2.201	0.211
2 vs. 3	-2.328	0.270
2 vs. 4	-2.341	0.242
3 vs. 6	-1.956	0.180
3 vs. 7	-2.898	0.477
3 vs. 8	-2.759	0.406
3 vs. 9	-2.905	0.484
4 vs. 7	-2.869	0.430
4 vs. 8	-2.670	0.324
4 vs. 9	-2.890	0.448
5 vs. 7	-2.616	0.294
5 vs. 8	-2.063	0.188
5 vs. 9	-2.680	0.305
6 vs. 7	-1.735	0.189
6 vs. 9	-2.231	0.211
7 vs. 9	-1.883	0.139

Conclusions

Treatments fertilized with 10 t ha^{-1} cattle manure annually + P_{36} N_{50} kg ha^{-1} (V_2), and with 40 t ha^{-1} cattle manure applied at 3 years + N_{50} P_{36} kg ha^{-1} (V_8) both have a floristic composition that overlaps, which shows a high similarity between phytocenoses. Mineral and organic inputs used in moderate rates have a positive effect on maintaining and improving grassland biodiversity.

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Silvopasture: a combination of grasslands and trees to green livestock production

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Abstract

Nowadays, livestock production is strongly dependent on inputs produced from outside the farm, such as fertilizers and concentrates. The combination of low-density trees and grasslands allows the feeding of animals in a cheap way, as tree branches can be used as fodder during periods of forage shortage. Moreover, increased productivity is ensured, as the land-equivalent ratio of 1 ha of silvopasture is between 1.2 and 1.6 ha of forest and crop monocrops, to produce the same amount of products. The presence of trees in grasslands at low density will promote biodiversity, carbon sequestration and nutrient recycling; therefore, a promotion of efficiency in the use of the resources is definitively enhanced. This paper reviews results from a series of experiments to show how silvopasture could promote production and environmental services to provide more sustainable land use options in livestock production.

Keywords: agroforestry, high quality wood, grassland biodiversity, carbon sequestration

Introduction

Agroforestry systems are sustainable land use production systems that were promoted by the last EU Common Agrarian Policy (CAP) (2007-2013) and which will also be an eligible system in the new CAP-period (2014-2020) (EURAF 2012). This system increases production per unit of land due to the best use of resources but, at the same time, it also increases ecosystem services. Biodiversity and carbon sequestration are promoted, but a reduction in nitrate and phosphorous leaching is also usually found. Therefore, an increase of this type of land use in Europe should be promoted. In this article, a summary of the main benefits for livestock production is explained, based on the findings of different experiments.

Production

Livestock dependence on inputs is evident in most intensive systems throughout Europe. Fertilizer use, as well as concentrate use based on external farm resources, allow easy land management for livestock farmers and simplify the systems, thereby improving the lifestyle of farmers who buy the farm inputs instead of producing them. More recently, however, due to rising costs the use of external farm inputs for production has been found not to improve farmers' lifestyle, as such an external-dependent system often causes enormous economic pressure. Furthermore, intensification based on external resources reduces the production of ecosystem services and is becoming unsustainable as transport costs are growing, in recent years causing an increase of resource and production costs and therefore reducing economic benefits. Several options have to be shown to farmers to increase profits, most of them based on traditional systems with current technical improvements. This is especially important if the increase of world temperatures is considered; higher temperatures can increase the period of the year when there are not enough food resources to feed animals. Enhanced temperatures in the world may increase the period of drought during the spring in some areas causing pasture shortages. The use of woody vegetation could be a solution to overcome this shortage period, as happens in the Mediterranean areas. *Morus alba* leaves and small branches are good examples used in most of the Central and South America countries to feed large livestock, as can be seen in the proceedings of the FAO electronic conference (FAO, 2000). *Robinia pseudoacacia* has also been tested for this purpose in North America (Snyder *et al*., 2007). In Europe, hard-harvested leaves of *Fraxinus excelsior* and *Betula pubescens* have been extensively used to feed animals during the wintertime. The cultivation of these species, based on the coppice management system, facilitates plant harvesting and chipping to be stored and used to feed animals during the shortage periods. Most of these species provide high levels of crude protein; e.g. the CP concentration of *Morus alba* reaching 24% (FAO, 2000). There are some places where these plants could be grown in lines surrounding the plots and used to feed animals directly. The use of trees or shrubs for feeding animals is expected to increase the biomass production due to it providing the best use of the resources (as tree and shrubs grow deeper in the soil and higher above the soil) as demonstrated values of 1.4-1.6 LER (Land Equivalent Ratio) found for the combination of tree for timber and crops (SAFE 2006).

Biodiversity

Agroforestry generates biodiversity because it increases the heterogeneity of land at the plot, farm and landscape level. The combination of woody with herbaceous vegetation also provides at least a double-layer system that could enhance the presence of animals, including birds, and also of insects that could be useful for reducing the need for pesticides. This could be one of the main reasons to promote the use of agroforestry in the 7% of the ecological-focus area proposed in the new CAP-period 2014-2020. The increase of birds and beetles in agroforestry systems, compared with other land uses, has been found in the UK (McAdam and McEvoy, 2008). Increased biodiversity (alpha and beta) has also been found by Moreno *et al*. (2007). The association of different types of invertebrates to different woody and herbaceous layers has been also described (Burgess, 1999; Rosa-García *et al*., 2013). Moreover, agroforestry could be one of the key land-use systems to promote the preservation of different autochthonous livestock around Europe (Mosquera-Losada *et al*., 2005).

Climate change mitigation

Globally, livestock are one of the largest emitters of methane from human-related activities, but livestock farming also contributes to emissions of nitrous oxide (a very potent greenhouse gas) and carbon dioxide, the most abundant greenhouse gas (EPA, 2012; FAO, 2006). The livestock sector is a major player, responsible for 18 percent of greenhouse gas emissions measured in CO_2-equivalent at a global level. The Kyoto Protocol establishes that land use, land use change and forestry (LULUCF) activities such as afforestation, reforestation, and deforestation (Article 3.3), and forest land management, cropland management, grazing land management, and revegetation (Article 3.4) can be used to meet the greenhouse gas (GHG) emission reduction goal (UN,1998); therefore, agroforestry is included. There are examples of promotion of agroforestry under different policy frameworks like that of the Global Environmental Facility (GEF) and the World Bank support to regional projects in Central America, which uses payment for environmental services as a tool to promote the conversion of degraded pastures towards more complex vegetation, like agroforestry in Costa Rica (FAO, 2006). China has also developed programmes to promote agroforestry to fulfil Millennium requirements. The main advantage of multilayer systems, including agroforestry, is the colonization of different soil depths by roots. More than 85% of terrestrial C is stored in terrestrial ecosystems. Several examples of C-sequestration promoting by European agroforestry systems are described in Mosquera-Losada *et al*. (2011).

Nutrient recycling

The promotion of nutrient recycling on grassland with planted trees, even at a low density, is based on the increased biodiversity caused by the heterogeneity generated by the trees. Different plant species are able to colonize different soil habitats and are therefore more efficient in nutrient uptake. The most important example is the comparison between the soil depths colonized by tree roots (deeper) and the herbaceous-layer groups described for the dehesa system (Howlett *et al*., 2011). In this sense, the findings described by Nair *et al.* (2007) comparing the uptake of P, are also important. Nutrient recycling, mainly N, is highly relevant in areas where the level of N coming from diffuse contamination by high N inputs has resulted in serious water pollution problems. Uptake of N in excess, by tree roots, is highly relevant because trees generate leaf litterfall, which can be used by grasslands after decomposition, as shown by the fact that levels of nutrients are higher below the tree the than further away (Moreno and Pulido, 2009; Pardini *et al*., 2010).

Conclusions

There are different underlying reasons explaining the need for promotion of agroforestry, which could be for both traditional or new-types of agroforestry systems, as a sustainable land use system in Europe. Biodiversity promotion and preservation, carbon mitigation and nutrient-cycling enhancement, as well as shortages of productivity caused by the effects of global warming could all be benefitted by this land-use option.

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Extensive grassland systems in Western Norway – effect of N-fertilization, clover and sheep grazing intensity

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Abstract

On the western coast of Norway abandonment of farming and a reduction in the number of farming animals result in plenty of farmland available for farmers remaining active. This development promotes an extensive farming system based on roughage production, grazing and use of less mineral fertilizer. We have studied how nitrogen (N) fertilization, seed mixtures, use of clover and sheep grazing intensity influence dry matter yield (DMY), forage nutritive quality, as well as the persistence of leys in a grazing-cut-grazing management regime. Decreasing N-fertilization from 140 to 96 kg $ha^{-1}yr^{-1}$ decreased both DMY and forage crude protein (CP) content in pure grass stands, whereas in grass-clover stands the DMY was not depressed and forage CP content was less affected. Including clover in the seed mixtures increased both DMY and CP at both N- levels compared to pure grass stands, whereas the net energy for lactation (NEl) in the forage decreased. Reduced grazing intensity on permanent ley during spring lowered the NEl and CP content of the forage. However, mowing the field after spring grazing compensated this. A low input grazing-cut-grazing management regime might become a way to make efficient use of farmland areas which are at risk of encroachment and still producing forage with a satisfying nutritive quality.

Keywords: CP, DMY, encroachment, forage nutritive quality, NEl

Introduction

Extensive management systems based on reduced fertilization and grazing intensity of grassland are often used when the land area per farm increases. However, maintaining the forage nutritive quality at an acceptable level might be a challenge with this strategy. The objective of the study was to examine the impact of N-fertilization, the use of clover, different seed mixtures and sheep grazing intensity on dry matter yield (DMY), forage nutritive quality and persistence of leys in grazing-cut-grazing systems.

Materials and methods

A field trial was conducted at Fureneset in Fjaler, western Norway (61°22'N, 5°24'E) during the years 2008-2011. The trial took place on silty sand with 11-13% loss on ignition in 0-5 cm depth and 10-12% in 5-20 cm depth. The following seed mixtures were applied (percentage based on weight): 1) 35% timothy (*Phleum pratense* L.), 35% meadow fescue (*Festuca pratensis* L.), 20% smooth meadow-grass (*Poa pratensis L.)* and 10% perennial ryegrass (*Lolium perenne* L.); 2) 10% red clover (*Trifolium pratense* L.), 10% white clover (*Trifolium repens* L.) and 80% of seed mixture 1; 3) 45% meadow fescue, 40% smooth meadow-grass and 15 % colonial bentgrass (*Agrostis capillaries* L.); 4) 20% white clover and 80% of seed mixture 3; 5) 60% perennial ryegrass and 40% ×*Festulolium*; 6) 10% red clover, 10% white clover and 80% of seed mixture 5. Two levels of a compound fertilizer (18-3-15 NPK) were applied: 96 and 140 kg N $ha^{-1}yr^{-1}$. Fertilizer was applied at a rate of 20 and 31 kg N in the spring, 45 and 70 kg after simulated grazing, and 31 and 39 kg after the summer harvest.

The experiment was designed as a split plot with four replicates. The fields were managed with a simulated grazing treatment with three cuts during the spring, harvesting in July, and simulated grazing with 1-2 cuts in the autumn. Before the harvest in July at least 45 timothy tillers were cut at soil level from three replicates of seed mixture 1 and 2 and development stage (mean stage by count, msc) was estimated according to a phenological scale based on the amount of tillers in the vegetative, elongative, regenerative or flowering stage (Moore *et al.*, 1991). In a separate experiment in Sørdalen in Fjaler (61°17'N, 5°12'E) sheep grazed a permanent ley during both spring and autumn in the years 2008-2010. This field was fertilized with 140 kg N $ha^{-1}yr^{-1}$ (30 kg in the spring, 80 kg after spring grazing and 30 kg after the summer harvest). Two grazing intensities, each combined with mowing and not mowing the fields just after the grazing period, were examined: normal (300-350 m^2 ewe^{-1}) and reduced (650 m^2 ewe^{-1}). At both Fureneset and Sørdalen DMY was recorded and the botanical composition was estimated visually just before the summer harvest. Net energy for lactation (NEl) and crude protein (CP) content in the harvested forage were estimated by NIRS analyses. A General Linear Model was applied for analysis of variance and to evaluate significance of the treatments.

Results and discussion

The grass-clover stands in general gave significantly higher DMY than the pure grass stands (P<0.001).The largest differences occurred at low N-fertilization, but there were also significantly higher DMY in grass-clover stands at high N-fertilization. Øyen & Aase (1987) found in Western Norway that a clover content of 30% gave the same N-effect as 100 kg N $ha^{-1}yr^{-1}$ applied to pure grass.

Table 1. DMY (t $ha^{-1}yr^{-1}$), NEl (Mj kg DM^{-1}), CP (% of DM) and field clover content according to seed mixture at low and high N-fertilization levels. Average for the years 2008-2011 at Fureneset.

Seed	DMY		NEl		CP		Clover	
Mixture	Low N	High N	Low N	High N	Low N	High N	Low N	High N
1	4.70	5.83	6.42	6.28	9.6	10.7		
2	7.86	8.05	5.93	5.93	14.9	15.4	36	31
3	4.97	6.60	6.14	6.14	11.1	12.3		
4	7.29	7.67	6.00	6.07	15.1	15.8	22	22
5	5.09	6.03	6.28	6.21	8.8	9.6		
6	7.55	8.30	5.93	5.93	13.7	14.9	31	29

There was a significant difference in NEl (P<0.05) between the seed mixtures 1 and 2 and also between the mixtures 5 and 6 at both fertilizer levels. In both cases NEl was lowest in the grass-clover stands. Between the seed mixtures 3 and 4 there were no significant differences for either low or high N-fertilization (Table 1). In the grass-clover stands (mixture 2) timothy was at a significantly (P=0.003) later phenological development stage at the cutting time compared to the pure grass stands (mixture 1). In the pure grass stands the content of CP was low, even with high N-fertilization, in particular for seed mixtures 1 and 5. Including clover in the mixtures resulted in significantly higher contents of CP (P<0.001). For pure grass stands the high N-fertilization treatment resulted in significantly higher concentrations of CP. There was a slight tendency for less clover in the fields with the highest N-fertilization, but none of the differences were significant.

The amount of red clover in the grass-clover stands decreased year by year, but there was still quite a high amount left after four years under intensive grazing/cutting. The content of white clover increased when it was mixed with the red clover, but decreased when it was the only clover.

Table 2. Impact of grazing intensity and mowing on DMY (t $ha^{-1}yr^{-1}$), NEl (Mj kg DM^{-1}) and CP (% of DM) in the years 2008-2010 at Sørdalen.

		2008			2009			2010		
Grazing intensity	Mowing	DMY	NEl	CP	DMY	NEl	CP	DMY	NEl	CP
Reduced	Unmown	4.13	5.49	11.9	4.82	5.19	10.2	3.66	5.81	13.5
	Mown	2.62	6.40	16.1	3.45	6.01	12.3	2.86	6.26	18.9
Normal	Unmown	3.11	5.95	13.7	4.20	5.52	11.1	2.73	6.22	17.8
	Mown	2.61	6.26	15.8	3.68	5.89	11.4	2.93	6.27	18.4

In Sørdalen reduced grazing intensity during spring increased DMY and reduced NEl and CP content in the forage at the summer harvest (Table 2). However, the impact was not significant every year. The NEl of the forage increased significantly by mowing the field after reduced grazing intensity in the spring, whereas the CP content increased significantly in 2010 only. Mowing the field after a normal grazing intensity did not have this effect. The results suggest that when grazing intensity is reduced, fodder from one harvest might be used during periods when animals do not require a high forage nutritive quality. Mowing may increase the nutritive quality in a way that the forage also might be used during periods when animals have higher demands.

Conclusion

Including 20% clover in the seed mixtures resulted in a higher DMY response than increasing total N-fertilization during the growing season from 96 to 140 kg N ha^{-1}. The CP increased considerably in grass-clover stands compared to the pure grasses, while NEl decreased. The different N-fertilization levels did not have any impact on the amount of clover in the leys. A low input grazing-cut-grazing management regime might become a way to make efficient use of farmland areas which are at risk of encroachment and still producing forage with a satisfying nutritive quality.

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Stresses on extensive grasslands in Iceland – A review

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Abstract

Grasslands in Iceland are either fertilized hayfields or unfertilized pastures. Stresses to grasses can be physical, chemical, biotic or anthropogenic. All kinds of stresses can be experienced in all seasons of the year and plants will be exposed to a number of stressors at one time, and interaction between them is highly likely. During summer, stresses caused by grazing and harvesting dominate, and also desiccation, soil compaction, and mineral toxicity or deficiency as well as weeds and pathogens may cause stresses and damage or kill grass plants. Grazing by livestock can be controlled by the farmers, and fortunately grasses tolerate that kind of stress better than other plant types. During winter the buds of grasses are often protected by withered grass and a snow layer and therefore do not experience extremely low air temperatures. The most common and serious plant damage on grasses during winter is ice encasement damage, where water freezes to ice after a warm spell and the ice cover lasts for a long time. Then metabolites are accumulated under the ice to lethal concentrations. Additionally, during winter there are several species of low-temperature fungi, so called snow moulds, which can attack and kill the grasses under the snow.

Keywords: hayfields, ice encasement, grazing, pastures, pests, stress

Stresses

Annual plants experience only summer stresses, while perennial plants are also stressed during winter. Stress is caused by different stressors and the plant is in a state of strain (Levitt, 1980). Strain can be characterized as physiological changes that occur in response to environmental stress. Orcutt and Nilsen (2000) have classified environmental stresses to plants into four classes; physical, chemical, biotic and anthropogenic stresses (Table 1). If the strain exceeds the tolerance of the plant cells the organism may become injured or even killed (Levitt, 1980). Plants have the ability to increase their tolerance to most strain types in a process called hardening. As seen in Table 1 the stressors are very diverse and the stress processes vary accordingly.

Icelandic grassland

Icelandic soils are andosols of volcanic origin and are therefore prone to erosion. Basically, Icelandic grasslands can be divided into two categories:
(1) Hayfields for production of winter feed for the livestock, fertilized and dominated by sown grass species. This category also includes permanent hayfields, for grazing and haymaking, sometimes fertilized and always dominated by indigenous grass species.
(2) Unfertilized pastures in the homeland and highland grazed by sheep and horses. Grasslands in both these categories, hayfields and pastures, are subject to different kinds of stresses. As most grass species are perennial they are stressed both summer and winter.

Table 1. List of possible stresses to plants. Modified from Orcutt and Nilsen (2000) and Levitt (1980). + sign indicates the intensity of damage: + = minor damage, ++ = medium damage, +++ = extensive damage.

			Summer stress		Winter stress	
Stresses	Stressors	Examples	Hayfields	Pastures	Hayfields	Pastures
Physical	Drought	Desiccation	++	+		
	Temperature	Heat, chilling, freezing			+	
	Radiation	x-rays, γ-rays				
	Anoxia	Flooding, icing	+	+	+++	+
	Wind	Erosion		++		+++
	Soil compaction	Trafficing	++	+	+	+
	Magnetism	Magnetic fields				
Chemical	Salinity	Sea flooding				
	Nutrient deficiency	N-shortage, P-shortage	++	+		
	Soil pH	Mn-toxicity, Fe-toxicity	++	+		
	Atmospheric gases	Ammonia toxicity	+			
	Oxidative	Free radicals	+	+	+	
Biotic	Competition	Weeds	++	+		
	Herbivory	Livestock, invertebrates	+++	+++	+	+
	Pathogens (parasites)	Fungi, viruses	++	+	+	
	Allelochemistry	Allelopathy	+			
	Harvesting	Grazing, haycutting	++	+		
Anthropo-genic	Atmospheric pollution	Ozone, Acid rain, NOx, Greenhouse gases	++	+		
	Pesticides, herbicides	Roundup	+			
	Heavy metals	Cd-toxicity, Pb-pollution	+	+		
	Fires	Forest or grass fire		+		

Summer stresses

Desiccation is only occasionally a problem in Iceland. Water surplus is more common and plants are subject to flooding, resulting in anoxia (McKersie and Leshem, 1994). The release or immobilization of nutrients is related to soil pH, which can result in mineral deficiency or toxicity. Icelandic soils are low in N and P content and the lack of these minerals is the most frequent cause of a reduced harvest. At a low pH, toxic levels of Mn, Fe and Al can be reached. Biotic stresses are probably most harmful to hayfields. This includes grazing by animals, which is more harmful at certain times of the growing season. Damage caused by invertebrates is not extensive, but the winter grain mite (*Penthaleus major*) and earlier the antler moth (*Cerapteryx graminis*) have caused damage. Pathogenic leaf-spot fungi such as *Rhynchosporium* spp. *Helminthosporium* spp. as well as rusts (*Puccinia* spp.) and powdery mildew (*Erysiphe graminis*) may reduce yield. Weeds can compete with grasses. The main weeds in Icelandic hayfields are buttercup (*Ranunculus acris*) and dandelion (*Taraxacum officinale*) and also nowadays cow parsley (*Anthriscus sylvestris*). It has been hypothesized that some of the weeds (e.g. *Poa annua*) use allelopathy to compete with sown grasses in hayfields. Anthropogenic stresses include the indirect impact of greenhouse gases on climate change. Pesticides and herbicides are only sporadically used in hay production in Iceland and are no real threat to production. The use of fertilizer has occasionally introduced heavy metals to the fertilized soil, but not a threat. On the other hand, hay cutting reduces the winter hardiness of cut plants compared with uncut plants. The stresses in pasture grasses are similar but in most cases milder than the stresses in hayfields. The most serious stress in pastures is wind erosion, related to the erodable nature of the andosols.

Winter stresses

Direct freezing stress occasionally causes damage, especially during spring if the winter hardiness has been reduced during winter by other stresses. The main stress to hayfields during winter is ice encasement, when plants are incorporated in ice for a longer or shorter period. Then the plants cannot respire naturally and harmful metabolites from anaerobic respiration are accumulated to toxic levels. Also, free radicals may be produced when plants return to air. The 'rule of thumb' is that hayfield grasses can tolerate up to three months of ice cover. Low-temperature fungi (snow moulds, e.g. *Microdochium*, *Typhula*, *Sclerotinia*) occasionally cause minor damage. Livestock can cause damage by grazing and compression of wet soil. The main winter damage to pastures is erosion of the easily eroded andosol soils. Ice is never as damaging in pastures as in hayfields, mainly because the uncut pasture plants are more winter hardy than the fertilized and harvested field crops.

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Species responses to different disturbance regimes and their short-term cessation in Mediterranean semi-arid grasslands

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Abstract

Mediterranean grasslands constitute an important ecological and economic resource that has been extensively influenced by human activities. The aim of the current study is to investigate the responses of annual and perennial grassland species to a number of disturbance regimes and also to its short-term cessation. In a fenced area, four treatments were applied, namely: prescribed burning, digging, cutting and a control, for three growing seasons, in a completely randomized block design with four blocks. The species' biomass was measured annually in May, and was separated into annual grasses, perennial grasses, annual legumes and forbs (which included all other species). One and seven years after the cessation of the treatments, the measurements were repeated. Annual grasses and legumes constituted the largest part of the total biomass in the first year of the experiment. Disturbance gradually reduced their biomass in favour of forbs. Seven years after cessation of the treatments, the annual and perennial grasses comprised more than 80% of the total biomass. Under conditions of disturbance, annual species had an advantage over perennials, whereas the cessation of disturbance enables perennial grasses to dominate.

Keywords: burning, digging, cutting, biomass recovery

Introduction

Mediterranean grasslands are dominated by annual species. The dominance of annuals has been associated with ruderal environments due to summer drought and human disturbances that radically affect successional trajectories and change species composition and their relative abundance. Different disturbance regimes may affect the ecosystem differently. Individual grassland species have evolved specific adaptations to burning that allow them to survive periodic fires, as fire directly affects both aboveground vegetation and the seed bank (Pausas *et al.*, 2006). Grazing, on the other hand, affects the vegetative community by altering the productivity and composition of grasslands (Noy-Meir *et al.,* 1989), whereas digging is regarded a more serious disturbance destroying all biomass. The aims of the current study are to investigate the contribution of annual and perennial grasses, legumes and forbs to total biomass under disturbance regimes, as well as to its short-term cessation.

Materials and methods

The research was conducted in Mediterranean grassland with semi-arid Mediterranean climate. A previously grazed area was fenced and an experiment established within the area excluded from grazing. The experiment was a completely randomized block design with four blocks and four treatments: prescribed burning, digging, cutting, and control. All plots were 3×3 m in size. Prescribed burning and digging (to about 10 cm depth) were applied in early autumn, in each of three years (1994, 1995 and 1996). In the cutting treatment, plants were cut once every year at 3 cm above ground level in late spring. Biomass was sampled five

times in all treatments at the end of the growing season in 1995-1998 and in 2004. The first three samplings were carried out during the period that disturbances were applied, and samplings in 1998 and 2004 were taken one and seven years after their cessation. Herbage was sampled in five quadrats (0.5×0.5 m) in each plot by cutting the aboveground vegetation. Grasses, legumes and forbs were hand separated and their dry biomass was weighed.
The Principle Response Curves (PRC) method described by Van den Brink and Ter Braak (1999) was used to identify the effect of the treatments on biomass composition over the experimental period. PRC focuses on the extent of differences among the individual experimental treatments at individual times (Leps and Smilauer, 2003). It relies on a partial Redundancy Analysis (RDA) in which the sampling times are used as covariables and the interactions between treatments and sampling times are used as the environmental variables. As the control should represent a reference point for each sampling time, no interactions with control are included. The result is a set of four principle response curves where the response curve of the control treatment corresponds to 0 value on the Y axis and is used as a reference point to evaluate the degree of change caused by each treatment in each sampling year.

Results and discussion

The results of the PRC analysis are shown in Figure 1. The significance of the first ordination axis (P=0.002) indicates a significant interaction between experimental treatments and time. Furthermore, the first axis summarizes 62% of the total variation explained, allowing interpretation of the effects of experimental treatments on biomass composition over the duration of the experiment.

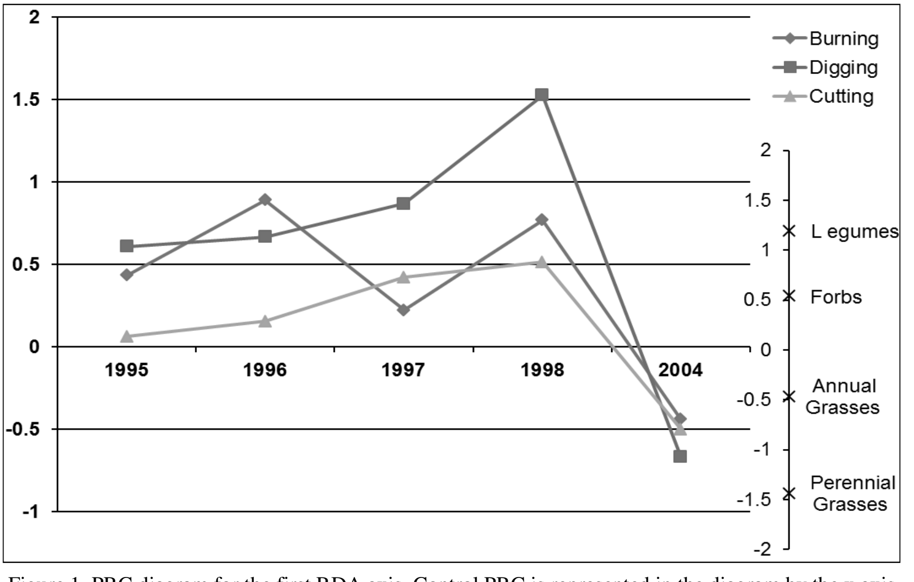

Figure 1. PRC diagram for the first RDA axis. Control PRC is represented in the diagram by the x axis.

The three disturbance treatments had similar effects since all PRC showed similar trajectories over the experimental period. Until 1998, one year after the cessation of the treatments, disturbance significantly favoured legumes and forbs, increasing their contributions to total biomass, while at the same time annual and especially perennial grasses have significantly lower proportional contributions compared with the control. Seven years after cessation of the disturbance treatments, annual and perennial grasses had recovered reaching percentages higher than the control, while legumes and forbs decreased to levels lower than the control. The treatment with the strongest effect was digging followed by burning and cutting.

The ecology of Mediterranean grasslands has been shaped both by climate and human disturbance. The dominance, under specific disturbance regime, depends on the reaction of every single species. Legumes have an advantage compared with annual grasses due to their soil seed bank. They form high permanent seed banks that enable them to better withstand disturbance (Sulas *et al*., 2000). In contrast, annual grasses have low carry-over of seeds from one year to the next and their abundance can be dramatically reduced if seed input is low (Evans and Young, 1989). The group classified as forbs includes a number of different species with different physiological characteristics; some forbs are favoured by specific disturbance regimes and can dominate the sward. Perennial grasses, on the other hand, rely on dormant meristems hidden near the soil surface, being protected from disturbance. Under conditions of disturbance, annual species have an advantage over perennials because of their higher morphological plasticity. However, cessation of disturbance enables perennial grasses to dominate.

Conclusions

The results suggest that disturbance in Mediterranean grasslands promotes the predominance of legumes, especially ones that have a persistent seed bank. Cessation of disturbance leads to the rapid recovery of annual grasses, and especially perennial grasses, which develop to constitute the highest proportion of biomass production.

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Quality characteristics of seed material harvested from Molinion litter meadows

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Abstract

Sufficient information about the quality characteristics of on-site harvested seed material from valuable donor sites for restoration of endangered grassland communities is a precondition for its successful use. In order to determine the necessary seed rate and the best-suited application method, information about thousand seed weight, purity and germination capacity of the material is essential. Seed mixtures from a herb-rich *Molinion* litter meadow, harvested with two different methods: i) on-site threshing (OST) and ii) seed stripping (SS), were assessed. The purity of stripped material exceeded 60% and was around 20% higher compared to OST. The germination capacity of the harvested pure seeds treated with and without pre-chilling was investigated. Slight differences between harvesting methods could be observed, whereas pre-chilling of the seed material caused a significant increase in germination capacity of the seed material. The total germination rate did not exceed 30%. Litter meadows contain a high percentage of dormant seeds. This refers to the practical experience that optimal restoration success can be reached if reseeding of *Molinion* meadows takes place at the end of the season.

Keywords: restoration, donor site, purity, germination capacity

Introduction

Nature conservation areas are valuable resources for the maintenance and promotion of biodiversity. About 15 to 25% of the areas used for agriculture in Europe can be included within this category, but only a small share of these areas are also designated as protected. The restoration and re-introduction of valuable local plant species from available donor sites is therefore seen as being of great political and ecological importance. The main objective of the restoration of semi-natural grassland areas lies in the establishment of ecologically valuable plant communities of regional origin. Suitable and usable restoration procedures are also important for the maintenance and safeguarding of the genetic diversity through the transfer of seed material. An essential prerequisite, however, is the careful selection of suitable donor areas of the greatest possible ecological value. In former times, the biomass of litter meadows was used as a straw substitute. This type of grassland dominated the bottom of many inner-alpine valleys. The re-establishment of the ecological valuable litter meadows has become an important issue, especially in the framework of the NATURA 2000 programme. There is an increasing demand for the enlargement of such rare areas, especially in the Styrian Enns valley. Basic knowledge concerning the seed quality of diaspore material harvested from suitable donor sites is essential for success.

Materials and methods

The experimental site is a *Molinion* litter meadow, cut once per year, located in the central part of Austria, (47°56'N, 14°19'E; 636 m a.s.l). During the year of harvest the mean annual air temperature was 6.7°C and annual precipitation was 968 mm. The pH-value is slightly acidic (6.0). Two different harvesting techniques were applied: i) a 'Wintersteiger Classic' plot combine thresher (OST) with a cutting width of 150 cm, and ii) a 'Prairie habitats 610' pull-type seed stripper (SS) with 120 cm width drawn at a speed of 3 km h^{-1} and used on 15 September 2009. After harvesting, the material was air dried and cleaned with a 6 mm sieve. The material was stored over 17 months in a cooling chamber at 2-5°C with 3-4 g m^{-3} absolute humidity. Homogenous samples of the harvested seed were taken and all assessments carried out according to the rules of the International Seed Testing Association (ISTA, 2011). For the purity assessment, ten seed samples per harvesting technique were divided into chaff and filled seeds. For determination of the thousand seed weight (TSW), 10×100 randomly chosen filled seeds were counted and weighed. For the germination capacity (GC) test 12×1.25 g samples per harvesting method were taken and sown in bulb trays (40×60×6 cm) on an organic growing substrate. To study the effect of pre-chilling, the bulb trays were stored outdoors under snow cover between 2 February and 22 March 2011. They were then transferred to the greenhouse, and compared to the same amount of fresh-sown samples. The duration of the germination trial was six weeks and the samples were counted once a week. The statistical analyses were done with the statistics language R 2.15.1. A Shapiro-Wilk Test was performed, showing a normal distribution of the data. Purity and the TSW were tested with an independent samples t-test to determine the 95% confidence interval. The two-way ANOVA was used to assess differences between pre-chilled and untreated seeds.

Results and discussion

Determination of the purity of harvested seed material is generally important to ascertain the volume of pure seeds in the material, which then defines the actual sowing volume of the entire material.

The rotating brush of the seed stripper did not have any strong mechanical impact on the vegetation. However, the huge amount of biomass caused repeated seizure of the rotating brush of the seed stripper. Therefore, mainly full ripe seeds were brushed out and the proportion of chaff, on average, did not exceed 32% (Table 1). Due to the more intensive mechanical impact of the combine plot thresher, the OST harvesting material contained nearly double the proportion of chaff but the total yield of pure seeds (187 kg ha^{-1}) was considerably higher than for the SS method (48 kg ha^{-1}). Consequently, the TSW of pure seeds was significantly lower in the OST variant.

Table 1. Results of TSW and purity of the harvested OST and SS material.

Harvesting method	Mean value	95% Confidence interval
OST – TSW (g)	0.94	0.830-1.046
SS – TSW (g)	1.83	1.687-1.975
OST - pure seeds (%)	45.36	40.15-50.56
SS - pure seeds (%)	68.09	62.99-73.19

Differences in mean TSW and pure seeds are significant (*t*-test, $P < 0.001$)

Table 2**.** Results of the two-way ANOVA of the germination capacity trial.

Treatment	Df	Sum Sq	Mean Sq	*F*-value	*P*-value	Partial Eta Sq
Harvesting method (HM)	1	98.30	98.30	3.88	0.055	0.081
Pre-chilling (PC)	1	1304.27	1304.27	51.50	< 0.001***	0.539
HM×PC	1	12.72	12.72	0.50	0.482	0.011
Residuals	44	1114.41	25.33			

Significance level: * P<0.05, ** P<0.01, *** P<0.001; R^2: 0.56; adjusted R^2: 0.53

The results of the two-way ANOVA showed no significant influence of the harvesting method on germination capacity (Table 2), whereas the positive effect of pre-chilling on germination capacity was highly significant. The germination capacity of untreated seeds of the *Molinion* litter meadow did not exceed 20%, independent of the harvesting method (Figure 1). The treatment pre-chill increased the spontaneous germination to more than 30%. Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage and Leubner-Metzger, 2006). This should guarantee that germination occurs when conditions for establishing a new plant generation are likely to be suitable. Due to the late cut of the litter meadows end of August/early September, the plant stands are dominated by late-maturing species. The short remaining vegetation period does not ensure suitable conditions for the establishment of young seedlings. Frost dormancy prevents germination in autumn, shifting it to the following spring with comparatively better conditions for seed establishment.

Together with the TSW and the data from purity assessment, the knowledge about germination capacity is important in order to determine the necessary seeding amount of harvested seed mixtures from donor sites. To ensure the rapid development of dense vegetation, 2-5g m^{-2} pure seeds (about 1000-2000 germinating seedlings, also depending on erosion risk) are recommended (Scotton *et al.*, 2012). For the restoration of a new litter meadow with our assessed seed material, a seeding amount of 5-7 g m^{-2} for SS and 8-10 g m^{-2} for OST material would be necessary, assuming a low erosion risk at the restoration site.

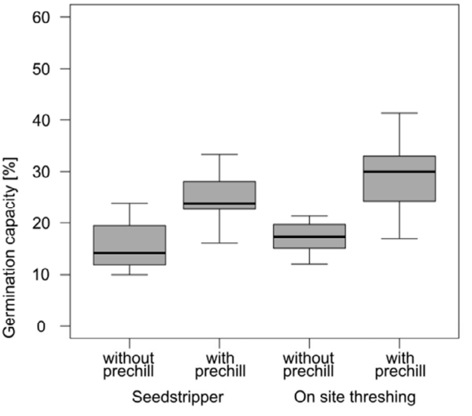

Figure 1. Germination capacity of untreated and pre-chilled seeds harvested from a *Molinion* litter meadow.

Conclusions

For the restoration of *Molinion* litter meadows with harvested seed mixtures, seeding rates of at least 5-8 g m^{-2}, depending on harvesting method, can be recommended. Due to the high share of frost germinators, the seeding of receptor sites should be undertaken in late autumn.

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Grassland renovation by natural self-seeding

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Abstract

To meet the increasing demand for forage quality from grassland, different strategies of re-seeding are used in practice. Commercial seed mixtures are usually sown with different over-seeding techniques such as slot-drill machines or combined harrows. Natural self-seeding could be an interesting alternative to improve grassland stands without any technical effort. Two field trials have been carried out by AREC Raumberg-Gumpenstein to investigate different methods of grassland renovation in mountainous regions with consideration of technique, frequency, seed mixtures and also natural self-seeding. The efficiency of natural self-seeding was studied by quantifying the total seed-amount, the species spectrum, and by testing the germination capacity of the seeds. Yield and forage quality were considered as well as the botanical composition of the treated plots.

Keywords: grassland re-seeding, seed mixtures, germination capacity, forage quality

Introduction

For most Austrian grassland and dairy farmers, the use of home-grown forage from meadows and pastures is seen as a substantial element of their farm management system. Different methods that aim to improve forage quality are therefore of great interest. In addition to aspects of fertilization, weed control and forage conservation, grassland renovation is one of the basic keys to success. Due to the specific climatic and topographical conditions, renovation of mountainous and alpine grassland presents special challenges, both from a technical and an ecological point of view. Simple over-seeding, slot row seeding and band rotavator seeding are the mostly used methods for grassland renovation in Austria. Natural self-seeding of grassland, which was the common method for grassland renovation in the past, has become less important through the significant increase of cutting and grazing frequency. This paper deals with the potential of natural self-seeding on grassland, concerning seed amount, species spectrum and germination capacity, and it also considers the preconditions that have to be met for a successful implementation of this method in practice.

Materials and methods

Field experiments were established at two different sites. These were, (i) established in 2005, Gumpenstein (700 m a.s.l; 1000 mm yearly precipitation; 6.8°C average temperature) and (ii) established in 2006, Piber (450 m a.s.l.; 880 mm yearly precipitation; 8.2°C average temperature). In addition to various reseeding techniques and different seed mixtures, a focus was also given to natural self-seeding (singular = only once in the first year, and regular = every two years). Once the dominating plants reached the optimal stage of maturity the plots (28 m^2 each) were cut and threshed with a combine harvester. The threshing material was then dried, cleaned, separated for species and tested for their germination capacity. Yield, forage quality and the botanical composition of the experimental plots was determined to assess the effect of this natural method of grassland renovation.

Results and discussion

The total yield of cleaned seeds ranged between 20 and 92 kg ha^{-1} yr^{-1} of which in all years of observation the highest proportions were dominated by grasses, followed by herbs and clover (Table 1). For technical grassland reseeding measures, 5 to 20 kg of quality seed mixtures ha^{-1} are normally used in practice, which is considerably less than the seed amount obtained by natural self-seeding. There were great differences in the total seed yield both between years and sites, which indicates that the outcome of natural self-seeding is difficult to predict. Key factors concerning the achieved seed yield, and its species spectrum, are the botanical composition of grassland and also the vegetation stage and the maturity scheme of the different plant species.

Table 1. Seed production by natural self-seeding of permanent grassland (kg pure seeds ha^{-1} yr^{-1}; data based on 6 replications, mean values and standard deviations).

Site	Year	Grass seeds		Clover seeds		Herb seeds		Total seeds	
		x̄	σ	x̄	σ	x̄	σ	x̄	σ
	2005	12.8	4.1	6.0	1.0	2.3	0.9	21.0	4.9
Gumpenstein	2007	38.9	10.5	1.5	1.2	3.6	2.3	44.0	10.9
	2009	67.2	42.1	-	-	24.8	13.7	92.0	52.1
	x̄	39.6		2.5		10.2		52.4	
	2006	26.8	18.8	0.6	0.5	3.7	1.9	31.1	18.6
Piber	2008	13.7	8.5	-	-	6.5	6.1	20.1	7.9
	2010	28.5	24.5	-	-	7.7	6.1	36.2	22.7
	x̄	23.0		0.2		6.0		29.1	

About 20 different grassland species could be identified in the threshing material at both sites, which is much more than the average number of species in commercial seed mixtures. *Poa pratensis* and *Dactylis glomerata* were the dominating grass species in the seed material at Gumpenstein but their proportions differed strongly over the years. *Trifolium repens* was the dominating clover species, whereas *Lamium album*, *Ranunculus acris*, *Pimpinella major* and *Chaerophyllum hirsutum* were the main herbs in the harvested seed mixtures. At Piber *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis* and *Phleum pratense* showed the highest proportion of total grass seeds; the dominating herbs were *Plantago lanceolata*, *Prunella vulgaris*, *Veronica chamaedrys* and *Galium album*, whereas legumes played a minor role.

Table 2. Germination capacity (according to ISTA, 2011) of selected species gathered by natural self-seeding at two different sites in Austria.

	Poa pratensis			*Dactylis glomerata*			*Festuca pratensis*			*Trifolium repens*		
	GCA*	x̄	σ	GCA*	x̄	σ	GCA*	x̄	σ	GCA*	x̄	σ
Gumpen-stein	80	87	9.8	80	56	14.2	85	78	27.7	85	39	8.7
	Poa pratensis			*Dactylis glomerata*			*Lolium perenne*			*Phleum pratense*		
	GCA*	x̄	σ	GCA*	x̄	σ	GCA*	x̄	σ	GCA*	x̄	σ
Piber	80	88	5.4	80	74.8	4.6	85	89	4.5	85	85	3.6

GCA* = required minimum germination capacity for species of quality seed mixtures in Austria, followed by mean value and standard deviation

The most relevant species were tested for their germination capacity (Table 2). Compared with the official Austrian seed quality guidelines (Krautzer *et al*., 2010) *Poa pratensis*, *Lolium perenne* and *Phleum pratense* partly even exceeded the required values, whereas *Dactylis glomerata*, *Festuca pratensis* and *Trifolium repens* failed. In the case of *Trifolium repens* a high proportion of hard seeds were responsible for the unusual low level of germination capacity. Compared with normal harvesting times in agricultural practice the plots of the natural self-seeding variants were harvested 6 to 8 weeks later to achieve sufficient yield of ripe seeds. This time lag negatively influenced both yield and forage quality of the particular growth and was resulting in partly significant lower average values of regular natural self-seeding for the total observation period compared to the untreated control and the technical re-seeding treatments at both sites (Table 3). Overall, singular self-seeding performed much better than regular self-seeding. At Gumpenstein the technical reseeding treatments performed slightly better than the untreated variant, whereas at Piber no positive effect could be observed.

Table 3. Yield and forage quality data of two reseeding experiments in Austria.

	Gumpenstein (average of 2005–10)			Piber (average of 2006–10)		
Parameters	DM[1]	CP[2]	GJ NEL[3]	DM	CP	GJ NEL
Treatments	t ha^{-1}	g kg DM^{-1}	ha^{-1}	t ha^{-1}	g kg DM^{-1}	ha^{-1}
Control	101.0^{ab}	132.2^{a}	51.3^{a}	70.9^{a}	115.4^{a}	39.2^{a}
Singular natural self-seeding[4]	95.4^{b}	131.5^{a}	46.8^{ab}	71.9^{a}	106.6^{a}	36.7^{a}
Regular natural self-seeding[5]	79.8^{c}	114.8^{b}	36.7^{b}	65.5^{a}	106.1^{a}	28.7^{b}
Technical seeding	106.6^{a}	135.4^{a}	53.9^{a}	69.4^{a}	112.8^{a}	36.6^{a}

[1]Dry Matter, [2]Crude Protein, [3]Gigajoule Net Energy Lactation, [4]natural self-seeding only once in the first year, [5]natural self-seeding every two years, $^{a, b, c}$ treatments with different letters are significantly different (P<0.05)

Concerning the impact of natural self-seeding on the botanical composition an increase of grasses could be noticed at both sites within the observation period. The proportion of legumes declined whereas that of herbs remained stable.

Conclusions

Natural self-seeding of grassland provides remarkable amounts of seeds with a mostly acceptable germination capacity. The species composition of seeds is depending on the floristic diversity of the vegetation but also on the time of self-seeding. A basic assumption to accept natural self-seeding of grassland is the absence of problematic weeds, as far as possible, to avoid a degradation of the vegetation. It has to be taken into account that this alternative method of grassland re-seeding causes both a significant yield reduction and low forage quality of the concerned growth. Regarding the increasing requirements for yield and forage quality in modern grassland farming, natural self-seeding can be regarded as a method primarily recommended for farming systems that follow a low intensity strategy.

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Mechanical treatment of rush (*Juncus* spp.) infestations in Western Norway

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Abstract

Infestations by rushes (*Juncus* spp.) have increased in Norway in recent decades, especially in coastal regions, causing serious reductions in forage quality and hampering meat and milk production. Mechanical treatments using a brush cutter in summer or autumn during two growing seasons reduced soft rush (*J. effusus* L.) plants considerably, whereas cutting during spring or early summer had little effect. When the rhizomatous plant part was removed, no regrowth was observed. A pasture topper was less effective than the brush cutter, as too much aerial stem was left, but was more practical for larger areas. In a nursery field, high photosynthetic activity was recorded during late winter-spring. The maximum concentration of sucrose, the principal reserve carbohydrate, was observed in spring and the minimum in mid-summer, demonstrating the most sensitive point in the life cycle of rushes in late season. Soft rush proved more tolerant to cutting and frost, which may explain its dominance in agricultural conditions compared with compact rush (*J. conglomeratus* L.).

Keywords: soft rush, compact rush, cutting, regrowth, brush cutter

Introduction

Soft rush (*Juncus effusus* L.) and compact rush (*J. conglomeratus* L.) are currently being investigated in terms of plant development in order to identify ways of tackling the spread of this weed in coastal parts of Norway. Effective herbicides are available, but herbicides are not allowed on organically managed grassland areas. When such areas are not suitable for common agricultural machines, other mechanical control solutions are needed. The aim of this experiment was to examine the effects of repeated cutting at two cutting heights and three dates for one cut per year, and combinations of these throughout the growing season, to identify the potential for reducing the regrowth both at single plant level and for larger areas.

Materials and methods

<u>Experiment 1</u>: Cutting date and height. This experiment was carried out on cultivated pastures with single plants of soft rush at two sites in west Norway: Sundfør, Tysvær (59°28'N, 5°27'E) and Straumsnes, Fjaler (61°20'N, 5°12'E). Six cutting options (Figure 1) and two cutting heights were examined, and in each treatment (cutting date and cutting height), three plants in each of three replicates were measured (in total 108 plants) at each site. The rush plant comprises a rhizomatous plate of 1-2 cm thickness, on which the roots and aerial shoots are attached. The cutting heights tested were at 2-3 cm above soil level (corresponding to the normal height of a pasture topper) or at -2 cm, i.e. removing the rhizomatous part. Plant size (diameter) and regrowth (% cover of aerial shoots within each tussock) were recorded in spring 2010 before the start of the experiment. The plant size was estimated using two diameter measurements if the tussock was not circular. Plant regrowth was measured in spring

2011 and in early summer 2012. A brush cutter (Stihl FS 450) fitted with a brush knife (41197134100B Stihl 300 mm) was used.

Experiment 2: Plot cutting. This experiment was carried out in Ferkingstad, Karmøy (59°13'N, 5°12'E) on 45-m^2 plots of soft rush comprising six cutting options (Table 1), with on average of 25 separate rush plants in each plot. To ensure similar cutting height of all plants within the plots, cutting was carried out from both sides of the plots. Plant size (diameter) and regrowth (% cover of aerial shoots within each tussock) were recorded before and after the treatment period in 2011. The cutting was performed with a pasture topper (Hanmey EFGC-175-Flail Mower, Midway Sales, Australia) with 1.75 m working width.

Results and discussion

Experiment 1. Cutting date and height. When the rhizome part was damaged, no regrowth appeared in year 2 and cutting below the soil surface thus proved very efficient. However, this method is practical only when rushes are present on a manageable scale and on areas not passable for agricultural machines. In both experimental years, three significantly different groups (P<0.0001) appeared for regrowth subsequent to cutting at soil level and these were ranked as follows: 'spring' > 'summer', 'autumn', 'summer + autumn' > 'summer + autumn', 'spring + summer + autumn', in which spring cutting had the highest regrowth, but with small changes in ranking within the middle group (Figure 1). Thus, cutting once a year should be performed either in summer or autumn.

Before the start of the experiment, the rush plants were larger at Tysvær (0.113 m^2) than at Fjaler (0.045 m^2), but with no significant differences between treatment groups. Averaged over all treatment dates, regrowth in year 2 was 12.0% and 14.5% for Tysvær and Fjaler, respectively, indicating that cutting reduces plants of different sizes on a general level.

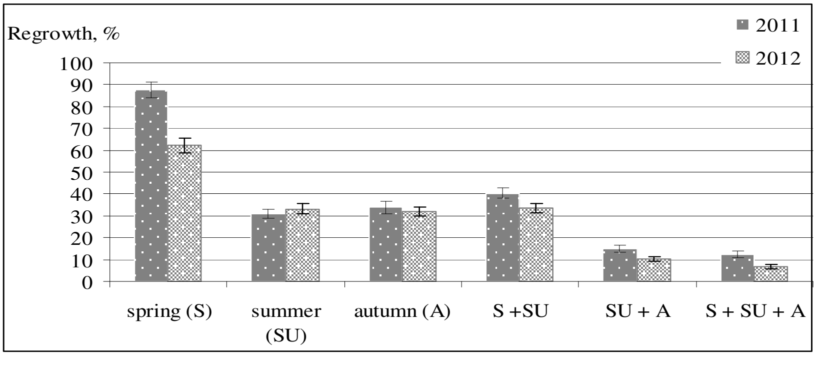

Figure 1. Regrowth (% aerial shoots; mean +/- standard error) of single plants as an effect of cutting date in two subsequent years (stubble height 2-3 cm).

Experiment 2. The studies on mechanical treatment constituted an applied approach in a project investigating the biology of soft and compact rush to identify sensitive periods of growth. Preliminary data on tussock size recorded yearly in early August in the nursery field trial of both rush species established in 2010, demonstrated a 15-fold increase between one- and three-year-old uncut plants of soft rush. When cut in June and August according to the normal dates of two grassland cuts, plant size was 50% smaller than that of uncut plants, but still represented a 13-fold increase, demonstrating the aggressiveness of soft rush. Compact rush demonstrated a considerably lower growth increase and less tolerance to cutting compared with soft rush (data not shown). Soft rush has been shown to have better tolerance to frost than compact rush (Folkestad *et al*., 2010), so tolerance to cutting and frost may explain the dominance of soft rush over compact rush in agricultural conditions.

Table 1. Regrowth (% aerial shoots) of untreated plants and after cutting during the growing season of 2011 using pasture topper (N = 25).

Cutting date	Untreated	June	July	September	October	June + Oct.
Regrowth, %	103	67	46	20	6	34

Measurements of photosynthetic activity (HandyPea fluorimeter, Hansatech Ltd., Kings Lynn, UK) in the nursery field trial on rush revealed little decrease in maximal PSII efficiency (Fv/Fm) during the period February-March 2011, indicating competitiveness compared with other ley species in the spring. Preliminary results on non-structural carbohydrates (HPLC, TLC, Anthrone method) in the lower part of aerial shoots (5 cm) and underground parts (roots, rhizomes) from the same nursery trial, demonstrated that the maximum concentration of sucrose (the major carbohydrate in both species), was observed in March (Kaczmarek-Derda *et al*., 2013), indicating that with scarce snow cover and relatively high winter temperatures, rush is able to acquire large amounts of reserves long before grass species in spring due to its photosynthetic activity. The minimum concentration of sucrose was observed in mid-summer (June-August), which corresponds with the results obtained for mechanical treatment of single plants. The low regrowth observed as a result of cutting at plot level in October may have been strongly influenced by low temperature during the following winter.

Conclusions

Mechanical treatment is an effective means of controlling rushes and can be lethal if the rhizomatous part is removed. Otherwise, cutting should be carried out in late summer or autumn, as this is the most sensitive period of the rush plant's diurnal cycle. Cutting during the very competitive phase in the spring should be avoided.

Acknowledgements

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Mechanical control of marsh ragwort (*Senecio aquaticus* Hill) by cutting

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Abstract

Marsh ragwort (*Senecio aquaticus*) is a monocarpic Asteraceae growing in the wet grasslands of Europe. It contains pyrrolizidine alkaloids and is therefore toxic to livestock. The aim of the study was to develop an environmentally sound method to control the species in meadows. Since 2008 two cutting regimes have been tested on permanent plots at three locations in Lower Austria. At each location two treatments were set up: cutting in June and September (treatment 1) and cutting in July and August (treatment 2). After three years, treatment 1 resulted in a higher annual seed production than treatment 2. Germination of marsh ragwort ceased significantly in treatment 2 in the second year. Population density decreased significantly under treatment 2, relative to treatment 1, at all locations. The population growth rate under treatment 2 was negative at two locations. Two cuts at peak flowering were able to decrease the population density of *S. aquaticus*. To achieve sustained results, treatment 2 must be applied for several years. Control efficacy depends on the accuracy of management, but also on the longevity of vegetative plants and the site-specific number of viable seeds in the soil seed bank.

Keywords: *Jacobaea aquaticus*, marsh ragwort, mowing, pyrrolizidine alkaloids, weed management, wet meadows

Introduction

Senecio aquaticus is a poisonous grassland plant occurring in many European countries. It contains various pyrrolizidine alkaloids, which damage the liver of livestock and cause cancer and infertility (Mattocks, 1968). Toxicity persists in hay and silage (Berendok *et al*., 2010). Management experiments for control have been carried out in Orkney, UK (Forbes, 1976), and in Switzerland (Suter and Lüscher, 2011). Detailed information about mechanical control is important, because the extensive grasslands, where *S. aquaticus* grows, is often subsidised by agri-environmental programs constraining control measures.

The population dynamics of *S. aquaticus* were investigated under two different cutting regimes: the local two-cut standard and an advanced regime that performed best for the control of *S. aquaticus* in a preliminary study (Bassler *et al.*, 2011).

Materials and methods

The study took place in the Hercynian part of Lower Austria (Waldviertel). The three locations are situated at altitudes from 470 to 800 m a.s.l. The climate is rather rough with mean annual temperatures of 6.8°C and mean annual precipitation of 660 mm. *S. aquaticus* grows in wet meadows, usually poorly or unfertilised and cut two or three times a year. Soils are acidic, developed from granite substrate.

Senecio aquaticus exhibits monocarpic flowering behaviour after 2 to 7 years of vegetative rosette growth. The flower shoots bear yellow disc and ray florets and elongate from July to August. After fruit-set (end of July to September) most plants die off.

In 2008 ten permanent plots of 1 m² were established in meadows at three sites, which were comparable in terms of management (cut twice a year, unfertilized), vegetation type and site conditions. Treatment 1 involved cuts in early June and early September, while treatment 2 involved cuts in early July and mid-August. Both treatments were replicated five times at each site. The following characters were counted each year from 2008 to 2011: number of seedlings, vegetative rosettes and flowering plants per m²; number of capitula containing mature seeds per plant. These values were used as response variables in an ANOVA with fixed factors year and treatment. Calculations (log transformation for statistics, regression analyses, t-tests, ANOVA, significance level α=0.05) were done in SPSS 15.0 (IBM, Somers, NY, USA).

Results and discussion

Figure 1a shows the population dynamics of marsh ragwort including seedlings at the three sites under two different treatments. Densities of populations mown in early June and early September (treatment 1) increased significantly from 125 (2008) to 348 (2011) individuals m^{-2} (means for all sites), whereas the density in populations mown in early July and mid-August (treatment 2) changed from a slight increase in 2009 to a decrease in the years 2010 and 2011. The three sites varied considerably: in Kleedorf the mean density of *S. aquaticus* in 2011 was not significantly different from the starting value in 2008 (137.2 and 131.0 individuals m^{-2}, resp.). At the Harbach site, the mean number of individuals m^{-2} decreased significantly from 137.8 in 2008 to 89.4 in 2011 and in Thaures from 184.2 to 69.8. The influence of the treatment on the population density was significant for every location (*P*=0.000 for all locations and log transformed values).

The number of capitula with mature seeds of plants cut in June and September (treatment 1) ranged from 3.80 to 13.07 (Figure 1c). Plants of this treatment were not harmed seriously, because the flowering stems elongated just after the first cut. Thus, seed set was almost finished before the second cut (Bassler *et al*., 2011). In treatment 2 (cuts in early July and mid-August), almost no mature seeds were produced because cutting took place twice a year just when plants were flowering. In the years 2008 and 2011, seed ripening was completely prevented, whereas in 2009 and 2010 in Harbach and Thaures very few seeds were produced (Figure 1c).

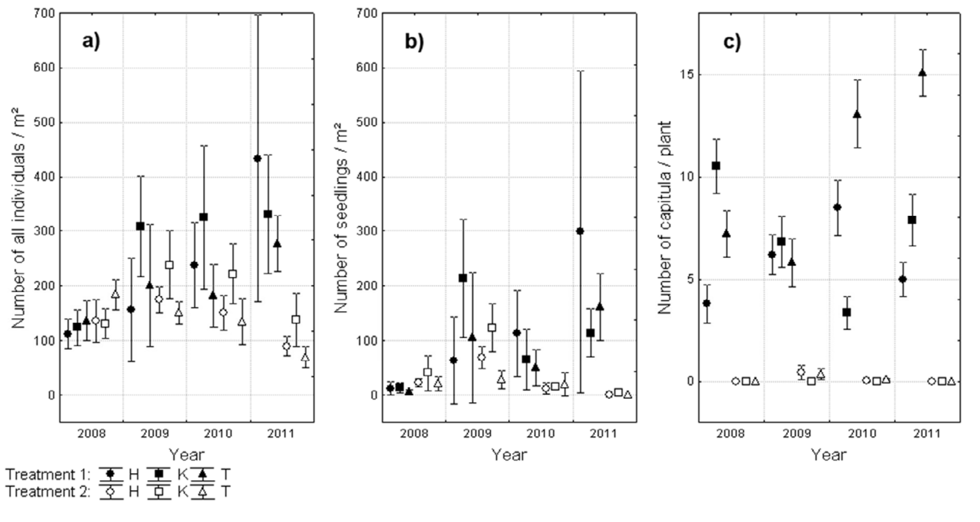

Figure 1. Number of individuals m^{-2} (a), of seedlings m^{-2} (b) and of capitula $individual^{-1}$ (c) of *Senecio aquaticus* in plots of treatment 1 (cut in June and September) and treatment 2 (cut in July and August) from 2008 to 2011 at the sites Harbach (H), Kleedorf (K) and Thaures (T); means +/- 0.95 confidence interval.

Germination is closely connected to seed production in the previous year. Different management started in summer 2008; thus the number of seedlings that germinated in winter 2007/2008 was not influenced by management (Figure 1b). In 2009, on average 28.8 to 123.2 seedlings were found per m^2 in treatment 2. These seedlings probably originated from the seed bank, because there was no seed production in 2008. In contrast to treatment 1, mean numbers of seedlings m^{-2} were already lower in 2009 at 2 sites. Prevention of seed ripening by cutting at peak flowering periods in the previous years resulted in a considerably lower germination in treatment 2 in 2010 (15.5 seedlings m^{-2}) and 2011 (1.4 seedlings m^{-2}). Factorial ANOVA revealed a significant influence of the treatment on germination (P<0.001). Edge effects (invasion of seeds from surrounding meadows) are possible, but not likely (Bassler *et al.*, 2011).

Conclusion

The widely applied cutting regime with one cut before flowering and one cut after seed set leads to accelerated population growth and should be avoided by farmers. Two cuts at peak flowering (early July and mid-August) prevent seed ripening almost totally and the population growth rate is considerably lower than in plots with cuts in early June and September. Unfortunately, the decrease of population density is very slow, because of initial germination from the seed bank and the relatively high age of plant individuals. In comparison to the results of other control options like ploughing, harrowing, herbicides, pulling/digging, one annual cut (Suter and Lüscher, 2011) or pulling (Plenk *et al.*, 2010), the efficacy of treatment 2 is rather good. Thus, a cutting regime best adapted to the phenological development of *S. aquaticus* seems to be an adequate method to reduce it in the medium or long term.

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Effect of nutrient availability on growth of *Urtica dioica* in cut grassland and on the concentrations of N, P, K, Ca and Mg in its biomass

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Abstract

The effects of N, P and K application on the growth of *Urtica dioica* were studied over five years in *Dactylis glomerata* grassland cut twice per year, and with different levels of fertilization. Nitrogen application over five years decreased the soil pH, while P and K application increased P and K availability in the soil. Cover of *U. dioica* increased from 1% initially to 7, 9, 58, 83 and 99% in the control, P, N, NP and NPK treatments, respectively. Concentrations of N, P and Ca in the aboveground biomass were very high in comparison to other grassland species. Nitrogen and phosphorus limitation of *U. dioica* growth was recorded if concentrations of N and P in the aboveground biomass were lower than 25 g N kg^{-1} and 4 g P kg^{-1} in the phenological stage of flowering. Our results show that two cuts per year are not sufficient to suppress expansion of *U. dioica* under conditions of high N and P availability.

Keywords: stinging or common nettle, soil chemical properties, nitrogen, phosphorus, potassium, plant nutrition

Introduction

Urtica dioica L. (stinging or common nettle) is a perennial herb that prefers slightly acidic to alkaline, moist and nutrient-rich soil (Ellenberg *et al*., 1991). The species can grow in conditions ranging from moderately shaded woodlands and hedgerows to open habitats such as floodplains, pastures and meadows (Taylor, 2009). Grime *et al*. (2007) described *U. dioica* as a ruderal species having an established competitor (C) strategy. It is a successful colonizer in grasslands and ruderal sites due to rhizome fragments and stolons. *Urtica dioica* is able to suppress the growth of other herbaceous plants and often forms monospecific stands on nutrient-rich sites (Grime *et al*., 2007). Herbage of *U. dioica* possesses a high nutritive value and was frequently used as an additive to human, poultry and pig foodstuff in the past. Although many authors mention high competitive ability of *U. dioica* and its ability to create dense stands on nutrient-rich soils, no information is available regarding how quickly dense stands develop after an increase in nutrient availability. In addition, dense and tall stands of *U. dioica* are characteristic of unmanaged or infrequently cut grasslands (Prach, 2008) and it is not clear whether dense stands can also develop in regularly managed grasslands such as hay meadows cut twice per year.

Materials and methods

A fertilizer experiment was established near the village of Mšec, 45 km north-west of Prague, in summer 2007 on a meadow with occasional occurrence of *U. dioica*. The main dominant species before the experiment was established were *Dactylis glomerata* (45%), *Festuca arundinacea* (12%), *Phleum pratense* (9%) and *Taraxacum* sp. (8%) (Hejcman *et al*., 2012). The meadow had been regularly cut two or three times per year and occasionally fertilized with farmyard manure before establishment of the experiment. A completely randomized block design was used with the unfertilized control and P, N, NP and NPK fertilizer treatments replicated four times. The application rates for N, P and K in each dressing were 150 kg N ha^{-1}, 40 kg P ha^{-1} and 100 kg K ha^{-1}, respectively. In each plot, one representative soil sample composed from five subsamples from the upper 0-10 cm soil layer was collected in October 2011. In each plot with the presence of *U. dioica*, we collected one representative sample of aboveground biomass of *U. dioica* during the second cut in August 2011. All chemical analyses were performed in an accredited national laboratory, Eko-Lab Žamberk. Cover of *U. dioica* was visually estimated, as percentage, before the harvests in June and August from 2008 to 2011.

Results and discussion

After five years of fertilizer application, the effect of fertilizer treatment on pH value and plant-available P, K and Ca was significant (Table 1). The soil reaction was strongly acidic in treatments with N application, and acidic in the control and P treatments. The concentrations of P and K were positively affected by P- and K-application. Similar results were recorded in other long-term experiments (Hejcman *et al.*, 2007; Hrevušová *et al.*, 2009). Plant-available Ca showed a clear trend to be the highest in the P treatment and this was probably due to the use of superphosphate with high Ca content.

Table 1. Effect of fertilizer treatments on soil chemical properties. Calculated by one-way ANOVA and using the Tukey post-hoc test, treatments with the same letter were not significantly different. F-value and P-value are results of one-way ANOVA. ± values indicate standard deviations.

Treatment	pH/$CaCl_2$	$N_{tot.}$ (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
F-value	10	0.4	32.7	13.6	7.1	1.6
P-value	<0.001	0.79	<0.001	<0.001	0.002	0.218
Control	5.22^{a} ± 0.4	2.90^{a} ± 0.2	0.18^{a} ± 0.05	0.16^{ab} ± 0.01	1.74^{ab} ± 0.4	0.12^{a} ± 0.03
P	5.25^{a} ± 0.2	3.13^{a} ± 0.3	0.45^{b} ± 0.06	0.20^{bc} ± 0.04	2.34^{a} ± 0.4	0.11^{a} ± 0.03
N	4.41^{b} ± 0.3	3.04^{a} ± 0.4	0.13^{a} ± 0.02	0.11^{a} ± 0.02	1.02^{b} ± 0.4	0.15^{a} ± 0.03
NP	4.45^{b} ± 0.2	3.05^{a} ± 0.1	0.36^{b} ± 0.02	0.11^{a} ± 0.05	1.49^{b} ± 0.2	0.11^{a} ± 0.02
NPK	4.44^{b} ± 0.2	3.16^{a} ± 0.4	0.35^{b} ± 0.06	0.26^{c} ± 0.05	1.49^{b} ± 0.4	0.11^{a} ± 0.03

Calculated by repeated measures ANOVA, the cover of *U. dioica* was significantly affected by treatment, time and by treatment × time interaction (Figure 1). Cover of *U. dioica* was relatively stable up to 5% in the control and P treatment during the experiment, but gradually increased in the N, NP and NPK treatments. The main message of the study is that two cuts per year were not sufficient to prevent expansion of *U. dioica* under high N application rate and optimum P availability in the soil. In addition to N supply, the expansion of *U. dioica* was supported by P and K application even though P availability in the soil was optimal for the growth of the desired forage species without additional P application. A positive effect of simultaneous N, P and K application on expansion of *U. dioica* was clear from its almost 100% cover in the NPK treatment in August 2011, even though its initial cover had been only 1% in September 2007 (Figure 1). *Urtica dioica* is thus not only an indicator of unmanaged grasslands as recorded by Prach (2008), but can also be recorded in grasslands cut twice per year if the nutrient availability is sufficiently high. Concentrations of N, P and Ca in the herbage of *U. dioica* were very high in comparison to other species (Table 2). N and P limitation of *U. dioica* growth can be recorded if concentrations of N and P in the aboveground biomass in the phenological stage of flowering are lower than 25 g N and 4 g P per kg, respectively.

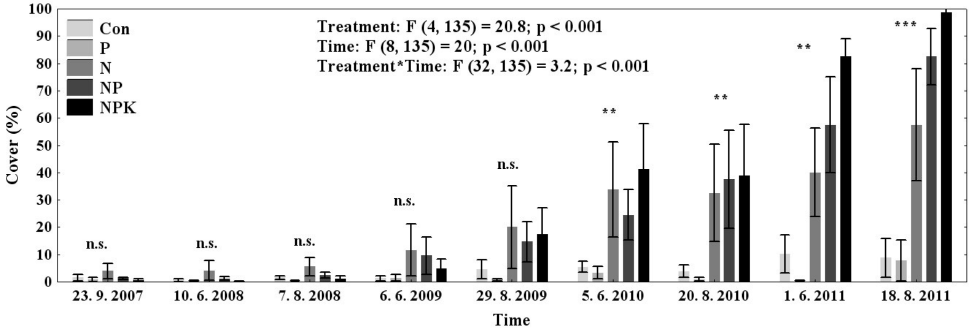

Figure 1. Cover of *Urtica dioica* under investigated treatments from the start of the experiment in September 2007 until the second cut in August 2011. Mean values and standard error of the mean (SE) are presented. Results of repeated measures ANOVA are provided in the graph. After obtaining significant results, one-way ANOVA analysis for data from each sampling date was performed. n.s. – not significant, ** or *** - result of one-way ANOVA was significant at a 0.01 or 0.001 probability level, respectively.

Table 2 Mean concentrations of N, P, K, Ca and Mg (g kg^{-1}) in the aboveground biomass of *Urtica dioica*. Calculated by one-way ANOVA and using the Tukey post-hoc test, treatments with the same letter were not significantly different.

Treatment	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
F-value	1.5	6.8	2.4	1.4	5.5
P-value	0.275	0.007	0.118	0.294	0.013
Control	$23.2^{a} \pm 3.3$	$4.3^{a} \pm 0.1$	$25.4^{a} \pm 0.7$	$25.1^{a} \pm 7.8$	$2.6^{ab} \pm 0.3$
P	21.2^{a}	4.7^{ab}	25.5^{a}	21.5^{a}	2.3^{ab}
N	$29.9^{a} \pm 3.7$	$3.9^{a} \pm 0.5$	$20.9^{a} \pm 3.1$	$28.0^{a} \pm 3.4$	$4.9^{ab} \pm 1.0$
NP	$29.1^{a} \pm 5.0$	$5.6^{b} \pm 0.5$	$20.3^{a} \pm 4.2$	$33.0^{a} \pm 5.0$	$5.1^{b} \pm 1.4$
NPK	$27.5^{a} \pm 4.4$	$4.7^{ab} \pm 0.5$	$26.3^{a} \pm 3.1$	$29.1^{a} \pm 5.8$	$2.8^{a} \pm 0.4$

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Session 3

Grasslands and Biofuels

Second generation biofuel: biomethane from co-digestion of grass and slurry

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Abstract

Grass is an excellent source of biomethane; it is a feedstock for anaerobic digestion with a high content of solids and it has a high specific methane capacity. However, mono-digestion of grass can be somewhat problematic and unstable over time. This is mainly due to a deficiency of essential trace elements. Co-digestion with slurry is a more stable digestion process. This is serendipitous, as beef and dairy farmers have slurry as a residue and typically operate grass-based farms. A model for biomethane to contribute significantly to meeting the renewable energy needs for Ireland from grass and slurry suggests a requirement of 100 biodigesters with a capacity of 75,000 t yr^{-1}, each treating 14,000 t yr^{-1} of grass silage (ca. 4,200 t dry solids (DS) yr^{-1} from 350 ha of grassland) and 61,000 t yr^{-1} of slurry (8% DS). Each biodigester would produce in the order of 2,150,000 m_n^3 yr^{-1} of biomethane (equivalent to 2,150,000 L yr^{-1}of diesel) which is sufficient to fuel 2000 cars travelling 15,000 km yr^{-1}. The proposed industry could generate 4% of expected energy use in transport by 2020. As a second-generation biofuel, it qualifies for a double credit under the Renewable Energy Directive of the European Union and thus would satisfy 80% of the 2020 European Union's target of using 10% of renewable energy in transport. The distribution system for the gaseous biofuel would be the existing natural gas grid system.

Keywords: grass, slurry, biofuel, biomethane

Introduction

First-generation biofuels are based on food crops and typically include ethanol produced from wheat or maize, or biodiesel produced from rapeseed oil. Second-generation biofuels are based on non-edible crops, including wood, straw, residues and perennial grass. The Renewable Energy Directive of the European Union (EC, 2009) requires 10% of all energy in transport to be renewable by 2020. As of October 2012, the use of food-based first-generation biofuels has been limited to 5% of energy used in transport, in order to stimulate the development of second-generation biofuels from non-edible feed stocks (EC, 2012), such as grasses. The Renewable Energy Directive also requires biofuels to emit a minimum of 60% less greenhouse gases than that of the fossil fuel they replace. First-generation liquid biofuels will struggle to satisfy this target. Typical values of targets listed in the Renewable Directive (EC, 2009) are 32% for wheat ethanol and 45% for rapeseed biodiesel. Using grasses as a source of biomethane negates the need for tillage, and allows for carbon sequestration. Korres *et al*. (2010) have shown that grass biomethane, when used as a transport fuel, can effect a 75% reduction in emissions when allowing for the fact that permanent grasslands can sequester 0.6 t C ha^{-1} yr^{-1}. Smyth *et al*. (2009) have shown that the energy production ha^{-1} (both gross and net) for grass biomethane is significantly higher than for first-generation liquid biofuels. This suggests that the quantity of land required to satisfy the energy required for transport is significantly less than that required if first-generation biofuels are used. Biogas has been used across Europe as a source of combined heat and power. More recently, the concept of upgrading biogas to biomethane and supplying it to the gas grid for use as a direct replacement for natural gas (in producing electricity, heat and transport fuel) has become popular. As of 2011, Germany had 83 plants which supplied biomethane into the gas grid (Biel, 2012).

This paper discusses the use of grass as a feedstock for anaerobic digestion, co-digestion of grass with slurry, design considerations in bioreactor design, and the potential supply of renewable energy from grass and slurry biomethane.

Grass silage as a potential feedstock for biomethane production

Lehtomäki *et al.* (2008a) suggest that 1500 m tonnes of agricultural material is available for digestion in the EU each year, half of which is crop material. While maize is the dominant crop in use, grass silage is used in over 50% of the anaerobic digesters operating in Germany and Austria (Prochnow *et al.*, 2009). Perennial ryegrass is the principal species of reseeded temperate grassland in Europe, but other species, such as Italian ryegrass, timothy, cocksfoot and tall fescue, are also common (Nizami *et al.*, 2009; McEniry and O'Kiely, 2013), while permanent pastures of mixed botanical composition are widespread in many regions. It has been reported that the digestion of a mixture of grasses can often have a higher methane potential than individual grasses (Prochnow *et al.*, 2005). Grass is ensiled to ensure a year-round supply of feedstock (Koch *et al.*, 2009). Good ensiling, where lactic acid production dominates the fermentation, will efficiently conserve grass feedstock for anaerobic digestion. In contrast, poor storage conditions can lead to losses of over 50% in potential methane yield (Pakarinen *et al.*, 2008). Ensiling very wet herbage results in a loss of leachate, but this can be collected and beneficially directed to the digester (McEniry *et al.*, 2011).

The solids content of the grass can vary from less than 15% to more than 50%, and so wet and dry digestion technologies can be employed (Lehtomäki *et al.*, 2007; Lehtomäki *et al.*, 2008a). However, grass has a tendency to float on the surface of the liquor in a reactor and form layers of scum in wet processes (Lehtomäki *et al.*, 2007; Thamsiriroj and Murphy, 2010). Wet digestion of grass silage may demand a high input of water for dilution, and hence the energy for pumping and heating the material may be increased (Lehtomäki *et al.*, 2008a; Jagadabhi *et al.*, 2011).

As a general rule, the specific methane yield of grass silage will increase the earlier the harvesting date, whereas the yield of biomass obtainable per unit grassland area will depend on the attainable growth of the crop (Prochnow *et al.*, 2009). This has been demonstrated in different studies where specific methane yields of first-harvest grasses (leafy/vegetative stage) are higher than their respective second harvests (stemmy/flowering stage) (Lehtomäki *et al.*, 2008b; Seppälä *et al.*, 2009). The increase in fibrous structure leads to a slower rate of degradation and also increases the required hydraulic retention time (Prochnow *et al.*, 2005; McEniry and O'Kiely, 2013). However, reed canary grass has been shown to give higher specific methane yields (up to 26%) with advancing crop maturity and decreasing water content (Lehtomäki *et al.*, 2008b).

The rate-limiting step in the digestion of grass silage is the hydrolysis of ligno-cellulosic components (Cirne *et al.*, 2007; Wang *et al.*, 2009; Jagadabhi *et al.*, 2011; Xie *et al.*, 2011b). The structure of grass silage is comprised primarily of cellulose, hemicellulose and lignin (up to 75% of its dry matter content) and, hence, microbes are restricted in accessing such fibrous material (Wang *et al.*, 2009; Xie *et al.*, 2011a). Various chemical, biological and physical pre- and post-treatment methods have been documented in order to increase digestibility (Jagadabhi *et al.*, 2008; Nizami *et al.*, 2009). In terms of physical pre-treatment, maceration of the grass silage is a possible option. A particle size of 1 cm has been reported to be ideal for anaerobic digestion (Kaparaju *et al.*, 2002). A study on the effects of combined thermal and alkali pre-treatment showed that application of 100°C combined with addition of 5% NaOH (5.0 g 100 g^{-1}) in the feed stock could enhance the biodegradability of such ligno-cellulosic materials and increase specific methane yields by as much as 38% (Xie *et al.*, 2011a). However, to achieve these high input temperatures, increased energy requirements will result, adding to the cost of the process (Xie *et al.,* 2011a). The effect of a biological pre-treatment additive on methane production at the ensiling stage has also been investigated. It was found that the biological additive, containing both lactic acid bacteria and enzymes (cellulose, pectinase and xylanase), did not enhance methane yields with various grass crops (Pakarinen *et al.*, 2008). Another study focused on the recirculation of alkali-treated solids from the digestate to assess the impact on methane production (Jagadabhi *et al.*, 2008). However, they were not found to be effective in destroying ligno-cellulosic structures.

The addition of cobalt as a trace element to a system digesting grass-clover silage has been shown to increase methane yields by increasing the conversion rate of acetate. A concentration of 0.2 mg L^{-1} was recommended (Jarvis *et al.*, 1997).

Co-digestion of grass silage with slurries

Another option is to co-digest grass silage with animal slurries (Lehtomäki *et al.*, 2007; Lehtomäki *et al.*, 2008a; Jagadabhi *et al.*, 2010). Mono-digestion of slurries can yield specific methane values as low as 100 L CH_4 kg^{-1} volatile solids (VS). The reason for the low yield is that the majority of the energy-rich substrates have been eliminated through the digestive tract of the animal (Weiland, 2003; Lehtomäki *et al.*, 2007). This is exacerbated by the low dry solids content. If the slurry is at 8% dry solids (DS) and 75% of the solids are volatile (VS), then the methane production may be as low as 6 m^3 t^{-1}. Co-digestion of slurries with grass silage increases yields of methane (Wang *et al.*, 2009); Koch *et al.*, 2009). The primary incentive for mono-digestion of grass silage is high volumetric yields of methane. It is, however, prone to process imbalance and hence co-digestion with slurry can alleviate some of these issues (Jagadabhi *et al.*, 2010). The addition of slurry to grass silage in a digester can stabilize pH, counteract ammonia inhibition and provide a more optimum C:N ratio for the process, all of which promote methanogenesis (Xie *et al.*, 2011b). Specific methane yields from grass silage are documented in the range of 200 to 500 L CH_4 kg^{-1} VS (Table 1). A grass silage at 30% DS and 92% VS can yield 60 to 138 m^3 t^{-1} wet weight (w/w) of methane. This is significantly in excess of that achieved from slurry. A question may be asked as to why the designer or developer would dilute the yield of methane through dilution with slurry. The rationale is that co-digestion can result in an increase in specific methane yield (L CH_4 kg^{-1} VS) relative to the calculated pro-rata yield, whilst providing a more stable process and utilizing two co-existing feed stocks.

A number of studies have investigated the grass-slurry ratio for co-digestion with varying results. In the literature there is a tendency to use the term ‘manure’ when describing what the authors would consider as slurry with a solids content of less than 10%. A study of cow manure (at 6.5% DS and 5.3%VS) and grass silage indicated that 30% grass silage on a VS basis was ideal to get the highest specific methane yield and an increase to 40% would have a negative impact by as much as 12% (Lehtomäki *et al.*, 2007; Jagadabhi *et al.*, 2008). Another study, focusing on pig manure and grass silage, indicated a ratio of 1:1 (VS pig manure: VS grass silage) to achieve an optimal specific methane yield (Xie *et al.*, 2011b). A further study on the co-digestion of *solid* pig manure and dried grass silage suggested that a 6:4 ratio of manure to silage (VS basis) gave the most promising results at a loading rate of 1 kg VS m^{-3} d^{-1} (Xie *et al.*, 2012b); this is a low loading rate. Cow manure and crop silage was optimized at 70-75% crop silage (Comino *et al.*, 2010).

If the source of slurry is dilute, one option is to concentrate the solid material before introducing it to the system as a co-substrate to ensure a higher solids loading and, in return, more biogas per unit volume than raw manure (Asam *et al.*, 2011; Xie *et al.*, 2011b; Xie *et al.*, 2012b). Also, as biomass transport costs are high, separating the solid content in the slurry can provide a cost-saving alternative. Specific methane yields of 0.302 to 0.304 m^3 CH_4 kg^{-1} VS were achieved by co-digesting solid pig manure and dried grass silage (Xie *et al.*, 2012b).

Specific methane yields from grass and slurry

Results from the literature on methane production from grass silage indicate that the species of grass, the fertility of the field, the application rates of fertilizer, the time of cut, and the ensiling process are potentially important. Issues of variability also are prevalent in slurries and manures. Is the slurry from pigs or cattle? Were the animals housed on concrete slats or on straw bedding? What were the animals fed? Is rain water mixed with the slurry? How much wash water was used? As a result of these issues it is difficult to directly compare results reported in the scientific literature. Table 1 outlines a selection of values from the literature on mono-digestion of grass whilst Table 2 outlines a selection of values from the literature on co-digestion of grass with slurries and manure.

Operational considerations in biogas production from grass silage

The organic loading rate (OLR) is a critical variable in an anaerobic digestion system. If the OLR is too high, it can lead to system failure through volatile fatty acid accumulation and/or ammonia inhibition (Xie *et al.*, 2012b). Specific studies related to the effect of OLR on co-digestion of manure and grass silage are limited. However, it has been reported that doubling the OLR from 2 to 4 kg VS m^{-3} d^{-1} decreased specific methane yields in the digestion of cow manure and grass silage (40% of VS) from 0.268 to 0.186 m^3 CH_4 kg^{-1} VS (Lehtomäki *et al.*, 2007). This was attributed to the retention time not allowing for effective degradation (Lehtomäki *et al.*, 2007). In a study of solid pig manure and dried grass silage, increasing the OLR from 1 to 3 kg VS m^{-3} d^{-1} also decreased the specific methane yields by an average of 38% (Xie *et al.*, 2012b).

Table 1. Selected results from a review of the scientific literature on mono-digestion of grass.

Substrate	Harvest/treatment	Reactor	Yield (m^3 CH_4 kg^{-1} VS)	OLR[7] (kg VS m^{-3} day^{-1})
Grass silage[1] (75% timothy, 25% meadow fescue)	Early flowering, bunker ensiled with additive	Leach bed-UASB[9]	0.197	NA
Grass silage[2]	Late summer, bunker silo with no additive	Loop Reactor	0.26	1.0-3.5
Ryegrass[3]	Cut in September	Batch	0.39-0.51	NA
Perennial ryegrass[4]	Fresh cut in May	Batch	0.36	NA
Grass mixture[4]	Second cut in May	Semi-continuous	0.30-0.32	0.7-1.4
Perennial ryegrass[5]	First cut in May, bale silage no additive	CSTR[8]	0.455	2.5
Perennial ryegrass[6]	First cut in May, bale silage no additive	Leach bed- UASB	0.341	1.9

[1]Lehtomäki *et al.*, 2008a; [2]Koch *et al.*, 2009; [3]Pakarinen *et al.*, 2008; [4]Mahnert *et al.*, 2005; [5]Thamsiriroj *et al.*, 2012; [6]Nizami and Murphy, 2011; [7]OLR, Organic loading rate; [8]CSTR, continuously stirred tank reactor; [9]UASB, upflow anaerobic sludge blanket

Table 2. Selected results from a review of the scientific literature on co-digestion of grass and slurry.

Substrate	Ratio VS $_{feed1}$:VS $_{feed2}$[5]	Reactor	Yield (m^3 CH_4 kg^{-1} VS)	OLR[6] (kg VS m^{-3} day^{-1})
Pig manure and grass silage[1]	3:1	Batch	0.304	NA
Pig manure and grass silage[1]	1:1	Batch	0.302	NA
Pig manure and grass silage[1]	1:3	Batch	0.267	NA
Cow manure and grass herbage[2]	2.3:1	CSTR[7]	0.268 (approx.)	2
Cow manure and grass herbage[2]	9:1	CSTR	0.143 (approx.)	2
Cow manure and grass herbage[2]	4:1	CSTR	0.178 (approx.)	2
Cow manure and grass herbage[2]	1.5:1	CSTR	0.250 (approx.)	2
Cow manure and grass herbage[2]	1.5:1	CSTR	0.233 (approx.)	3
Cow manure and grass herbage[2]	1.5:1	CSTR	0.186 (approx.)	4
Cow manure and grass herbage[3]	2.3:1	CSTR	0.180-0.185	2
Cattle slurry and grass herbage[4]	1:3	Semi-continuous	0.286 (averaged)	0.7-1.4

[1]Xie at al., 2012a; [2]Lehtomäki *et al*., 2008a; [3]Jagadabh *et al*, 2008; [4]Mahnert *et al*., 2005; [5]VS, volatile solids; [6]OLR, Organic loading rate; [7]CSTR, continuously stirred tank reactor

The tendency of grass silage to float at the top liquor level of a wet continuously stirred tank reactor (CSTR) can be very problematic for stable digestion (Thamsiriroj and Murphy, 2010). Without an efficient mixing system, grass particles form a floating mass that accumulates, dries and becomes a barrier between the liquor and the gas in the digester. Maceration of grass silage to a suitably small particle size reduces the tendency of grass to float; it is also advisable that the mixing system breaks the surface and pulls the floating grass sods into the liquor (Thamsiriroj and Murphy, 2010).

The recirculation of liquid effluent in a CSTR, treating alfalfa silage, was shown to increase pH and alkalinity initially, but it subsequently resulted in an accumulation of organic and inorganic material which inhibited hydrolysis and methanogenesis (Nordberg *et al*., 2007). It was found that the inhibition could be overcome by replacing 50% of the liquid effluent with water which diluted the inhibiting effect (Nordberg *et al*., 2007). Recirculation of liquid effluent was also examined in the continuous digestion of liquor of grass silage. Results illustrated that combining an increased OLR with effluent recycling allowed higher methane yields to be achieved. This was attributed to the recycled material acting as a source of inoculum and thus helping the bacteria adapt to the process (Abu-Dahrieh *et al*., 2011). Thamsiriroj *et al*., (2012) found that recirculation of leachate from the second step of a two-step wet-stirred system, mono-digesting grass silage, removed any need for the dilution of grass, and allowed a sharing of the work by the two digesters. Overall, an OLR of 2.5 kg VS $m^{-3}d^{-1}$ was achieved with a methane production of 455 L kg^{-1} VS; this equates to over 90% destruction of volatiles. Raising the OLR to 3 kg VS m^{-3} d^{-1} led to system failure.

Alternative designs to the CSTR are available. Leach-bed reactors in conjunction with an upflow anaerobic sludge blanket (UASB) reactor may be used for the digestion of grass silage in a two-phase process. These systems operate by loading grass silage into the batch leach beds, which are subsequently sprinkled with leachate recirculated from the UASB and the closed cycle continues (Lehtomäki *et al*., 2008a; Jagadabhi *et al*., 2011; Nizami and Murphy, 2011). Each kg of VS destroyed in the grass silage equates to 1.4 kg of chemical oxygen demand (COD). Each kg of COD destroyed in the UASB generates 350 L of CH_4 (Nizami *et al*., 2011). Thus the maximum production of methane is 490 L CH_4 kg^{-1} VS destroyed. The overriding advantages of two-phase systems are a reduced hydraulic retention time and a higher methane content in the produced biogas (Yu *et al*., 2002; Nizami *et al*., 2012). Nizami *et al.* (2012) produced a biogas with a methane composition of 71% from a leach bed-UASB system, whereas they produced a biogas with a 52% methane composition from the same grass silage in a two-step wet digestion system. The leach bed-UASB system improves the hydrolysis rate, and the replacement of leachate has been shown to prevent the accumulation of organic substances which can further aid hydrolysis (Jagadabhi *et al*., 2011). This was also illustrated in another study where the highest hydrolysis yield, acidification yield and VS removal was achieved with a 1:1 (v/v) leachate dilution in the digestion of grass silage (Xie *et al*., 2012a).

Another alternative is a single-phase dry-batch system. It has been demonstrated in one-stage leach-bed reactors, mono-digesting grass, that COD solubilization and volatile fatty acid production can be enhanced through the replacement of leachate with fresh water. This dilution dilutes inhibitory products introduced throughout the different digestion stages (Jagadabhi *et al*., 2010). The same study also illustrated that micro-aeration of the leach-bed system can result in greater volatile fatty acid production.

Bioresource of grass and slurry biomethane in Ireland

Ireland has approximately 4.4 m ha of agricultural land of which 91% is under grassland; this proportion is very high in a European context. The majority of this grassland area is used by beef and dairy cattle. Almost 90% of the output from the beef and dairy sector is exported (Smyth and Murphy, 2011). Ireland has targets, documented in *Food Harvest 2020* (Department of Agriculture, Food and the Marine, 2010), to increase dairy production by 50% and beef production by 20% by 2020.

McEniry *et al*. (2012) state that, allowing for increased food production, Ireland would still have an annual grassland resource of 0.39 m t DS yr^{-1} in excess of its livestock requirements. If it is considered that 12 t DS ha^{-1} would be achievable in Ireland, this corresponds to 32,500 ha equivalent, or 0.7% of the agricultural land. Furthermore, by increasing the efficiency of fertilizer application and grass utilization by grazing cattle, this resource could extend to 12.2 million t DS yr^{-1} (McEniry *et al*., 2012). The resource, if desired, could be considerable and may not impact on food production. Singh *et al*. (2010) documented that there was over 35 m t yr^{-1} of slurry production in Ireland in 2007 (30.5 m t from cattle, 2.35 m t from pigs, 1.84 m t from poultry and 0.19 m t from sheep).

Table 3 outlines the biomethane production associated with co-digestion of grass silage and slurry at a ratio of 1:1 (on a volatile-solid basis). The grass production is excess grass production, allowing for the needs of *Food Harvest 2020* on a conservative basis (McEniry *et al*., 2012), corresponding to a land equivalent of 0.7% of agricultural land. The energy produced is equivalent to 7.7 PJ yr^{-1}, which is 3.5% of the thermal energy forecast for 2020 and 4% of energy used in transport that is forecast for 2020 (Murphy and Thamsiriroj, 2011).

Table 3. Energy production from digesting excess grass silage (allowing for *Food Harvest 2020*) with slurry at a ratio of 1:1 on a volatile solids basis.

Feed stocks			Energy output	
Grass silage 92% VS; 30% DS	Slurry 75% VS; 8% DS	Total (1:1 VS basis)	Methane 300 m_n^3 CH_4/t VS	LHV of methane 35.9 MJ/m_n^3
1.3 Mt yr^{-1}	5.975 Mt yr^{-1}	7.275 Mt yr^{-1}		
0.39 Mt DS yr^{-1}	0.478 Mt DS yr^{-1}	0.868 Mt DS yr^{-1}		
0.359 Mt VS yr^{-1}	0.359 Mt VS yr^{-1}	0.718 Mt VS yr^{-1}	215 M m_n^3 CH_4	7.7 PJ yr^{-1}

Considering transport fuel as a vector for biomethane, the Renewable Energy Directive (EC, 2009) allows a double credit for biofuels from residues and ligno-cellulosic feed stocks. Thus, the energy associated with the biomethane is equivalent to 8% of the renewable energy supply in transport or 80% of the target, whilst requiring less than 0.7% of agricultural land. For example, if wheat ethanol were used to meet this level of target, then 233,333 ha of land (5.3% of the agricultural land area) would be required.

If an average size of digester of 75,000 t yr^{-1} was chosen then this industry would equate to 100 digesters, each treating 14,000 t yr^{-1} of grass silage (ca. 4,200 t DS yr^{-1} from 350 ha of grassland) and 61,000 t yr^{-1} of slurry. Each biodigester would produce approximately

2,150,000 m_n^3 yr^{-1} of biomethane (equivalent to 2,150,000 L of diesel yr^{-1}). Each facility would be sufficient to fuel 2000 cars travelling 15,000 km yr^{-1}. Biomethane may be distributed via the existing natural gas grid.

Conclusions

Grass silage is an excellent feedstock for anaerobic digestion. For a biogas system, it is a high solids-content feedstock and, as such, minimizes heat demand in the digester system. The specific methane capacity can be very high, typically in excess of 400 L CH_4 kg^{-1} VS. However, over time, mono-digestion of grass has been shown to be problematic due to lack of essential trace elements. Co-digestion of grass silage and slurry is more stable. Grass silage is associated with livestock; in particular cattle. Slurry and silage co-exist on most beef or dairy farms.

This paper suggests a model of co-digestion at a rate of 1:1 on a volatile solids basis (VS_{grass}:VS_{slurry}). This equates to approximately a ratio of 1:4.6 (grass silage to slurry) on a wet volume basis. The biomethane produced from co-digestion of excess grass in Ireland (approximately 0.7% of agriculture land) with slurry would generate 4% of the expected energy in transport. This would be deemed a second-generation gaseous biofuel and, as such, qualifies for a double credit in accordance with the methodology of the Renewable Energy Directive of the European Union. Grass and slurry biomethane can thus satisfy 80% of the target for renewable energy supply in transport of 10% by 2020 using only 0.7% of agriculture land and not interfering with food production.

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Permanent grasslands for bioenergy: factors affecting management and conversion efficiency

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Abstract

In Europe it is common practice to use grass as a bioenergy resource both in anaerobic digestion and combustion. The efficiency can be influenced by various factors during grassland management, harvest, postharvest, and conversion. Grassland management factors that have major impacts on biomass yields and characteristics are the period of first cut, cutting frequency and fertilization. For anaerobic digestion, intensive grassland management with three or more cuts per year and an early first cut is favourable, while for combustion an extensive management with one late cut per year and low level of fertilization is preferable. Harvest and postharvest processes are important to preserve the yield and quality of the biomass and allow for improving physical and chemical biomass properties. Efficiency of grass conversion to biogas depends on type of digester and technical solutions to specific problems with the grass feedstock, such as floating, wrapping around moving devices, sedimentation of soil particles in the digesters and increased abrasion. The characteristics of grass as a solid biofuel require various specific and costly adaptations of the combustion technologies. Providing grass with a high fuel quality by appropriate grassland management and harvest is the key for the efficiency of the combustion process. Net energy yields, greenhouse gas savings, greenhouse gas mitigation costs and profitability, as parameters used to characterize efficiency of bioenergy production from grassland, mainly depend on the conversion route, the biomass yields and the amount of resource input.

Keywords: permanent grassland, anaerobic digestion, combustion

Introduction

Permanent grasslands cover an area of 179 million ha in Europe, i.e. 38% of the agricultural land (FAOSTAT, 2012). However, less and less grass is needed in animal husbandry due to decreasing ruminant numbers in nearly all developed countries of the world, and because of increased livestock performance and changing ruminant diets with higher proportions of concentrates (Prochnow *et al.*, 2009a). An alternative use for grasslands is as a biomass source for energy production. The potential grassland area that can be used for bioenergy in the EU-27 without utilizing land that is needed for animal feed is estimated at 9.2-14.9 million ha, i.e. 13-22% of the permanent grassland. Thus, grasslands could contribute a share of 16-19% of the energy crops potential and 6-7% of the total bioenergy potential of the EU-27 (Thrän *et al.*, 2005; Prochnow *et al.*, 2008). The global bioenergy potential of semi-natural grasslands is roughly estimated at 386 million ha (Field *et al.*, 2008) to 500 million ha (Tilman *et al.*, 2006).
Among the various options for energy production from grass, anaerobic digestion and combustion are practised in Europe. While grass is a common feedstock for anaerobic digestion in Germany (Weiland, 2006), Austria (Hopfner-Sixt and Amon, 2007), Belgium

(Gerin *et al.*, 2008) and Sweden (Gunnarsson *et al.*, 2008), combustion is reported to be a broadly established practice in Finland (Pahkala *et al.*, 2008). Potential future pathways of using grass might be the production of lignocellulosic bioethanol, synthetic biofuels or synthetic natural gas.

To be competitive with fossil energy and other land uses, bioenergy production from grassland must be highly efficient. Efficiency in general is an economic term describing the relation of output to input, and aimed at the quantification how much resource input is needed per unit of useful output (e.g. Mühlbradt, 2007). In the case of bioenergy production from grass the resource input is usually expressed in units of land, energy input, greenhouse gas emissions or costs. The useful output is calculated in units of energy output, avoided greenhouse gas emissions or revenues. Usual criteria to characterize the efficiency of bioenergy production from grass are the net energy yield as the difference between energy input and energy output (GJ ha^{-1}), net greenhouse gas emissions/greenhouse gas savings as the difference between avoided and emitted greenhouse gases (kg CO_2-eq ha^{-1}, kg CO_2-eq MJ^{-1}), profit as the difference between revenues and costs (€ ha^{-1}, € t^{-1}, ct kWh^{-1}) or greenhouse gas mitigation costs (€ t^{-1} CO_2-eq). Further useful outputs of grassland use exist in terms of biodiversity, protection of soil from erosion, groundwater formation, overall appearance of the landscape or contribution to the rural economy and development. So far, these outputs have not been included in quantitative efficiency calculations. The efficiency of bioenergy production from grassland can be influenced by various factors during grassland management, harvest, postharvest and conversion.

Grassland management

Grassland management affects the biomass yields and characteristics, thus influencing the suitability of the grass for specific utilization pathways and the efficiency.

For anaerobic digestion, the goal is to obtain a high methane yield per area unit (m^3 ha^{-1}). The methane yield per hectare is calculated from the organic dry matter (ODM) yield (kg ODM ha^{-1}) and the methane yield per mass unit of grass feedstock (L kg^{-1} ODM). A feedstock with a low content of lignocellulose and high concentrations of crude fat and crude protein is desirable to obtain high methane yields per mass unit. Both the biomass yields and the methane yields per feedstock mass unit are highly variable for grassland. Reported methane yields range from 80 to 641 L kg^{-1} ODM (for overview see Prochnow *et al.*, 2009).

For combustion, the aim of grassland management is to achieve a high energy yield per unit area (GJ ha^{-1}). This is influenced by the dry matter (DM) yield (kg DM ha^{-1}) and the calorific value (MJ kg^{-1} DM). The calorific value of grass biomass varies slightly between 16.3 and 19.5 MJ kg^{-1} DM (for overview see Prochnow *et al.*, 2009b). For combustion, even prior to the energy yield is the importance of chemical composition of the grass. A highly lignified solid biofuel with low ash contents, N contents $\leq 6\%$ w/w, S and Cl contents $\leq$ w/w, low K contents and high ash melting temperature is favoured for combustion (Lewandowski and Kicherer, 1997; Obernberger *et al*., 2006).

The management factors that have major impacts on biomass yields and characteristics are the period of first cut, the cutting frequency and fertilization. For anaerobic digestion, the period of first cut is of primary importance for feedstock-specific methane yields. Several systematic experiments have shown that feedstock-specific methane yields decrease with advancing stage of vegetation (Prochnow *et al.*, 2005; Amon *et al.*, 2007a, 2007b; Massé *et al.*, 2010, 2012; Herrmann *et al.*, submitted). As a rule, intensive grassland management with higher cutting frequency yielded more methane both on a mass basis and on a hectare basis than extensive management with one or two cuts (Baserga and Egger, 1997; Amon *et al.*, 2007a; Gerstl, 2008). Increasing N fertilization levels is reported to decrease feedstock-specific methane yields, but to increase biomass yields, so that in total the methane yields per hectare increase (Massé *et al.*, 2012). Altogether, it can be summarized that methane yields are enhanced by increasing the management intensity. Feedstock-specific methane yields rise due to earlier cutting dates, whereas area-specific methane yields mainly increase due to higher biomass yields.

In contrast, an extensive grassland management system with one late cut and low level of fertilization is preferable for combustion. Various systematic investigations with grassland biomass consistently come to the result that biofuel quality improves with delayed harvest (Landström *et al.*, 1996; Burvall, 1997; Hadders and Olsson, 1997; Kasper, 1997; Christian *et al.*, 2002; 2006; Lemus *et al.*, 2002; Lewandowski *et al.*, 2003; Xiong *et al.*, 2008; Tahir *et al.*, 2010; Tonn *et al.*, 2010; Heinsoo *et al.*, 2011; Kludze *et al.*, 2012). Concentrations of undesired elements such as N, S, K and Cl decrease significantly with advancing age of vegetation and may fall below the thresholds with harvest in late winter or early spring. Furthermore, ash contents decrease and the ash-melting temperature rises. Similar to delayed harvesting, reduced fertilization also improves fuel quality, but at the expense of decreases in biomass yield (for overview see Prochnow *et al.*, 2009b). In this conflict, priority is usually given to fuel quality, since the options to adapt combustion technology are expensive and/or technically limited.

The reported effects of the type of vegetation on yields vary for both anaerobic digestion and combustion. Pure stands of various grass species showed no significant differences in methane yields (Baserga and Egger, 1997; Mähnert *et al.*, 2002) while another experiment resulted in higher methane yields from switchgrass (*Panicum virgatum* L.) than from reed canary grass (*Phalaris arundinacea* L.) (Massé *et al.*, 2011). In the comparison of three semi-natural biotopes, acute sedge (*Carex acuta* L.) vegetation showed 25% lower methane yields than purple moor grass (*Molinia caerulea* (L.) Moench) and meadow foxtail (*Alopecurus pratensis* L.) vegetation (Herrmann *et al.*, submitted). Solid biofuel quality was found by some authors (Kasper, 1997; Florine *et al.*, 2006) to be independent of the type of grassland vegetation, whereas other authors obtained different chemical fuel composition of grass from different vegetation (Tonn *et al.*, 2010; Khalsa *et al.*, 2012; McEniry *et al.*, 2012).

Harvest and postharvest

In the process chain, the process steps of harvest, transport, storage and pre-treatment form the link between grassland management and biomass conversion. Each one can influence the physical and chemical biomass properties. It is important to preserve the biomass in terms of yield and quality during these process steps.

For anaerobic digestion, grass usually is harvested with choppers, self-loading forage wagons or balers and preserved as silage. With ensiling and anaerobic digestion two complex biochemical processes are combined. Ensiling changes feedstock properties of the grass. Especially the particle size of the grass and the use of ensiling additives influences the ensiling process and silage quality, which in turn affects the subsequent anaerobic digestion directly or indirectly.

Particle size reduction for anaerobic digestion may enhance methane yields by providing more surface to microorganisms and opening cell structures. Varying responses to decreasing particle size were observed for grass, ranging from negligible effects (Baserga and Egger, 1997) to maximum methane yields at medium particle lengths (Kaparaju *et al.*, 2002) or constantly increasing methane yields (Weiß and Brückner, 2008; Jin *et al.*, 2012). Basically, particle size reduction is only reasonable if the achievable additional benefits exceed the additional expenditures. In farm experiments on reducing particle size during chopping of biogas crops, net greenhouse gas emissions decreased slightly with reduced particle size, while net energy yield and profit only increased significantly in 30 to 40% of the harvests (Herrmann *et al.*, 2012). Hence, chopping lengths below 7 to 8 mm are not recommended.

A number of experiments have been carried out to investigate the influence of silage additives on the ensiling process and subsequent anaerobic digestion of grass (Idler *et al.*, 2007; Pakarinen *et al.*, 2008; Plöchl *et al.*, 2009; Herrmann *et al.*, submitted). There is no obvious trend in the influence of silage additives on anaerobic digestion. Nevertheless, their application prevents losses of the silage and thus maintains the value of the resource.

For combustion, grass is usually dried in the field, baled and preserved as hay. Field drying reduces the moisture content of the grass to below 20%, which ensures storability and enhances the calorific value and energy density of the solid biofuel. During the period of field drying, undesirable elements may be leached from the grass (Kasper, 1997; Tonn *et al.*, 2011). Rainfall of 30-40 mm can improve fuel quality significantly; however, the required weather conditions might occur rarely, for example in southeast Germany not even once a year on average (Tonn *et al.*, 2012).

In order to achieve a high efficiency in the harvest, transport and storage of grass for use as solid biofuel, it is crucial to manage the weather risk so as to reach the required low moisture contents, to reduce biomass losses, to achieve highly compacted and stable bales and to establish efficient bale logistics.

Depending on the firing technology to be used, further biomass processing is necessary, such as comminution via chopping, cutting, shredding or milling, and compression to briquettes or pellets.

A combination of anaerobic digestion and combustion is the integrated generation of solid fuel and biogas. Here the grass biomass is processed by hydrothermal conditioning and mechanical dehydration, which produces a press fluid used for anaerobic digestion and a press cake with favourable characteristics for combustion (Wachendorf *et al.*, 2009). Currently, this technology is still at the pilot scale (Hensgen *et al.*, 2012).

Conversion

For biogas production, grass usually is co-digested with slurry and other crop feedstock in wet-process digesters (e.g. Weiland, 2006; Hopfner-Sixt and Amon, 2007). Mono-digestion is reported to be problematic in a two-stage, wet continuously stirred tank reactor due to accumulation of undigested floating grass and resulting difficulties with the agitation system (Thamsiriroj and Murphy, 2010), as well as inhibition of acetogenesis, leading to accumulation of lactic acid, and decreases in pH and methane production (Thamsiriroj *et al.*, 2012). Further problems when using grass as a feedstock may include increased abrasion of digester parts and grass contamination with soil requiring more frequent removal of sediment from the digester. Depending on the digestion system methane yields from the same grass silage may vary over a wide range: a two-stage continuously stirred tank reactor achieved 451 L kg^{-1} ODM_{added} over a 50-day retention period, while a sequentially fed leach bed reactor connected to an upflow anaerobic sludge blanket yielded 341 L kg^{-1} ODM_{added} with a 30-day retention time (Nizami *et al.*, 2012).

Grass combustion is possible as stand-alone biomass-firing or co-firing with other fuels such as coal, natural gas, peat or wood. The characteristics of grass as a solid biofuel require various specific and costly adaptations of the different combustion technologies (for an overview see Prochnow *et al.*, 2009b). Providing grass with a high fuel quality by appropriate grassland management and harvest is the key for the efficiency of the combustion process.

Efficiency parameters

Net energy yields reported for bioenergy production from permanent grassland range from 25 to 81 GJ ha^{-1} yr^{-1} (Table 1). Both for anaerobic digestion and for combustion, high biomass yields lead to high net energy yields and vice versa. At equal biomass yields, net energy yields are higher for combustion than for anaerobic digestion. In anaerobic digestion, the percentage of heat use is crucial for net energy yields at the same grassland management intensity and the same biomass yields. For semi-natural grassland with low biomass yield the hydrothermal conditioning and mechanical dehydration with subsequent anaerobic digestion of the press fluid and combustion of the press cake achieves highest net energy yields as add-on system to an agricultural biogas plant.

Table 1. Net energy yields, greenhouse gas savings and greenhouse gas mitigation costs of bioenergy production from permanent grassland.

Bioenergy pathway	Biomass yield t DM ha^{-1} yr^{-1}	Net energy yield GJ ha^{-1} yr^{-1}	Greenhouse gas savings t CO_2-eq ha^{-1} yr^{-1}	Greenhouse gas mitigation costs € t^{-1} CO_2-eq	Reference
Biogas					
- intensive grassland, co-digestion with slurry	12.0	69–78	–	–	Smyth *et al.* (2009)
- intensive grassland (three cuts), co-digestion with slurry, electricity use only	10.0	68	4.1	250	Rösch *et al.* (2009)
- intensive grassland (three cuts), co-digestion with slurry, electricity and 50% heat use	10.0	–	5.6	175	
- semi-natural grassland, dry fermentation	3.8	25	1.4–1.8	–	Bühle *et al.* (2012)
Hay combustion					
- low-input permanent grassland, bales	3.9	59	4.4–4.5	70	Rösch *et al.*
- low-input permanent grassland, pellets	3.9	65	5.1	80	(2009)
- semi-natural grassland, pellets	3.8	45	2.9	–	Bühle *et al.* (2012)
- miscanthus (*Miscanthus x giganteus*)	11.7	81	25.1	–	Styles and Jones (2007)
Hydrothermal conditioning and mechanical dehydration with anaerobic digestion of the press fluid and combustion of the press cake					
- as stand-alone system	3.8	44	3.0	–	Bühle *et al.*
- as add-on system to agricultural biogas plant	3.8	54	3.6	–	(2012)

Greenhouse gas savings vary from 1.4 to 25.1 t CO_2-eq ha^{-1} yr^{-1} (Table 1). They correspond with the net energy yields and are influenced by the same factors. The extraordinarily high upper value is explained by the inclusion of soil carbon sequestration after the establishment of miscanthus as perennial energy grass on former arable land, while all the other examples refer to permanent grassland without land use change.

Greenhouse gas mitigation costs are calculated from 70 to 250 € t^{-1} CO_2-eq (Table 1). With 70 to 80 € t^{-1} CO_2-eq, they are much lower for combustion of solid biofuel from low-input grassland than for anaerobic digestion of feedstock from intensive grassland with 175 to 250 € t^{-1} CO_2-eq.

Profitability mainly depends on biomass yields, the amount of resource input, conversion routes, sale prices for heat and electricity and subsidies (Smyth *et al*., 2010; Blokhina *et al*., 2011; McEniry *et al*., 2011; Blumenstein *et al*., 2012). Several studies show that anaerobic digestion and combustion of grass can be economically viable under certain conditions (Rösch *et al*., 2009; Smyth *et al*., 2010; Blokhina *et al*., 2011).

Conclusions

Grass can be used for bioenergy production via anaerobic digestion and combustion. Efficiency in terms of net energy yields, greenhouse-gas emission savings and greenhouse-gas mitigation costs is mainly influenced by biomass yield and composition, resource input, utilization pathway, conversion technology. Profitability, in addition, depends on market prices for energy and the regulatory framework.

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Oral Presentations

Biochemical methane potential of timothy, tall fescue and red clover silages harvested at different stages of maturity

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Abstract

Red clover (n=6), timothy-meadow fescue (n=3) and tall fescue (n=3) swards were harvested at different maturities and ensiled. Silage digestibility was analysed *in vitro* with pepsin-cellulase and *in vivo* using sheep. Further, biochemical methane potential (BMP) of the silages was measured. BMP of grass species was higher (344 L CH_4 kg^{-1} volatile solids, VS) than that of red clover (291 L CH_4 kg^{-1} VS). Although there was a strong correlation (R^2=0.81) between digestibility and BMP in grass species, the reduction in BMP was only 5% when digestibility declined from 700 to 600 g kg^{-1} dry matter. Our results suggest that silage for a biogas plant could be harvested later than for dairy cattle, thus improving the utilization of machinery capacity and increasing the grass yield.

Keywords: biogas, BMP, digestibility, grass maturity, silage, timing

Introduction

In order to make grass silage a competitive raw material for biogas production, there is a need to reduce its production costs. At Finnish farm level, the production costs of grass silage vary significantly, as the best quarter of the farms produce their silage with half of the costs of the poorest quarter (Peltonen, 2010). The 2008 statistics of ProAgria (agricultural advisory service in Finland) suggest that machinery costs make up one third of the silage production costs, being 290 and 470 € ha^{-1} for the best and poorest quarters, respectively (Peltonen, 2010). One possibility to reduce machinery cost is to divide the capital cost over a larger amount of silage harvested. If the optimal harvest time for a biogas plant differs from the optimum of a dairy herd, the same machinery could be utilized for both purposes.

Silage samples harvested at different stages of maturity were measured for BMP, chemical composition and digestibility. The relationship between digestibility and BMP was combined with previous knowledge on grass yield and digestibility development in order to assess how delaying the first cut affects the total methane yield per hectare.

Materials and methods

The silage samples analysed included the primary growth silages of timothy/meadow fescue (*Phleum pratense*/*Festuca pratensis*) and tall fescue (*Festuca arundinacea)* harvested at three different stages of maturity, two primary growth silages of red clover (*Trifolium pratense*) and four regrowth silages of red clover, also differing at stages of maturity. The detailed descriptions of the grass silages and of the red clover silages (harvested 2003 in Jokioinen) have been presented by Särkijärvi *et al.,* (2012) and Kuoppala *et al.,* (2006), respectively. The chemical composition and digestibility were measured as described in the original papers. BMP of the silages was measured using the automated methane potential test system (Bioprocess Control AB, Lund, Sweden).

A regression model $Y_{ijk} = A_i + B_j \cdot X + e_{ijk}$ was fitted to the data separately for each independent continuous numeric variable, using REG procedure of SAS. In the model, Y_{ijk} is BMP, A_i is intercept in the model, B_j is slope, X is independent variable in the data and e_{ijk} is the error term. The tested X_j variables were D-value (digestible organic matter concentration in dry matter (DM), measured either *in vitro* using pepsin-cellulase method or *in vivo* using sheep), neutral detergent fibre (NDF), acid detergent fibre (ADF), indigestible neutral detergent fibre (iNDF) and lignin concentration. Units are presented on DM bases as this is the practice of the D-value predicting services available at the moment in Finland.

The combined effects of changes in grass maturity and yield on total methane yield were demonstrated assuming that the start of harvest of primary growth varied. Weather data from 2010 and Finnish forage yield and quality models were used. In the calculation, 200 hectares were harvested twice and the assumed harvest capacity was 306,000 kg day^{-1} resulting in harvest periods of 7 to 15 days. Timing of first cut was restricted so that the D-value of first cut stayed between 570 and 730 g kg^{-1} DM, and the start of the second cut started 30 days after the finish of first cut but not earlier than 30 July.

Results and discussion

The average BMP of grass silages was 344 L CH_4 kg^{-1} VS and BMP decreased with increasing maturity. This is in line with the results of McEniry and O'Kiely (2013) although, due to differences in the methodology, the absolute BMP values are not directly comparable.

In Finland, the timing of primary growth harvest is optimized by utilizing the high correlation between temperature accumulation and grass digestibility. Moreover, an advisory service is available to predict local D-value. To be able to use the same service it is encouraging that the *in vitro* D-value predicted the BMP with a high coefficient of determination (R^2=0.79, Figure 1, when both variables were presented on the basis of organic matter (VS) R^2 was 0.81). In spite of the high correlation, the slope between BMP and D-value had a small value. In practice, e.g. when D-value changed from 700 to 600 g kg^{-1} DM, the BMP decreased only 5%. Further, ADF appears to be a promising candidate for predicting BMP of grass silage (R^2 was 0.87 when BMP was presented on VS basis).

The average BMP of clover silages was 291 L CH_4 kg^{-1} VS. Unlike grass silages, the differences in chemical composition or digestibility of the clover silages did not explain the differences in BMP results in these data. Due to the low number of samples, the conclusions related to the absence of correlations are not justified.

Table 1. Predicting biochemical methane potential (L CH_4 kg^{-1} dry matter) of primary growth grass silage from silage chemical composition and digestibility. $Y_{ijk} = A_i + B_j X + e_{ijk}$, where A_i is intercept in the model, B_j is slope, X is independent variable in the data and e_{ijk} is the error term, number of observations was six. Units g kg^{-1} dry matter.

Variable	A	S.E.	*P*-value	B	S.E.	*P*-value	RMSE	Adjusted R^2
in vitro D-value	201	29.5	0.002	0.178	0.0461	0.018	4.5	0.736
ADF	429	37.8	< 0.001	-0.364	0.1195	0.038	5.3	0.623
iNDF	332	6.5	< 0.001	-0.147	0.0518	0.047	5.6	0.587
in vivo D-value	237	27.8	0.001	0.125	0.0447	0.049	5.6	0.579
crude protein	267	18.8	< 0.001	0.381	0.1489	0.063	6.0	0.526
NDF	420	55.1	0.002	-0.180	0.0942	0.128	7.0	0.347
lignin	336	15.8	< 0.001	-0.716	0.5001	0.226	7.9	0.173

D-value = digestible organic matter g kg^{-1} dry matter, ADF = acid detergent fibre, iNDF = indigestible fibre, NDF = neutral detergent fibre, S.E. = standard error, RMSE= Root mean square error

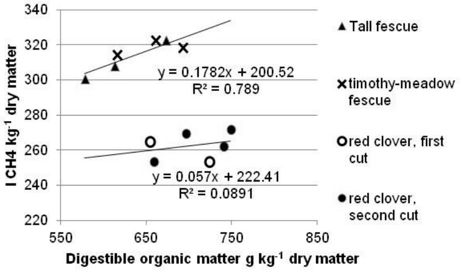

Figure 1. Correlation between D-value and biochemical methane potential.

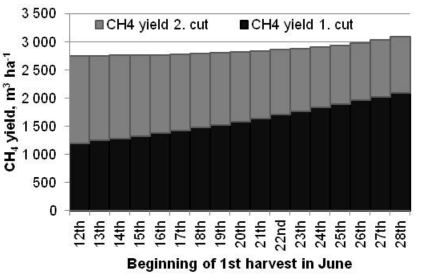

Figure 2. Methane yield accumulation depending on the beginning of the first harvest.

When combined with the yield accumulation data, it is concluded that towards the end of June the yield increases rapidly compared to the decrease in BMP (Figure 2). Based on these calculations, postponing the first harvest appears to be beneficial for a biogas plant.
These results were calculated based on BMP measured using a 45-day batch incubation. The role of specific BMP and digestion rate are more pronounced with shorter incubation times, which might potentially change the conclusion related to the optimization of harvest timing.

Conclusions

Increasing maturity reduces specific BMP of harvested grass silages. Our results suggest, however, that postponing the first harvest appears to be beneficial for a biogas plant as the increase in yield exceeds the reduction in specific BMP. This result is important on areas where dairy herds and biogas plants would be using the same harvest machinery.

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EU sustainability criteria for biofuels potentially restrict ley crop production on marginal land for use as biogas substrate

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Abstract

Ley crops can be grown to provide a substrate for biogas vehicle-fuel production on a range of soils including marginal land. According to EU regulations, such biofuel currently has to achieve an emission reduction of 35%, but tightened goals of 50 and 60% will come into effect as early as 2017. In two field trials (one on marginal soil, one on productive soil) ley crop mixtures were tested and the biomass DM yield was determined. In a life-cycle assessment approach, the emissions of the production chain for biogas-vehicle-fuel were estimated for a range of biomass DM yields. The results show that the emission intensity per energy unit of fuel produced is an asymptotic function of the DM yield. Currently, marginal lands not competing with food production can provide biofuels fulfilling the emission reduction requirements. However, a tightening of the goals to 50 or 60% is likely to cause a shift in biomass production towards better soils, potentially causing competition with food and feed production. Alternatively, the CO_2 emissions from biomass production of marginal soils need to be further reduced, e.g. by increased nitrogen fixation or reduced machinery use.

Keywords: biogas, substrate, systems analysis, energy balance, greenhouse gas emissions

Introduction

Grasslands can provide a number of ecosystem services. When used for biogas production, biomass from grasses and feed legumes is often the main desired ecosystem service. Furthermore, grassland can fix atmospheric nitrogen, sequester carbon, improve soil texture, increase biodiversity at the field and landscape level, and provide other ecosystem services in parallel. Several of these ecosystem services have a direct impact on the greenhouse gas (GHG) balance, i.e. biomass yield affects the amount of petroleum replaced by biogas; nitrogen fixation affects the amount of GHG emissions from mineral nitrogen required in cultivation; root growth and litter formation affects carbon sequestration.
The basic sustainability criteria for renewable vehicle-fuels require biofuels to reduce carbon dioxide (CO_2) emissions by at least 35% compared to petroleum (EC, 2009). This goal will be increased to 50% by 2017, and further to 60% in 2018 (for installations constructed after 1 Jan 2017) (EC, 2009). The aim of this study was to estimate the minimum amount of biomass per hectare of ley crops required to comply with these future requirements.

Materials and methodology

In two field trials in southern Sweden, biomass DM yields were determined (Table 1). Dry matter yields were calculated from the fresh weight and the moisture content (MC) of the samples. The MC was determined as weight loss in a drying oven at 105°C until constant weight. Differences in biomass yield were determined by ANOVA using Minitab statistical software. In order to test the dependence of GHG reduction efficiency of ley crops, models developed for calculating the energy balance for biogas production from ley crop biomass in southern Sweden (Gissén *et al.*, 2012) and the correlating emissions of CO_2-equivalents (Börjesson *et al.* 2012) were used. The models account for GHG emissions from cultivation, transport, storage and anaerobic digestion of the biomass. According to EU regulations, carbon sequestration arising from land use change and the use of the digestate produced were not accounted for (EC, 2009). It was assumed that the ley crops were fertilized with digestate complemented with mineral NPK fertilizer (Gissén *et al.*, 2012). Changes in CO_2-equivalent emissions (per hectare) resulting from cultivation, storage and transport of the biomass were simulated in a detailed model (Gissén *et al.*, 2012). Underlying assumptions were a relatively small N fertilization of 45 kg ha^{-1} plus a yield-dependent part assuming a biomass nitrogen content of 15 g kg^{-1}. Emissions resulting from digestate use in cultivation and biogas production were assumed to change linearly with changes in biomass DM yield.

Table 1. Main parameters of the two field trials in this study.

		Site 1		Site 2		
Land type		Marginal		Productive		
Location		55°49’N 13°22’E		56°06’N 12°57’E		
Harvest system	[harvests yr^{-1}]	1	2	1	2	3
N-fertilization	[kg ha^{-1}]	0, 60	0, 60	100	100(+60[a])	100(+60[a])
Harvest dates		17/09	25/06; 17/09	15/08	25/06; 18/09	5/06; 31/07; 18/09
Replicate samples	[n]	4	4	45	45	45

[a] after first harvest

Results and discussion

As expected, the biomass yields in the field trials were higher under high intensity cultivation (Figure 1). However, even the low input field trials resulted in considerable biomass yields. In both trials, two harvests per year yielded the most biomass, although yields from the one-harvest system on productive soils yielded more DM biomass than the two-harvest system on marginal land.

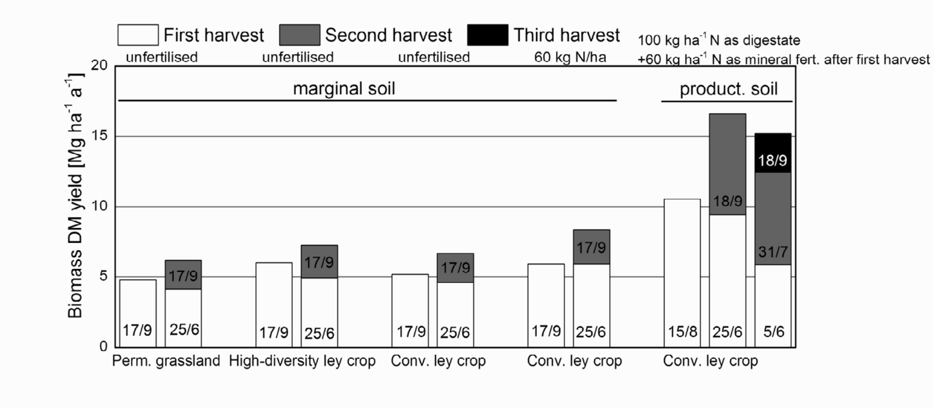

Figure 1. Biomass dry matter yields of different ley crop mixtures from marginal and productive soil in southern Sweden (numbers denote harvest dates).

The CO_2-equivalent emission intensity of biogas vehicle-fuel produced from ley-crop biomass is shown for a range of biomass DM yields (Figure 2). With increasing biomass DM yield, the emission of CO_2-equivalents decreases asymptotically. The share of emissions per energy unit caused by biogenic nitrous oxide and the anaerobic production process increases due to their linear relationship to the biomass DM yield in the model, though their intensity is constant.

This is realistic, since these emissions are related to the use of digestate and mineral fertilizer in cultivation. The share of emissions originating from cultivation, harvest, storage and transport decreased with increasing DM yield. These seemingly yield-related categories contain area-dependent operations (e.g. soil preparation) and lower indirect environmental costs (e.g. better utilization of machinery capacity). The net GHG emission intensity reaches currently acceptable limits at biomass DM yields above 3 Mg ha^{-1} yr^{-1} (Figure 2) value that can be achieved even on marginal lands with very low or no fertilization. When the emission requirements will be tightened in 2017, DM yields of about 5.5 Mg ha^{-1} yr^{-1} will be required in this scenario to fulfil emission-reduction goals of 50%. Since the curve has an asymptotic shape, a larger increase in DM yield per percentage unit of emission reduction is required. The 60% goal therefore implies a requirement of 10.5 Mg ha^{-1} yr^{-1} DM yield, which may cause a shift to the use of better land and increased food-energy competition.

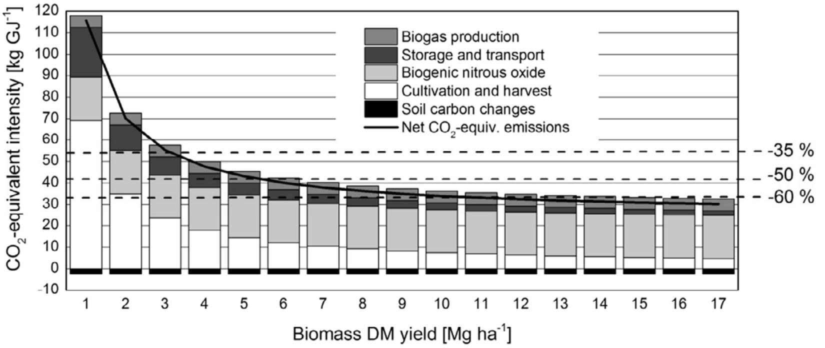

Figure 2. Intensity of CO_2-equivalents as emitted during cultivation and harvest, digestate use for fertilizing the crop, storage and transport of biomass and biogas production. Carbon sequestered by digestate use is shown as negative emissions. The bold line shows the net CO_2-equivalent emissions; the dashed lines show the emission reduction goals set by the EU (EC, 2009).

Conclusions

DM yields of ley crops vary strongly, and are dependent on the amount of inputs (mainly nitrogen fertilizer) and soil quality. Tightened emission reduction requirements may cause undesired use of land with better soils, while fallow marginal land might be practically excluded from production of renewable energy. This may cause an even tighter competition towards food and feed production. Alternatively, the CO_2 emissions from biomass production of marginal soils need to be further reduced, e.g. by increased nitrogen fixation or reduced machinery use.

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Effects of agricultural biogas-production facilities on land use and land-use change in Lower Saxony

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Abstract

In Germany, the production of biogas has expanded rapidly during recent years due to its promotion by the Renewable Energy Law. In consequence, the area cropped with maize, the main feed stock for the production of biogas, has expanded. In the meantime a pronounced conversion of grassland into arable land has been observed. These two phenomena, expansion of maize and conversion of grassland, are frequently linked in the public debate on the effects of the promotion of renewable energies. Here we report on a detailed analysis of the agricultural land-use and land-use changes for Lower Saxony based on plot-specific data of the Integrated Accounting and Control System. The data show that maize is the dominant crop after the conversion (nearly 60% of the converted area). However, only 15% of this maize can be attributed unambiguously to the production of biogas. The majority of the maize can be attributed to intensive dairy farms. Based on a logit model, we conclude that the likelihood of a conversion of grassland to arable land depends on its distance from a biogas plant: it is highest if it is less than 2 km away from the facility and it declines markedly at distances beyond 8 km. These results back up the opinion that the promotion of biogas puts additional pressure on grassland mainly by indirect effects, e.g. by increasing land rents.

Keywords: biogas, grassland conversion, renewable energy law

Introduction

Between 2000 and 2010, the grassland area in Germany declined by 393,000 ha (or 7%) while in the same time period the area cultivated with maize expanded by 692,000 ha (DeStatis, various years). The expansion of maize is primarily attributed to the effects of the German Renewable Energy Law, which, until recently, had heavily promoted the cultivation of maize as a substrate for the production of biogas. However, the question remains whether these two trends (decline of grassland and expansion of the maize area) just correlate or are causally linked. In order to explain gross land-use change between arable and grassland, we analyse spatially referenced data, and additional maps on soil, slope and protected areas. These data are supplemented by information on the location and capacity of biogas plants. We analysed the situation for Lower Saxony (north-west Germany) an area where the above-mentioned trends are very pronounced. The analysis allows for insights into the dependencies of grassland conversion on the presence of biogas plants.

In Lower Saxony 26,700 ha of grassland were converted into arable land just in 2006 and 2007. However, the area cultivated with maize expanded by 67,800 ha. Maize was the dominant crop in the year following the conversion to arable (66%). However, only 11% of this conversion could be attributed unambiguously to maize for the production of biogas. This result is in line with data published by Osterburg *et al*. (2011) who observed that over 75% of the grassland losses occurred in farms that are not involved in the production of biogas.

However, the analysis would be incomplete without considering the indirect effects of the production of biogas. The production of biogas creates additional demand for commodities (silage maize) and leads to higher tenure charges for arable land (Emmann *et al.*, 2011). Higher tenure costs might force farmers who use silage maize as a feedstock for their cattle to convert their grassland to substitute for the arable land that they lost to the producers of biogas feedstock.

We calculate two Logit-models. In the first we explain the likelihood of production of biogas feedstock, and in the second the likelihood of the conversion of arable land to grassland.

Table 1. Factors explaining the cultivation of biogas feedstock and conversion of grassland[1].

	Cultivation of biogas feedstock			Conversion of grassland		
		95% Wald			95% Wald	
Explanatory variable	exp(β)	Confidence Limits		exp(β)	Confidence Limits	
Constant	0.01			0.031		
Protected Areas						
Protected Habitat (Regional legislation)	0.27	0.12	0.65	0.09	0.05	0.15
Nature Protection Area	0.73	0.63	0.85	0.04	0.03	0.05
Landscape Protection Area	0.87	0.85	0.90	0.59	0.57	0.62
according to habitats Directive	1.20	1.11	1.30	0.21	0.19	0.24
according to Birds Directive	1.10	1.03	1.17			
Water Protection Area	0.93	0.91	0.95			
Soil type						
Hochmoor	3.36	3.23	3.50	1.16	1.11	1.21
Hochmoor (Deckkultur)	3.13	2.23	4.38			
Niedermoor	3.12	3.00	3.24			
Tiefumbruch (ehemaliges Moor)	3.41	3.27	3.56	3.17	2.95	3.41
Tiefumbruchboden	1.41	1.35	1.49	3.77	3.43	4.14
Gley	1.89	1.83	1.95	1.76	1.68	1.85
Pseudogley	1.68	1.61	1.75	1.40	1.31	1.50
Kolluvisol	2.23	1.91	2.59			
Braunerde	1.48	1.44	1.52	1.29	1.19	1.39
Braunauenboden	1.11	1.05	1.17	0.51	0.45	0.59
Schwarzerde	0.87	0.81	0.94			
Pelosol	0.43	0.34	0.53			
Plaggenesch				2.28	2.10	2.47
Podsol	1.90	1.86	1.95	2.46	2.38	2.55
Regosol	1.32	1.03	1.69	0.35	0.23	0.52
Ranker	0.06	0.02	0.16			
Rendzina	0.19	0.13	0.29	0.27	0.17	0.44
Distance to the closest biogas plant (in km)						
<1	6.08	5.75	6.42			
1- <2	4.92	4.69	5.17	1.12	1.08	1.17
2- <3	3.78	3.60	3.97	1.11	1.07	1.15
3- <4	2.98	2.83	3.12			
4- <5	2.62	2.50	2.76			
5- <6	2.09	1.98	2.19			
6- <7	1.70	1.61	1.79			
7- <8	1.53	1.45	1.62			
8- <9	1.40	1.32	1.48	0.80	0.75	0.86
9- <10						
10- <11	0.56	0.49	0.63	0.78	0.73	0.83
>11	0.53	0.49	0.58			
Model statistics						
n (cases)		286,271			282,612	
n (weights)		1,860,067			738,907	
ROC		0.642			0.636	
Pseudo-R-Square		0.103			0.035	

[1] Only values for variables whose *P*-value is below 0.0001 are shown

Materials and methods

The analysis was based on spatially disaggregated parcel data of the Integrated Administration and Control System (IACS) of Lower Saxony. The parcels were analysed in a Geographic Information System in order to detect land-use changes and grassland losses between 2005 and 2007 (Osterburg, 2010). Osterburg (2010) also provides a full overview on the used auxiliary data sources (soil, location of biogas production facilities, protected areas).
For the statistical analysis we conducted a Logit-regressions based on parcel level data of the IACS. The variables were selected using a forward-selection algorithm using a threshold value of $P<0.0001$. In Logit models, a coefficient above 1 implies that an increase in the respective explanatory variable increases the likelihood of the dependent variable, while a value below 1 implies a lower likelihood (Gujarati, 2003, p. 561 ff.). To assess the quality of the models we use the Receiver Operating Characteristic (ROC). A ROC value of 0.5 implies that the model is purely stochastic, while a value of 1 indicates a perfect fit of the data. The observations were weighted with their respective area.

Results and discussion

Despite the fact that the explanatory power of both models is fairly low, some variables have a highly significant impact (Table 1). In most protected areas the likelihood that a grassland area will be converted to arable land is lower than outside (except for water protection areas and areas protected under the Birds Directive). Biogas feedstock is especially cultivated on soils that have a high organic content (Hochmoor, Gley, etc.) and seldom on shallow soils (Ranker, Rendzina, etc.). Grassland is particularly prone to conversion if it is located on soils that have a long history of intensive human influence (Tiefumbruch, Plaggenesch) and also Podsols. Feedstock for biogas production is primarily produced close to the plant. The influence disappears only at distances greater than 9 km. For grassland conversion the influence of nearby biogas plants is weaker. The likelihood is increased by roughly 10% in the band between 1 and 3 km. At distances between 8 and 11 km we detect a roughly 20% lower-than-average likelihood.

Conclusion

Our results confirm the assumption that the likelihood of a conversion of grassland to arable land depends on the distance from a biogas plant. It is the highest if it is less than 2 km from the facility and it declines markedly at distances beyond 8 km. This result backs the opinion that the promotion of biogas puts additional pressure on grassland mainly by indirect effects, e.g. by increasing tenure charges. However, the explanatory power of the current models is weak. We intend to improve the power by including other factors such as capacities of biogas plants, the conversion to housing, stocking density and by extending the time series to 2012.

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Ethanol production from grass by *Thermoanaerobacter* B2 isolated from a hot spring in Iceland

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Abstract

The ethanol production capacity from sugars and timothy (*Phleum pratense*) hydrolysates by *Thermoanaerobacter* strain B2 was studied in batch cultures. The strain converts various carbohydrates to ethanol (major product), acetate, hydrogen and carbon dioxide (minor). Maximum ethanol yields were 1.50 mol ethanol mol^{-1} glucose and 1.32 mol ethanol mol^{-1} xylose. A stepwise increase in initial glucose concentrations (5-20 mM) led to proportional increases in ethanol production and complete glucose degradation. At >20 mM glucose concentration, incomplete degradation occurred and end-product formation levelled off. Ethanol production from timothy was investigated. Ethanol yields of untreated timothy hydrolysates resulted in 1.7 mM g^{-1} but were increased by using acid pre-treatment to 4.2 mM g^{-1}.

Keywords: *Thermoanaerobacter*, timothy, ethanol, lignocelluose, hydrolysates

Introduction

Complex (lignocellulosic) biomass has been put forward as a feasible alternative for ethanol production instead of simple biomass like mono- and disaccharides and starch due to its abundance in nature and the large quantities generated as waste from agricultural activities (Sanchex and Cardona, 2008). Lignocellulosic biomass is primarily composed of cellulose, hemicellulose and lignin. The latter is a random heteropolymer of lignols which cannot be used for ethanol production and has to be removed before fermentation. This is the main reason for unsuccessful implementation of complex lignocellulosic biomass as a starting material for bioethanol as expensive pre-treatments are needed for lignin removal and hydrolysis of cellulose and hemicellulose to sugars.

The use of thermophilic bacteria with broad substrate range and high yields may be a good option for ethanol production from complex biomasses. There are several advantages in using thermophilic bacteria: the increased temperature deters contamination from mesophilic bacteria and fungi, possible self-distillation of ethanol avoiding the generally low ethanol tolerance problem with those bacteria, and broad substrate spectrum.

The present study focuses on a recently isolated thermophilic bacterium, *Thermoanaerobacter* strain B2. The physiological characteristics of the strain were investigated to explore the ethanol production capacity both from simple sugars as well as from lignocellulosic biomass.

Materials and methods

Strain B2 was isolated from a hot spring in Grensdalur (temperature 70.1°C; pH 7.5) by using enrichments on glucose (20 mM) and end-point dilutions according to Baldursson and Orlygsson (2007). The medium used was the anaerobic medium as described by Sveinsdottir *et al*. (2009). Experiments were done in anaerobic serum bottles with butyl rubber stoppers, closed with aluminium caps. Substrate spectrum of the strain was done by using 20 mM concentration or, in the case of polymers, 3 g L^{-1}. The concentration of hydrolysates was 4.5 g L^{-1} and they were pretreated with acid and alkali as well as enzymes according to

Almarsdottir *et al*. (2012). Cellulose (Whatman paper) was used in same concentration for comparison. The timothy was a primary growth from a well fertilized mowing field cut at early boot stage. Phylogenetic analysis (16S rRNA) and analysis of end-product formation (ethanol, acetate and hydrogen) was done according to methods described in Baldursson and Orlygsson (2007).

Results and discussion

Partial 16S rRNA analysis revealed that the strain is most closely related to the genus *Thermoanaerobacter* with its closest neighbour being *T. ethanolicus* (98.7% similarity). The strain has an optimal temperature of 65.0°C and pH optimum of 7.0. One of the main reasons for increased interest in using thermophilic bacteria for second-generation ethanol production is because of their broad substrate spectrum. Therefore, it was decided to cultivate the strain on the most common sugars present in lignocellulosic biomass as well as several others (Figure 1). Clearly, the strain is a very powerful ethanol producer; it produces about 1.5 mol ethanol mol glucose^{-1} and 1.32 mol ethanol mol xylose^{-1} or 75.0 and 80.0% of theoretical yields, respectively.

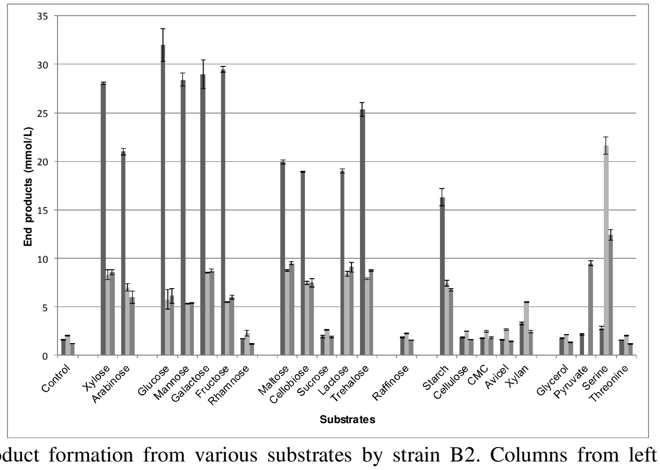

Figure 1. End product formation from various substrates by strain B2. Columns from left to right; ethanol, acetate and hydrogen. Control is growth with no external carbon source, only yeast extract (2 g L^{-1}). Bars represent standard deviation.

The substrate spectrum of the strain shows a broad capacity in degrading pentoses (xylose, arabinose), hexoses (glucose, mannose, galactose, fructose, rhamnose), disaccharides (maltose, cellobiose, lactose, trehalose) as well as starch, pyruvate and serine, but not cellulosic substrates. This is similar to other known *Thermoanaerobacter* species that are good ethanol producers within this genus (Lacid and Lawford, 1988; Brynjarsdottir *et al*., 2012). The strain was sensitive to relatively low initial glucose concentrations. By gradually increasing substrate loadings from 5 mM to 100 mM a clear inhibition was observed at 30 mM leading to incomplete glucose degradation and leveling off of end-product formation. The pH was measured after fermentation and decreased from low glucose loadings (pH 6.7) to high (≥100mM) loadings (pH 5.2). This, together with the fact that end-product formation levels off at high substrate loadings, indicate that this inhibition is more likely to be caused by the low pH rather than the high substrate loadings.

The strain is producing maximally 33.0 mM (6.9 mM g^{-1}; controls are subtracted) of ethanol from 4.5 g L^{-1} of cellulose hydrolysates (Table 1). These yields are considerable lower as compared to glucose degradation alone (1.50 mol ethanol mol^{-1} glucose; 8.6 mM g^{-1} glucose), which can be explained of incomplete glucose degradation in the cellulose hydrolysates, but 6.4 mM of glucose were analysed in the fermentation broth after fermentation. The addition of acid did not increase the end-product formation yields on cellulose. Ethanol production in timothy hydrolysates without pre-treatment was 1.7 mM g^{-1} hydrolysate but increased to 4.2 mM g^{-1} with acid pre-treatment. These yields are in a similar range as compared with many thermophilic bacteria (Sveinsdottir *et al.*, 2009; Almarsdottir *et al.*, 2012) although not as high as reported by *Thermoanaerobacter* BG1L1 (Georgieva *et al.*, 2008; Georgieva and Ahring, 2008). This bacterium has been reported to have very high ethanol yields (8.5 to 9.2 mM g^{-1}) from corn stover and wheat straw hydrolysates (calculated from sugar equivalents present in the hydrolysates).

Table 1. Production of end products from hydrolysates (4.5 g L^{-1}) from cellulose and timothy hydrolysates with and without acid pretreatment. Data represent the average of two replicated experiments (+/- standard deviation). Ethanol yields in mM g^{-1} DM are given within brackets with control (growth without hydrolysate but with 2g L^{-1} of yeast extract) subtracted.

	End product formation (mmol L^{-1})		
Biomass and pretreatment	Ethanol	Acetate	Hydrogen
Control	1.8 ± 0.1 (0.0)	2.0 ± 0.2 (0.0)	1.1 ± 0.0 (0.0)
Cellulose	33.0 ± 0.8 (6.9)	10.3 ± 0.4 (1.8)	12.0 ± 1.4 (2.3)
Cellulose, acid	32.6 ± 0.8 (6.8)	10.5 ± 0.3 (1.8)	12.7 ± 1.2 (2.6)
Timothy	9.5 ± 0.1 (1.7)	4.4 ± 0.1 (0.5)	7.4 ± 0.2 (1.4)
Timothy, acid	24.0 ± 0.5 (4.2)	7.0 ± 0.3 (1.1)	11.5 ± 0.4 (2.3)

Conclusions

Ethanol production was studied by *Thermoanaerobacter* B2 isolated from hot spring in Iceland. The main findings of the strain were: 1) good ethanol yields on glucose and xylose (1.50 and 1.32 mol mol^{-1} glucose and xylose respectively), 2) broad substrate spectrum, 3) low sensitivity towards high substrate concentrations, and 4) high ethanol yields on cellulose and timothy hydrolysates at low concentrations. Optimization experiments in continuous cultures are likely to solve its low substrate tolerance.

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Poster Presentations

Farm-scale co-digestion of manure and grass silage for improved energy yield and nutrient recycling

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Abstract

This paper presents results of farm-scale anaerobic co-digestion of dairy cow slurry and grass silage (feed ratio of 93:7 w/w) and experiences from fertilizing grass plots with digestate. The small amount of grass silage fed with the slurry increased the biogas production by 60% as compared with that from digesting slurry alone. The co-digestion also resulted in higher amount of ammonium nitrogen in the digestate than in the raw slurry. No significant differences were detected between fertilizing grass with digestate or raw slurry.

Keywords: anaerobic digestion, biogas, grass silage, manure, nutrients, renewable energy

Introduction

Agriculture relies heavily on depleting resources, such as fossil energy and phosphorus. Moreover, its environmental effects can be notable due to energy consumption and emissions associated with production and use of mineral fertilizers and direct emissions on farms. There is a need to reduce agricultural dependence on non-renewable resources and to decrease emissions.

Anaerobic digestion turns organic matter into energy-rich biogas, retains the nutrients in the substrates for reuse and improves nitrogen solubility, thus improving its availability to plants in digestate (e.g. Mata-Alvarez, 2003). It also enables improved control over gaseous emissions (NH_3, CH_4, N_2O; Amon *et al.*, 2006) and reduced nutrient losses to waters through use of proper spreading methods and timing (injection, vegetative phase; Grönroos *et al.*, 2011).

Biogas can be produced from animal manure, but the energy yield is low and the N:P ratio in digestate is possibly unfavourable for plants. Another potential substrate is grass silage. Monodigestion of silage, however, requires technology development. Supplementation of the process with trace elements may also be needed in order to meet the needs of the microbial consortia. Thus, co-digestion with manure may hold several benefits. Energy yield will be improved, as compared with digesting manure alone, and the digestate N:P ratio may become more favourable for fertilizer use and thus also for closing the nutrient cycle within a farm.

This paper presents results on farm-scale anaerobic co-digestion of dairy cow slurry and grass silage (feed ratio of 93:7 w/w) during process start-up and experiences in utilizing the resulting digestate in grass silage production on field plots.

Materials and methods

The farm-scale biogas plant (built 2009) of MTT Agrifood Research Finland is located in Maaninka, Finland (63°08'N, 27°19'E). It is designed to co-digest 3,500 m^3 of dairy cattle slurry (total solids, TS 6-10%) and 300 t of fresh grass or grass silage per year. The slurry is pumped from a 100 m^3 pre-storage tank into a 300 m^3 reactor made of concrete elements (two propeller mixers, 37°C) and the grass is fed via a separate feeding screw. The digestate flows by gravity into a 300 m^3 post-digestion tank (one propeller mixer, no heating). The tanks are located below ground and covered with plastic hoods, one for gas storage and another for protection. The digestate produced is stored in open manure tanks, while the biogas produced is utilized as heat and power (63 kW combined heat and power (CHP) device + 85 kW boiler).

The biogas plant was fed daily with 10 m^3 of slurry (feeding at 8 h intervals) and 800 kg of grass silage (once a day). The hydraulic retention time (HRT; reactor + post-digestion) 25 + 25 d and the desired organic loading rate (OLR) of 3 kg of volatile solids (VS) m^{-3} d^{-1} was reached in 12 weeks after the start-up. At this point, the slurry from the new cowshed had a TS of 7.1%, being 4.9-6.7% in the previous weeks. The average VS content was 5.8%. The grass silage used was 55% timothy grass (*Phleum pratense*) and 45% meadow fescue (*Festuca pratensis*) with an average 27.5% TS, 25.7% VS and digestibility of 765 g kg DM^{-1}.

The fertilizer value of the resulting digestate was compared with raw slurry and with mineral N in a field experiment during 2009-2011. Both slurry and digestate were injected into the soil at a depth of 5-7 cm with a plot-sized slurry spreader. The grass was a 70:30 w/w mixture of timothy (cv. Tuure) and meadow fescue (cv. Ilmari). During establishment, whole crop barley (cv. Voitto) was used as a cover crop. During grass years, harvesting was done twice at silage stage. In the establishment year, slurry and digestate were used at 21-30 t ha^{-1} (equivalent of 29 kg ha^{-1} soluble N (Nsol) and 55 kg ha^{-1} total N (Ntot) for the slurry and 47 and 84 kg ha^{-1} for the digestate, respectively). During grass years, mineral N (100 kg N ha^{-1} as NH_4NO_3) was spread in the spring and organic fertilizers for second cut only (28-30 t ha^{-1} = 51-62 kg Nsol ha^{-1} and 7891 kg Ntot ha^{-1}). In addition, six levels of mineral N (NH_4 NO_3) were used to calculate N fertilizer replacement value for Nsol of the organic fertilizers. The plot size was 1.50×10 m and they were arranged in a randomized complete block design with four replicates. The nutrient content of organic fertilizers and yields were determined and nutrient balances calculated. Soil samples (profiles of 0-2 cm, 2-10 cm and 10-25 cm) were taken each autumn. Soil P (PAc) was analysed according to Vuorinen and Mäkitie (1955) and all other chemical analyses were made according to standard methods. The treatments in fertilizer experiments were compared using ANOVA (Mixed procedure, SAS 9.2) and pairwise comparisons were performed using contrasts.

Results and discussion

The farm-scale biogas plant produced 17 m^3 CH_4 t^{-1} fresh material (FM) or 238 m^3 CH_4 tVS^{-1}, once it reached the design load. This was 91% of the expected biological methane potential (BMP, Table 1). Approximately 20% of the biogas was produced in the post-digestion tank, emphasizing its importance in utilizing total biogas produced and in reducing CH_4 otherwise emitted from the digestate. When comparing these results to monodigestion of slurry (TS 7.3%) in the same farm-scale plant (data not shown), methane production increased by approximately 60% with the small amount of grass silage added.

Table 1. Average characteristics of the slurry and grass silage during the design OLR of the farm-scale biogas plant and of the resulting digestate.

Material	TS %	VS %	Ntot kg t^{-1}	NH_4-N kg t^{-1}	BMP Nm^3 CH_4 $t^{-1}VS$
Slurry	7.1	5.8	3.2	1.6	227
Silage	27.5	25.3	6.5	0.4	364
Total feed	8.6	7.3	3.4	1.6	262
Digestate	3.6	2.8	2.1	1.5	-

The digestate TS and VS were 3.6% and 2.8%, respectively (Table 1). It contained relatively more mineral N than the original feed; 72% of Ntot was as NH_4-N, whereas the raw slurry

and the mixture of slurry and silage had NH_4-N/ Ntot ratios of 52% and 46%, respectively. Therefore, the digestate contained on average 59% more ammonium nitrogen as compared with the substrates (38% as compared with slurry alone), increasing its fertilizer value.
There were no differences in grass dry matter production between slurry and digestate except in the first cut of 2011, for which slurry gave 9% higher yield than digestate (7680 vs. 7040 kg ha^{-1}, P=0.036). In the same harvest, the content of crude protein (P=0.025) and of P were lower for slurry than digestate (P=0.064). Otherwise there were no significant differences in yield parameters. Over the three years, N fertilizer replacement value for Nsol was 94% for slurry and 91% for digestate. Based on soil and grass analyses, the effect of differences in applied P and K amounts between the treatments were most likely minor. During grass years, nutrient balances were generally negative in the first cut with mineral N, and positive in the second cut with organic fertilizers. When calculated over the three years, the annual N balances were negative for both slurry and digestate (-61 and -26 kg N ha^{-1}, respectively) which was mostly due to high yield in the first cut utilizing the nutrients left from the previous year. Similarly, the annual P balances were -35 kg P ha^{-1} for slurry and -25 kg P ha^{-1} for digestate. Both N and P balances show that organic fertilizers may not cause nutrient accumulation into the soil when moderate amounts together with injection are used for productive grass. This was further verified with only minor changes in soil NO_3-N and PAc concentrations in surface layer in autumn samples (data not shown).

Conclusions

A small addition of grass silage increases the biogas production of a farm-scale biogas plant significantly as compared to digesting slurry alone. Such a smallish quantity of silage is most likely available in cattle farms without a need to increase current production or modifying methods. Silage addition also improves the N:P ratio of the digestate, making it more attractive as an organic fertilizer than raw manure. The use of organic fertilizers for grass is an effective way to recycle nutrients if moderate amounts are used. In general there were only slight differences between raw slurry and digestate in the value of replacing inorganic fertilizers and in effects on N and P balances or soil parameters.

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The productivity of *Miscanthus* under Lithuania's climate conditions: a two-year study

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Abstract

Biomass cultivation for bioenergy should fulfil many economic, energy and environmental requirements. As a result, in many countries considerable attention has been drawn to agricultural plants with a potential to be used as raw material for various energy purposes. When searching for non-food plants for combustion, priority in warm and moderate climate zones is given to *Miscanthus giganteus.* The aim of this study was to explore the feasibility to grow *Miscanthus giganteus, Miscanthus sachariflorus* and *Miscanthus sinensis* under the climatic conditions of Lithuania and to evaluate their productivity. Rhizomes and *in vitro* propagated plantlets from Denmark, United Kingdom, Poland and Lithuania were chosen for the establishment of the experiment. The initial findings of the experiment suggest that *Miscanthus* planted using the *in vitro* plantlets did not survive the first winter, whereas that planted by rhizome division exhibited a better cold-season resistance. *Miscanthus giganteus* produced higher biomass yield than *Miscanthus sachariflorus*.

Keywords: *Miscanthus*, cold resistance, productivity

Introduction

In most warm-climate zone countries, biomass of *Miscanthus* is a promising raw material for energy production. This herbaceous plant produces high biomass yield of the quality appropriate for burning. The biomass quality can be controlled by cultivation technologies (Lewandowski *et al*., 2000; Heaton *et al*., 2008). In southern Europe, the biomass yield of *Miscantus* is in excess of 30 t ha^{-1} (Zub *et al*., 2011). In northern countries, its yield amounts to 7-15 t ha^{-1} but *Miscanthus* is still of interest as an energy crop. *Miscanthus* reaches the highest biomass potential in 3-5 years (Lewandowski *et al*., 2000), and this is mostly influenced by the sward formation within the first two years (Jezowski, 2008; Jezowski, 2011).
Research conducted in Europe has shown evidence of poor *Miscanthus* overwintering in the first year, but it has been noted that under conditions of changing climate this energy crop has a potential to be grown over almost all of Europe (Clifton-Brown *et al.,* 2004; Clifton-Brown and Lewandowski, 2008). The aim of this study was to explore the feasibility to grow *Miscanthus giganteus, Miscanthus sachariflorus* and *Miscanthus sinensis* and to evaluate their productivity under the climatic conditions of Lithuania.

Materials and methods

A field experiment was established in 2010 in Central Lithuania in a reclaimed river-bed territory. It was set up in 15 m^2 plots. The experiment was laid out in a randomized block design with ten treatments and four replicates. The soil of the experimental site is light, sand on sand, with stone and gravel admixture, classified as *Eutri-Cambic Arenosol.* The concentration of total nitrogen in the plough layer was 1.2 mg kg^{-1}, available phosphorus 120 mg kg^{-1} and available potassium 148 mg kg^{-1}. The planting density of *Miscanthus* was 2 plants m^{-2}. The countries of origin of *in vitro* propagated plantlets of *Miscanthus sachariflorus, M. sinensis and M. giganteus* were Denmark (DK), Lithuania (LT) and Poland (PL). The rhizomes for the experiment were chosen from DK and the United Kingdom (UK). The nursery was planted in the middle of May and harvested at the beginning of December. After harvesting, the plots were mulched with a 10-cm thick layer of wheat straw. The number of plants and shoots per m^2 were estimated before harvesting. The results were subjected to standard analysis of variance.

Results and discussion

Our findings suggest that in the first year the number of plants per unit area differed between *Miscanthus* genotypes and planting material. The highest plant density was recorded in the plots that were planted with DK *in-vitro* propagated plantlets. In the same year, the planting material had different effects on the plant density. Out of the 30 planted DK *Miscanthus giganteus* rhizomes, 79% became established, while out of the 30 *in-vitro* propagated plantlets 96% became established (Table 1). This rate is similar to that obtained in previous *Miscanthus* studies for the same soil in 2007, where out of 348 seedlings planted 88% became established (Kryzeviciene *et al.,* 2011).

In the first year of *Miscanthus* growth, *in-vitro* propagated plantlets exhibited better above-ground development compared with those planted by rhizome division. The number of shoots of *in-vitro* propagated plantlets varied from 6.3 to 13.6 per plant and their height ranged between 74.3 and 88.7 cm. The number of shoots per plant of *Miscanthus* planted by rhizome division was 3.0-3.9. However, the shoot height was significantly higher compared with that of plants grown from *in-vitro* propagated plantlets (Table 1).

Table 1. Number of plants and shoots and plant height of *Miscanthus* in the first two years of growth.

	Country of origin	Planting material	Number of plants per m^2		Number of shoots per plant		Plant height cm	
			2010	2011	2010	2011	2010	2011
Miscanthus sachariflorus	Denmark	*in vitro* plantlets	1.77	1.67	8.7	8.0	99.9	133.6
Miscanthus sinensis	Lithuania	*in vitro* plantlets	1.20	0.00	6.3	-	74.3	-
Miscanthus giganteus	Poland	*in vitro* plantlets	1.58	0.24	7.4	5.6	78.2	69.0
	Denmark	rhizomes	1.35	1.47	3.9	16.4	116.5	204.8
		in vitro plantlets	1.92	0.40	13.6	9.2	88.7	115.4
	UK	rhizomes	0.82	1.02	3.0	16.2	88.2	185.8
		LSD_{05}			1.8	5.9	15.6	28.9

Miscanthus genotypes usually differ in their re-growth ability and cold resistance (Clifton-Brown and Lewandowski, 2008). In our research, over the first winter the number of *in-vitro* propagated plants (*M. sinensis* and *M. giganteus*) decreased markedly (Table 1). The average number of DK *M. giganteus* was 0.40 plants m^{-2}, PL *M. giganteus* was 0.24 plants m^{-2}, and LT *M. sinensis* plants were winter killed. The highest density of *in-vitro* propagated plantlets was determined for *M. sachariflorus* or 1.67 plants m^{-2}. In the second year of *Miscanthus* growth, *M. giganteus* (rhizomes, DK) exhibited the highest shoot density per plant and the highest height compared with the other species investigated.

Miscanthus biomass yield (dry matter) in the second year was very low and differed between species and type of planting material. The biomass yield of the stand planted with *in-vitro* propagated plantlets was lower than the stands planted with rhizomes. The highest yields were recorded for the DK *Miscanthus giganteus*, planted as rhizomes (Figure 1).

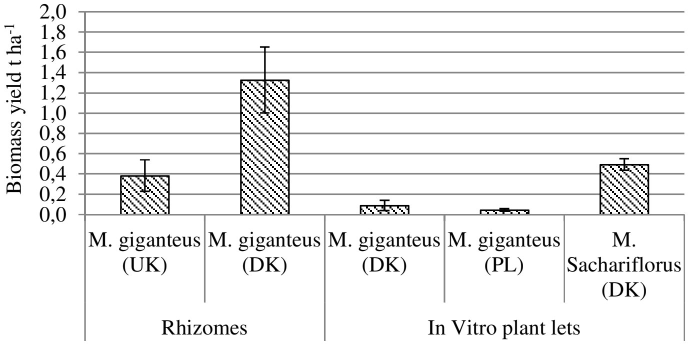

Figure 1. Biomass (dry matter) yield of different *Miscanthus* species and genotypes.

During the two experimental years, all species and genotypes of *Miscanthus* did not produce high biomass yield. However there were differences in biomass values. The DK *Miscanthus giganteus* produced significantly higher biomass yield compared to that of UK and PL. The results of previous experiments suggest that full biomass yield potential can be achieved in the third year of plant growth (Kryzeviciene *et al*., 2011) and according to this, it is expected that the biomass yield will increase. The *Miscanthus* overwintering ability and cold resistance gave some promising results for future research.

Conclusions

The results of the first two experimental years suggested that *Miscanthus* productivity and cold resistance differed depending on the hybrid origin and planting material. Further research is needed to study *Miscanthus* performance in a wider range of soils and environments.

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Evaluation of biomass yield and quality of energy crops in Lithuania

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Abstract

The objective of this research was to study biomass potential and quality of 14 plant species grown for bioenergy. The tested plants were divided into groups. In the imported plants group, the highest dry matter yield (9.58 t ha^{-1}) was produced by *Sida hermaphrodita* when cut once. Other plants of this group were less productive: dry biomass yield of *Silphium perfoliatum* was 7.29 t ha^{-1}, and *Polygonum japonica* and *Polygonum sachalinensis* were 8.74 and 5.13 t ha^{-1}, respectively. Of the non-traditional plants, the highest biomass content was accumulated by *Artemisia dubia* (11.10 t ha^{-1}) and *Helianthus tuberosus* (8.56 t ha^{-1}) and *Artemisia dubia* biomass was 3.3 times as high as that of *Artemisia vulgaris*. In the group of local plants, unfertilized *Galega orientalis* produced 10.98 t ha^{-1} dry biomass over 3 cuts. The lowest ash contents (2.80-3.95 g kg^{-1}) were accumulated in the biomass of *Miscanthus* and *Artemisia vulgaris*. Higher sulphur contents accumulated in traditional grasses that had been cut more frequently. The lowest sulphur contents were accumulated in *Polygonum, Artemisia, Miscanthus, Silphium* and *Sida* plants. The choice of plants intended for bioenergetics will depend on specific local conditions, needs and circumstances.

Keywords: energy crops, biomass, productivity, quality, fertilization

Introduction

Plant biomass is a renewable energy resource. In Lithuania there is little research into the choice and cultivation of plants for energy needs, and the yield of dry biomass of perennial tall grasses is 6-9 t ha^{-1} and up to 12 t ha^{-1} only in favourable years (Jasinskas *et al*., 2008). As well as indigenous C_3 plants, imported C_4 plants that possess very high energy potential are promising material for bioenergy production. Until now, imported plants (*Sida hermaphrodita*, *Polygonum japonica, Polygonum sachalinensis*, *Miscanthus giganteum*) have been used only for ornamental purposes. Continuing the search for potential energy crops and expecting that global climate change processes and climate warming will become conducive to warmth-loving C_4 type plants, we tested them as energy crops. In Lithuania, plants are often damaged by late frosts in spring and co-occurring soil droughts and hot weather spells. As a result, research on imported plants in specific conditions is indispensable.

The search for and development of renewable energy sources in Lithuania as well as rational use of their resources has therefore become increasingly relevant. The aim of this study was to explore growth and development features of perennial plants differing in origin, type and species and to estimate biomass yield as influenced by various rates of nitrogen fertilization.

Materials and methods

The trials were conducted in Dotnuva (lat. 55º24'N, 23º52'E), in a reclaimed river bed territory during 2010-2012. The soil is light, sand on sand, with small stone and gravel admixture, *Eutri-Cambic Arenosols* (*ARb-eu*). A plantation of energy plants was established in 2007. Three groups of energy plants were investigated: (i) imported herbaceous plants – *Miscanthus giganteus*, *Sida hermaphrodita*, *Silphium perfoliatum* L., *Polygonum sachalinensis* L., *Polygonum japonica* hybrid; (ii) non-traditional plants – *Artemisia vulgaris*

L., *Artemisia dubia* Wall., *Helianthus tuberosus* L.), *Panicum virgatum* L.; and (iii) indigenous (local) plants as a control – *Dactylis glomerata* L., *Phalaris arundinacea* L., *Galega orientalis* Lam., *Medicago sativa* L., *Onobrychis viciifolia* L. Imported plants (except *Miscanthus*) were planted at a density of 1 per square meter. The *Miscanthus* hybrid was obtained from Austria and grown up to 2-4 leaf stage by vegetative propagation (rhizome division) and planted at two plants per square meter. *Artemisia dubia* was planted using the vegetative propagation method of rhizome division. *Helianthus* was planted as tubers (2 tubers per square meter with 1 m inter-row spacing). The indigenous (local) perennial grasses were sown by a drill with 15 cm inter-row spacing. The experiment was designed as a randomized complete block with three replicates. Plot size was 10×10 m. Harvested plot size was $10 m^2$. Before planting 60 kg P ha^{-1} and 60 K kg ha^{-1} fertilization were applied to the experimental area. In the first year, 60 N kg ha^{-1} were applied as a starter to stimulate growth. In the second year, the nitrogen fertilization scheme (except on legumes) was 0N, 60N and 120N kg ha^{-1}. Nitrogen fertilization was continued each year in early spring. Indigenous grasses were cut 3 times, *Panicum virgatum* twice. Other plants were cut once in October. Total C, N and S content of samples was determined simultaneously by dry combustion (Dumas method) using Vario *EL* III *CNS*-autoanalyser. The results were processed using STAT_ENG for EXCEL v. 1.55.

Results and discussion

The dry matter (DM) yield of plant biomass is an important factor for bioenergy production. The data averaged over two years showed that the highest DM yields were produced by *Artemisia dubia* and *Galega orentalis*, 11.10 and 10.98 t ha^{-1}, respectively (Table 1). The amounts of biomass produced by the best yielding herbaceous plants were similar to those produced by short-rotation forest plant *Salix vinimalis* (Tahvanainen, 1999). The *Artemisia dubia* at cutting time contained 49.9% DM. Compared with *Galega orentalis*, *Artemisia dubia* developed more rapidly and produced a high stand density, which prevented weeds from being established. *Galega* is superior to *Artemisia* in two aspects: it does not need nitrogen fertilization and is easier to establish. Another two promising plants for bioenergy are *Sida hermaphrodita* and *Phalaris arundinacea.* Their DM yield when fertilized with 60 and 120 kg N ha^{-1} was 9.58 t and 9.89 t ha^{-1} respectively. *Sida* plants cut once were the tallest (271 cm) of all the plants tested. They are suitable for direct combustion, as their biomass accumulates 51.0% of DM. In terms of DM yield, the other tested plants *Helianthus* and *Miscanthus* (8.56-7.14 t ha^{-1}), significantly lagged behind the above-discussed plants. *Miscanthus* produced around 7.0 t ha^{-1} in our research. In another study the highest yield (11.53 t ha^{-1}) was obtained in the second year of use (Kryzeviciene *et al*., 2011). Due to the cold winters of 2010 and 2011, *Miscanthus* markedly thinned out. In the spring of 2010, the plants that survived winter accounted for 54% of the initial number planted.

When cut 3 times, traditional legumes (*Galega, Onobrychis, Medicago*) accumulated the largest content of nitrogen in the dry biomass. For the anaerobic biomethane process to be optimal, the carbon to nitrogen ratio (C:N) in biomass is one of the main quality indicators. Literature sources indicate various ranges of C:N values. It is maintained that its optimal value commonly ranges between 20 and 30, however, some authors have shown this value to range from 15 to 30 (Osman *et al.*, 2006). In our study, in the biomass of traditional grasses the range of carbon to nitrogen ratio varied from 15.3 *(Galega)* to 51.5 (*Phalaris).* Thus, according to this indicator, *Phalaris* is better suited for combustion than for biogas production. *Sida, Silphium* and *Artemisia* accumulated the lowest levels of nitrogen in the DM, which confirms their suitability for direct combustion. Higher sulphur contents accumulated in traditional grasses that had been cut more frequently. The lowest sulphur contents were accumulated in *Polygonum, Artemisia, Miscanthus, Silphium* and *Sida* plants.

Ash is unwanted in the biomass intended for combustion. The lowest ash contents (2.80-3.95 g kg^{-1}) accumulated in the biomass of *Miscanthus* and *Artemisia.*

Table1. The potential of biomass dry matter (DM) yield and chemical composition of herbaceous species (from data averaged over two years).

Species	Nitrogen fertilization kg ha^{-1}	DM yield t ha^{-1}	Standard error SE	DM before cut %	Chemical composition g kg^{-1}			
					C	N	S	Ash
Imported species								
Sida hermaphrodita	60	9.58	1.10	51.0	471	4.11	0.46	42.3
Polygonum japonica hybr.	0	8.74	0.74	36.2	474	5.27	0.34	48.4
Silphium perfoliatum	60	7.29	0.58	38.5	451	4.11	0.40	91.8
Miscanthus giganteus	120	7.14	0.63	52.1	482	5.15	0.40	28.0
Polygonum sachalinensis	0	5.13	0.55	35.8	470	2.66	0.37	66.1
Non-traditional species								
Artemisia dubia	60	11.10	0.70	49.9	489	3.55	0.43	41.7
Helianthus tuberosus	60	8.56	0.53	40.7	464	5.05	0.50	49.7
Panicum virgatum	120	4.62	0.25	26.2	488	8.31	0.78	56.2
Artemisia vulgaris	60	3.40	0.45	59.5	484	4.55	0.48	39.5
Local species								
Galega orientalis	0	10.98	0.54	18.2	461	30.1	0.84	93.2
Phalaris arundinacea	120	9.89	0.33	31.0	459	8.91	2.73	78.1
Medicago sativa	0	6.16	0.93	25.3	470	23.3	0.88	76.7
Dactylis glomerata	120	6.14	0.20	21.2	455	17.2	1.24	93.5
Onobrychis viciifolia	0	5.21	0.80	33.9	471	25.9	1.70	80.7

Conclusions

The largest amount of biomass, 9.58-11.10 t DM ha^{-1}, which can be used for the production of various types of bioenergy, was accumulated by *Artemisia dubia, Galega orientalis, Phalaris arundinacea* and *Sida hermaphrodita*. In terms of their biomass output, the imported plants were not superior to the local ones under the Lithuanian climate conditions. The choice of plants intended for bioenergy will depend on specific local conditions, needs and circumstances.

Acknowledgements

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Energy balance of grass based biogas production systems on a coastal marsh soil

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Abstract

Due to specific environmental conditions in the coastal marsh region of Schleswig-Holstein, grass silage could represent a valuable resource for biogas production in Northern Germany. Therefore, a 2-year field trial was conducted at a coastal site to perform an energy balance for quantifying and evaluating the impact of cropping system, nitrogen (N) level and N fertilizer type on the energy balance. Considering the whole chain of biogas production, grassland revealed a lower net energy yield and lower energy efficiency than maize monoculture and the rotation. For grassland the comparison of fertilizer types showed clear differences in net energy yield and energy efficiency.

Keywords: biogas, grassland, maize, net energy yield, coastal marsh soil

Introduction

Expansion of biogas production in Germany has been paralleled by a strong increase in the acreage for maize production, even at marginal sites such as the coastal marsh region of Schleswig-Holstein. This region has specific environmental conditions less favourable for maize cultivation such as heavy clay soils, high groundwater level, low temperatures and high wind speed. Thus, the competitiveness of grass against maize for biogas production in terms of energy yield potential and energy efficiency might be increased. Although various studies on energy balances of biogas production are available, most are estimates based on literature and not applicable to northern Germany. The aim of the present study was to calculate energy balances for regionally adapted cropping systems as a first step of a life cycle assessment.

Materials and methods

A 2-year field trial (2009-2010) was conducted on a heavy clay soil site at the west coast of Schleswig-Holstein. The long-term annual precipitation at the experimental site averages 830 mm with a mean annual temperature of about 8.7°C. The soil is classified as a gleyic Fluvisol (calcaric) of silt-clayey structure. Three different cropping systems were investigated, i.e. a 4 - cut perennial ryegrass ley (GR), a maize – winter-wheat – Italian ryegrass – rotation (MWG) and a maize – maize rotation (MM). Due to a late harvest of the maize in autumn 2008 sowing of winter wheat was prevented, which resulted in seeding of spring wheat in April 2009. Nitrogen fertilization was varied in three levels (control, moderate, high), either applied as calcium-ammonium-nitrate (CAN) or biogas residue from co-fermentation. The N-rate differed between the crops and is specified in Table 1. The energy balance was calculated according to the life cycle inventory analysis provided by the International Organization for Standardisation guidelines 14044 (ISO 2006) based on method and assumptions of Claus *et al.* (2011) for the input calculation. The total energy output calculation ($output_{el}$, $output_{th}$, nutrients in biogas residue) was based on the dry matter (DM) yields, the specific-methane yields (L_N CH_4 kg^{-1} DM), and N-, P-, and K-uptake of the investigated cropping systems (MWG, GR, MM). The net energy yield (NEY) was obtained as the difference between the total energy output and energy input. For conversion, a combined heat and power plant (500 kW, mono-digestion), with an electric efficiency of 40%, a thermal efficiency of 41.5% and heat utilization of 45% was assumed. Heat and electricity demand for plant operation were assumed to be 20% of the generated electricity for heat and 7.5% for electricity. The impact of cropping system, N fertilizer type and N amount on net energy yield was investigated using SAS 9.2 Proc Mixed by assuming these factors as fix and replicate as random. Multiple comparisons were conducted by t-test and subsequent Bonferroni-Holm adjustment.

Table 1. Nitrogen fertilization rates (kg N ha^{-1}).

	Perennial ryegrass	Maize	Spring wheat	Winter wheat	Italian ryegrass
Control	0	0	0	0	0
Moderate	360	150	180	240	80
High	480	200	240	320	80

Results and discussion

The net energy yield (NEY; GJ ha^{-1}) showed a substantial variation from -0.8 to 79.6 GJ ha^{-1} and was significantly affected by an interaction of cropping system, N fertilizer type and N amount. MM achieved highest NEY in all combinations of N fertilizer type and N amount (Figure 1). GR showed lowest NEY in the control treatments, whereas in the moderate and high N level it showed a similar NEY as MWG. The N fertilizer type affected GR and MWG in the moderate and high N-level mainly, where CAN achieved higher NEY than biogas residue. The control plots always yielded less than the moderate N levels but no further increase could be observed from the moderate to high N level.

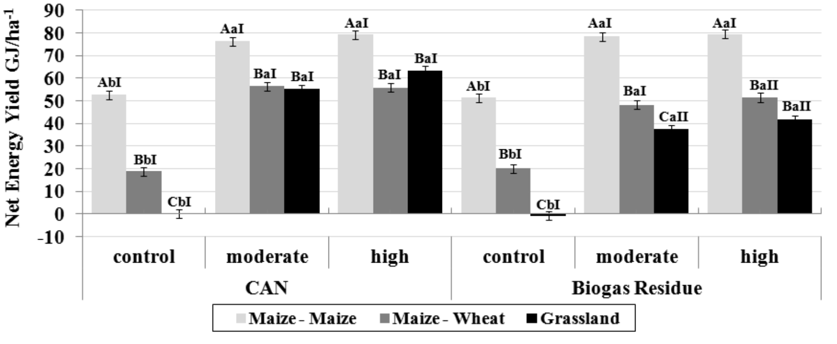

Figure 1. Net energy yield (GJ ha^{-1}) as influenced by cropping system, N fertilizer type (CAN, biogas residue) and N amount (control, moderate, high). Capitals: sig. differences between cropping systems, within N amount and N fertilizer type; lower case: sig. differences between N amount, within N fertilizer type and cropping system; Roman num.: significant differences between N fertilizer types, within cropping system and N amount).

Better performance of MM can be attributed to high DM yield (15.5 t DM ha^{-1}), see Quakernack *et al.* (2012), and a slightly higher specific methane yield (MM: 371 L_N CH_4 kg^{-1} ODM; MWG: 340 CH_4 kg^{-1} ODM; GR: 352 CH_4 kg^{-1} ODM). It should, however, be noted that the maize yield in the coastal marsh area is considerably lower than in other regions (Herrmann *et al.*, 2012). Springtime droughts occur regularly in the marsh region and may therefore strongly affect maize yield performance and can even cause a complete maize failure. The lower NEY of MWR compared to MM can be explained by the necessity to sow summer wheat in spring 2009 instead of winter wheat in autumn 2008, which led to a comparably low average DM yield (CAN: 15.4 t DM ha^{-1}, biogas residue: 13.3 t DM ha^{-1}). Hence this cropping system is also characterized by an increased yield risk. In contrast, GR has a high yield stability but low yield when fertilized with biogas residue (9.7 t DM ha^{-1}) compared to CAN (16.3 t DM ha^{-1}), partly due to higher N losses via ammonia volatilization (Quakernack *et al*., 2012) and a lower N-use efficiency.

Table 2. Energy output (EO), energy input (EI) and net energy yield (NEY) (GJ ha^{-1} yr^{-1}), based on the moderate N level.

Cropping system	CAN				Biogas residue			
	N input	EO	EI	NEY	N input	EO	EI	NEY
	kg N ha^{-1}	GJ ha^{-1}	GJ ha^{-1}	GJ ha^{-1}	kg N ha^{-1}	GJ ha^{-1}	GJ ha^{-1}	GJ ha^{-1}
Maize - maize	150	108.9	32.5	76.4	152	105.4	27.0	78.4
Maize - wheat	220	97.4	40.9	56.5	245	77.5	29.2	48.3
Grassland	360	98.5	43.3	55.2	360	64.5	27.0	37.5

Apart from higher DM and methane yield, low energy input of MM contributed to its better performance (Table 2). Compared with MWG and GR, MM required fewer field operations. However, the soil was not ploughed; a cultivator was used, which may explain the overall low level of energy input compared with the two other sites, where soils were ploughed (Claus *et al.,* 2012). Impact of fertilizer type on energy input and the resulting NEY was more pronounced than the cropping system effect, especially for the perennial ryegrass ley (Table 2). In general, mineral-N fertilization induces a higher energy input due to differences in energy for fertilizer production. Energy efficiency (energy output/input) therefore was generally lower for CAN (MM: 3.3; MWG: 2.4; GR: 2.3) than for biogas residue (MM: 3.9; MWG: 2.7; GR: 2.4). Results of this study are in agreement with a study conducted in two other landscapes of northern Germany (Claus *et al.*, 2012), where MM likewise performed better, in terms of net energy yield and energy efficiency, compared with MWG and GR.

Conclusions

Net energy yield and energy efficiency of biogas production are substantially affected by environmental conditions, cropping system and N-fertilizer type. However, results were based on a 2-year study only, which does not allow to fully account for long-term effects, as for instance residual N effects or a proper yield risk assessment. A comprehensive assessment of biogas cropping systems in a regional context furthermore requires the consideration of additional environmental impacts. Therefore a CO_2 balance will be performed in a next step to evaluate the climate change mitigation potential of the tested cropping systems.

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The effects of different fertilizers and cutting frequencies on yield of three energy crops

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Abstract

Environmental protection and decreasing resources of non-renewable energy lead to increasing interest in energy acquisition from renewable resources. At the same time the successful management of high amounts of waste products is very important. Successful use of grass biomass in livestock production and utilization for energy purposes depends on identifying species and cultivars with high yield potential and applying the appropriate fertilization. Field experiments were carried out in central Latvia (56°42'N, 25°08'E) to estimate sward condition and biomass yield of reed canary grass (*Phalaris arundinacea* L.), festulolium (×*Festulolium pabulare*) and galega (*Galega orientalis* Lam.). The fertilization treatments were control (no fertilization), sewage sludge, biogas digestate and wood ash. Application rates of organic fertilization were calculated in order to provide similar amount of plant available potassium (K) from each fertilizer. Trial results showed a significant dry matter (DM) yield dependence on the grass species and the applied fertilizer. The highest grass DM yields were produced when using sewage sludge.

Keywords: fertilization, perennial grasses, biomass yield, by-products, renewable resources

Introduction

The demand for biomass in the bioenergy and fibre industries has changed the traditional utilization of grasses in many countries. Biomass from permanent grasslands might be a valuable renewable resource (Lewandowski *et al*., 2003; Sanderson and Adler, 2008). Compared with growing other crops for agro-fuel production, grasslands can be produced on marginal agricultural land, and they do not require high inputs of fertilizers or pesticides (Kryzeviciene *et al*., 2008; Peeters, 2008). On the other hand, cultivating perennial grasses for energy purposes in Latvia will enable us to use the arable lands that are now left fallow, and it will reduce the dependence on imports of fossil fuels. In energy crop fertilization it is appropriate to use by-products – digestate, wood ash and sewage sludge – that contain significant amount of nutrients (Insam *et al.,* 2009; Makadi *et al.,* 2012). In that way, the problem of waste-product disposal can be solved and nutrient recycling can be promoted. The successful development of a bioenergy industry depends on identifying species and cultivars that are well adapted to low-input biomass production with high yield potential. Reed canary grass, festulolium, and galega are well known as productive grass and legume species suitable for biogas or fuel-pellet production (Tilvikiene *et al*., 2010; Adamovics *et al*., 2011). The objective of the current research was to study the effect of different bio-energy products and municipal waste products (digestate, wood ash and sewage sludge) used as fertilizers, the choice of grass species, and the intensity of management on the productivity of perennial grasses.

Materials and methods

Field trials were performed at the Research Institute of Agriculture in Skriveri on Phaeozems (soil pH_{KCl} – 6.1, plant available phosphorus (P_2O_5) – 277.1 mg kg^{-1}, potassium (K_2O) – 136.8

mg kg^{-1}, organic matter content 23 g kg^{-1}). The trials were sown in July 2011 without a cover crop, in randomized block design with four replications and with a 20 m^2 harvest plot size. Three potential energy-crop grass species were investigated for dry matter (DM) yield: reed canary grass, festulolium (tall fescue type) and galega. Two intensities of management were applied: (a) 3 cuts per vegetation period, and (b) 1 cut in October 2012. The following fertilization treatments were used: control (no fertilization), sewage sludge, biogas digestate and wood ash. Doses of fertilizer were calculated in order to provide a similar amount of plant available potassium (K) from digestate and wood ash (Table 1). The low content of potassium in sewage sludge did not allow the input of 120 kg ha^{-1} K_2O per year to be achieved; therefore calculation of the applied sewage sludge dose was conducted according to the input of the medium-low nitrogen (N) rate (N150). Fertilizer was spread and incorporated into the soil before sowing of energy crops. The experimental data were statistically analysed by three-way analysis of variance with four levels of fertilizer, 2 levels of management and 3 levels of species, and all interactions were accounted for.

Table 1. The nutrient content and applied amount of fertilizers.

Fertilizer	Nutrient content in the fertilizer DM, g kg^{-1}			Applied nutrients per year, kg ha^{-1}		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Control	0	0	0	0	0	0
Ash	0.73	14.7	88.2	1	20	120
Digestate	29.2	22.4	53.8	65	50	120
Sewage sludge	46.3	71.0	2.5	150	230	8

Results and discussion

Each of the applied fertilizer rates provided a significant (P<0.05) increase of mean dry matter yield for the investigated species (Table 2). The highest average DM yield was achieved by using sewage sludge. This can be explained by the amount of nitrogen in this fertilizer and by the responsiveness of the fast-growing festulolium to nitrogenous fertilizer.

Table 2. Annual dry matter production of reed canary grass, festulolium and galega.

Species (F_C)	Management (F_B)	Fertilizer (F_A)				
		Control	Ash	Digestate	Sewage sludge	Mean
Phalaris arundinacea	3 cuts	4.99	5.62	6.43	6.27	
	1 cut	5.85	6.34	7.42	7.00	6.24
Festulolium	3 cuts	5.29	6.57	6.09	8.15	
	1 cut	6.43	7.25	6.86	10.15	7.10
Galega orientalis	3 cuts	5.51	6.92	6.92	7.90	
	1 cut	6.00	7.01	7.73	7.49	6.94
Mean		5.68	6.62	6.91	7.83	

$LSD_{0.05}$ for DM yield: $F_A = 0.81$; $F_B = 0.57$; $F_C = 0.70$; $F_{AB} = 1.14$; $F_{AC} = 1.40$; $F_{BC} = 0.99$

The dry matter yields achieved by applying wood ash and digestate fertilizers did not differ significantly in 2012, when the weather conditions were characterized by high precipitation. In the wood ash and digestate treatments, the higher yields were produced by galega, a nitrogen-fixing legume plant.

Analysis of variance showed that influence of species factor on dry matter yields was significant (P<0.05). The highest average dry matter yield was given by festulolium. This can be explained by its quick-growing qualities in the first production year. The average dry matter yield of reed canary grass was lowest. The number of harvests per year provided for significant (P<0.05) differences of dry matter yield. The highest average dry matter yield

(7.13 t ha^{-1}) was achieved with cutting once in a season at crop senescence, in comparison with a 3-cutting frequency (6.39 t ha^{-1}).

Conclusion

The productivity of perennial grass biomass was dependent on the applied fertilizer type, grass species and cutting frequency. In the first production year the highest dry matter yield was produced by festulolium. Higher DM yields of all grasses were provided by fertilizing with sewage sludge and by mowing once in a season at the crop-senescence growth stage.

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Sulphur and carbon content in energy crop pellets

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Abstract

The use of plant biomass for the production of energy is one of the solutions for avoiding future ecological disasters and to compensate for the growing shortage of fossil energy resources. Pellets prepared for this research had different proportions (1:3, 1:1, 3:1 w/w) of reed canary grass (*Phalaris arundinacea* L. (henceforth RCG)) variety 'Marathon', energy wood – osier (*Salix viminalis* L.) and poplar (*Populus tremula* L.). Afterwards, the sulphur (S) and carbon (C) content in each pellet was measured. The lowest S contents were recorded for RCG (at 0.13%), while the highest was for osier (0.40%) and poplar (0.53%). The lowest S content among the various energy crop proportions was observed for mixtures of RCG with osier (3:1) at 0.11%, and poplar, at 0.115%. The C content in poplar comprises 55.27%, in osier 53.92%, and in RCG 51.55%. The highest C contents were recorded for RCG with osier 1:3 (53.48%) and with poplar (53.41%).

Keywords: *Phalaris arundinacea* L., *Salix viminalis* L., *Populus tremula* L., sulphur and carbon, pellets

Introduction

In many countries, experts suggest the growing of various energy crops as an alternative energy source for heat production (Heaton, 2004; Saballos, 2008). Reed canary grass (RCG) is one of the energy crops widely grown in Nordic countries. The calorific value of RCG pellets comprises up to 96% of wood pellet value, while combustion of grass releases almost 90% less greenhouse gases than fuel oil, coal or natural gas. Higher S content facilitates emission of SO_2 into the atmosphere as well as corrosion of chimneys and other metal constructions. Quantitatively C is the most important element in living organisms. Therefore, when organic material is burnt, gaseous C compounds, especially CO_2, are formed. Greenhouse gases are fundamentally affecting global warming and consequently the climate on the earth (Kļavinš *et al*., 2008). This study aims at quantifying S and C content in pellets with various proportions of RCG and osier or poplar, as well as correlation between component proportions.

Materials and methods

Pellets were produced with various proportions (w/w – 1:3, 1:1, 3:1) of RCG variety 'Marathon' (N fertilizer dose of 90 kg N ha^{-1}) and energy wood (osier or poplar). Those pellets had the two components (RCG + osier or poplar) in the following proportions: 1:3, 1:1, 3:1. Within the pellet manufacturing process, plant biomass was chopped and ground in the laboratory mill EM-ZA UHL 4.2 and the powder produced afterwards was formed into a pellet with the hand press "IKA WERKE".
The pellets were made from 100% natural ingredients – chopped wood (osier or poplar) and chopped RCG biomass. Amount of elements S and C in pellet samples was measured using the analyser 'Eltra CS-500 Analizator'.

Results and discussion

The lowest S concentrations were recorded for RCG or 0.113% of dry matter (DM), based on 100 g of DM, while the highest (exceeding regulatory standards (EN 14961-2)) were for osier (0.40%) and poplar (0.53%). The lowest S content among various energy crop proportions was observed for RCG in the proportion 3:1 with osier, or 0.11%, and 0.115% with poplar. The C content was 55.27%, 53.92% and 51.55% in poplar, osier and RCG, respectively. The highest C content was recorded for the RCG in proportion 1:3 with osier, or 53.48%, and 53.41% with poplar. Biomass pellets made of poplar and RCG indicated a negative correlation (*r*=-0.85) between proportion of RCG and S content in flue gasses (Figure 1).

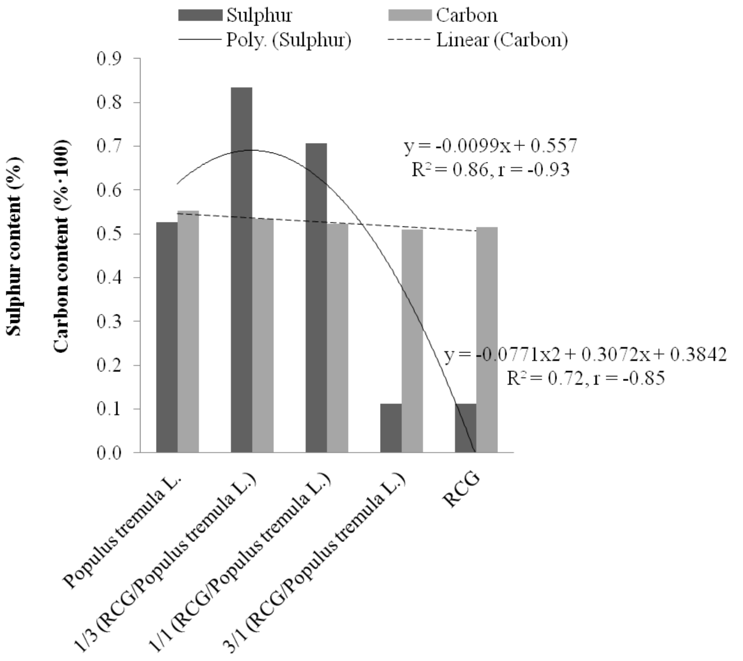

Figure 1. S and C content in RCG and poplar pellets.

A positive trend, with respect to the S content in flue gases, was observed when the RCG content in pellets accounted for 75% (3:1 RCG:poplar), i.e. it was 4.7 times or 0.112% smaller as compared to that in flue gases emitted when burning pellets solely from poplar.

Biomass pellets made of osier and RCG indicated a negative correlation (*r*=-0.82) between the proportion of RCG and S content in waste gases (Figure 2).

A positive trend with respect to the S content in flue gasses was observed if the RCG content in pellets accounted for 75% (3:1 RCG:osier); i.e. it was 3.5 times or 0.114% smaller as compared to that in flue gasses emitted when burning pellets solely from osier.

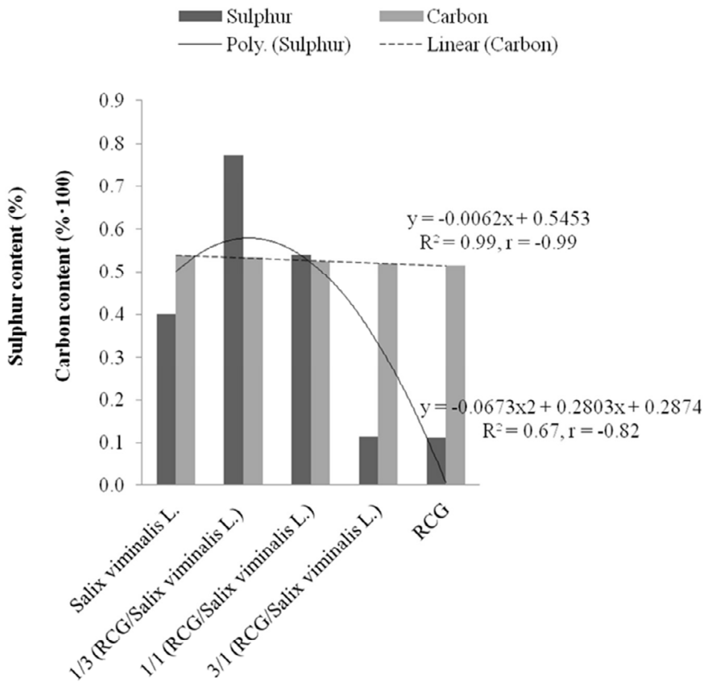

Figure 2. S and C content in RCG and osier pellets.

Conclusions

For pellets made from reed canary grass and osier or poplar (in the proportion 3:1) the sulphur content in flue gasses diminished by 0.29% and 0.42%, respectively, while the carbon content decreased by 1.93% and 4.21%. The proportion of components that is most suitable for pellet production was found at the combination 3:1 (RCG + timber). Calorific value of pellets solely from RCG is roughly equal to wood pellets; nevertheless RCG pellets are more environmentally friendly as they contain less sulphur.

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Are K and Na levels in biomass of reed canary grass influenced by grass maturity and in-field leaching?

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Abstract

For improving the properties of grass biomass for combustion, it is important to reduce its concentration of mineral elements, primarily alkaline metals. The objective of this research was to study the effect of grass maturity on K and Na concentrations in the biomass and the leachability of K and Na from the biomass under field conditions. The experiment was carried out on plots with reed canary grass fertilized with mineral fertilizer at a rate of $N_{60}P_{30}K_{60}$. The harvest was performed at three stages of reed canary grass development: (i) heading, (ii) dough and (iii) ripe seed. After cutting, the biomass was left on the growing site for 14 days. The sampling was done at harvest and 7 and 14 days afterwards. Samples were dried and analysed for K and Na concentrations. Potassium concentration decreased in biomass with the maturing of the reed canary grass, and it was partly leachable. Sodium concentration in the biomass was low; it did not change during the maturing of grass and did not leach during the post-mowing period. For lowering the K concentration in the biomass, the harvesting should be performed at seed ripening stage and the yield should be left on the field for one week.

Keywords: bioenergy, biomass, combustion, fuel quality, leaching, reed canary grass

Introduction

Under Nordic conditions, delayed-harvest technology is used for combustible biomass production in grassland because it removes alkaline metals (Landström *et al.*, 1996; Xiong, 2009). In Estonia the harvesting of herbage biomass for combustion is practical only during the summer period, as the weather in late autumn and early spring is mild and wet, resulting in the moisture content of the biomass being too high for harvesting. Therefore, for the conditions of the Estonian climate it is important to develop a technology that enables the production of biomass with low concentrations of alkaline metals during the summer months. Earlier studies have shown that the content of the most undesired elements in the biomass decreases with advancing age of the vegetation (Prochnow *et al.*, 2009) and that some elements are leachable from the biomass (Tonn *et al.*, 2011). The hypothesis of the present study was that the potassium (K) and sodium (Na) concentrations of the biomass can be reduced if mowing is performed at the later stage of grass development and if the mown biomass is left on the site for a short period after mowing. The aim of the present research was to study (i) K and Na concentrations of the biomass in relation to the development stage of reed canary grass (*Phalaris arundinacea* L.) at mowing, and (ii) leaching of K and Na from the mown biomass.

Materials and methods

The experiment was carried out in Estonia (58°21'N, 26°31'E; elevation 51 m a.s.l.) in 2012. The experiment was conducted on plots of reed canary grass fertilized with mineral fertilizer at a rate of $N_{60}P_{30}K_{60}$ at the beginning of the vegetative period. A one-cut harvest system was used. The grass was mown at three stages of development: (i) heading (at the beginning of July), (ii) dough (at the beginning of August) and (iii) ripe seed (mid-August). Swards harvested at different times were located next to each other as narrow strips. After mowing, the biomass was left on the growing site for 14 days. Sampling of the herbage biomass was taken at mowing and 7 and 14 days later. One average sample of 1 kg was taken per 4 replicates. Samples were dried and analysed for K and Na. Meteorological conditions were monitored with Metos Model MCR300 weather stations (Table 1). We used Pearson correlation to test the precipitation impact on K and Na content in the biomass. Calculations were performed using the statistical package Statistica 64 (StatSoft.Inc).The probability level was set at $P<0.05$.

Table 1. Weather conditions during the experiment.

Stage of development of grass at mowing	Sum of precipitation (mm)		Average temperature (°C)	
	1-7 days after cut	8-14 days after cut	1-7 days after harvest	8-14 days after harvest
Heading	0.1	61.8	19.8	15.3
Dough	4.2	15.4	18.1	13.4
Ripe seed	21.6	28.0	15.7	13.9

Results

Potassium concentration of the biomass depended on the stage of development of the reed canary grass at mowing and it decreased with increasing maturing of the grass (Table 2). Sodium content of the biomass was low and did not depend on the factors studied in the experiment. Within the 14 days that it was left on the growing site, its K concentration decreased remarkably.

Table 2. Potassium and sodium concentration of biomass (g kg^{-1} DM).

Sampling	Stage of development of grass at mowing					
	Heading		Dough		Ripe seed	
	K	Na	K	Na	K	Na
At mowing	17.8	0.28	11.8	0.34	10.1	0.28
7 days after mowing	15.8	0.28	10.3	0.30	5.1	0.27
14 days after mowing	7.6	0.28	8.4	0.30	4.8	0.27

The magnitude of change was highest if mowing was performed at the earlier development stages of the grass. Potassium leaching from the biomass was positively influenced by the amount of precipitation ($r = 0.92$, $P<0.05$). The reduction of K concentration of the biomass during the 14-day period on the growing site was as follows: 57.3% at the heading stage after 61.9 mm precipitation; 28.8% at the dough stage after 19.6 mm precipitation and 52.5% at the ripe seed stage after 49.6 mm.

Discussion

Of the studied elements only the K concentration in the biomass changed during the experiment. Sodium concentration of the biomass was very low: it did not depend on the stage of maturity of the reed canary grass at mowing and did not leach from the biomass in the post-mowing period. Several studies have shown that potassium concentration decreases with maturing of grass (Tonn *et al.*, 2011). In our experiment, K concentration in the biomass was 7.7 g kg^{-1} DM less if the harvest was performed at the ripe-seed stage as compared with the heading stage. The largest difference in the biomass K concentration was between heading and dough stages, whereupon the reduction slowed.

Potassium concentration of the biomass was reduced further by leaching that occurred after the crop was mown. Similarly to the findings of Tonn *et al.* (2011), the magnitude of the reduction depended on the stage of grass maturity and it was less if the grass was mown at later maturity stage. Only part of the total potassium in the biomass leached. At later stages of maturity the initial K concentration of the biomass was less, which could explain why K concentration was not reduced as much as at the heading stage. Most K leaching from the biomass at ripe-seed stage occurred during the first seven days even though there was more rain during the following seven days.
Results presented by Tonn *et al.* (2011) showed a positive relationship between the amount of rain and K leaching. The same was true in our experiment, but our results also demonstrated that the amount of rain was not the only factor that affects leaching under field conditions. The reduction of K in the biomass had already taken place due to the 0.1 mm of rain during days 1-7 if the grass was mown at the heading stage, whereas the impact of 28 mm of rain on the grass biomass mown at the ripe-seed stage during 8-14 days after harvest was small. These results indicate that leaching is also influenced by grass maturity but, in addition, it may be impacted on by the dry and wet cycles caused by the variability of weather conditions and soil moisture content.

Conclusion

Sodium concentration in the biomass was low; it did not change during the maturing of grass and was not easily leached. Potassium concentration decreases with the maturing of grass and is also reduced by leaching if the biomass is left on the stubble after mowing. To lower K concentration in biomass it is expedient to mow at the seed ripening stage and to leave the biomass on the stubble for one week.

Acknowledgments

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Herbage from extensively managed grasslands for biogas production: methane yield of stands and individual species

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Abstract

The experiment aimed at identifying the capacity of herbage from species-rich, extensively managed meadows to serve as a substrate for biogas production. We determined methane yield of *Arrhenatherum*-dominated (approx. 26 species per 9 m^2) and *Lolium*-dominated swards (approx. 15 species per 9 m^2) using the Hohenheim biogas yield test. Both sward types received two levels of fertilizer application (0 and 80 kg N ha^{-1}) and were cut at three harvest dates (mid-May, late May, and early June). In addition, we determined the substrate-specific methane yield of a range of individual grass species which are common in extensively managed grassland, but which have so far received little attention for their potential for biogas production: *Arrhenatherum elatius*, *Trisetum flavescens*, *Holcus lanatus*, *Festuca rubra* and *Dactylis glomerata*. We compared these with methane yields obtained from *Lolium perenne*. Our results revealed values of substrate-specific methane yield comparable to those of *L. perenne* for some species common in species-rich stands. Substrate-specific methane yield of the two sward types was similar; however, dry matter yield of the *Arrhenatherum*-dominated stands was significantly lower than that of *Lolium*-dominated stands, which resulted in comparatively lower methane yield per hectare of species-rich stands.

Keywords: substrate specific methane yield, *Arrhenatherum*, *Trisetum*, *Holcus*

Introduction

Species-rich, high nature value meadows receiving low fertilizer inputs and harvested infrequently and late in the year often produce herbage of low forage quality. They can, however, deliver a substrate for bio-energy production. For utilization through combustion, depending on their species composition and hence on their nutrient content, their herbage can cause difficulties in processing due to unfavourable melting behaviour of ash, which may necessitate preparative treatment (Tonn *et al.*, 2011). The aim of the present study was to identify whether the utilization as a substrate for the production of biogas offers a viable alternative valorisation for herbage of this type of meadows. Within a field experiment, we quantified the specific methane yield and the methane yield per hectare of an *Arrhenatherum*-dominated sward and contrasted them to those of a *Lolium*-dominated sward. We additionally determined substrate-specific methane yields of individual grass species common in extensively managed meadows some of which have so far received little consideration for biogas production. We further analysed the effect of fertilization and harvest date, which are known to be a prevalent determinant of methane yield of grasslands (e.g. Weiland, 2006).

Materials and methods

For the present study, a field experiment was conducted at the experimental site of the Department of Crop Sciences of Göttingen University, at Göttingen, Lower Saxony,

Germany. The replicated split-plot design comprised a total of four plots covering an area of 18 m^2 (6×3 m) each, with two replications of the main plots in randomized blocks. The main factor was sward composition, with two levels: 1) an *Arrhenatherum*-dominated sward with approx. 26 species per 9 m^2 (yield proportions of 24, 16, 8, and 7% of the species *Arrhenatherum elatius*, *Holcus lanatus*, *Dactylis glomerata* and *Trisetum flavescens*, respectively), and 2) a *Lolium*-dominated sward with approx. 15 species per 9 m^2 (yield proportions of 36, 22, 9 and 7% of the species *Lolium perenne, Poa trivialis*, *Elymus repens* and *Poa pratensis*, respectively). The subordinate factor was fertilization, with two levels: 1) no fertilization, and 2) one application of calcium ammonium nitrate of 80 kg N ha^{-1}. Fertilization was applied in late April and randomly assigned to half (3×3 m) of each plot.
Samples of the primary growth in spring were taken at three harvest dates: mid-May, late May and early June. At each date, 108 cm² each from three random locations on each subplot was harvested to obtain a mixed sample. Additionally, approx. 30 g of fresh biomass of a number of individual plant species was collected. In the species-rich plots, samples of *A. elatius*, *T. flavescens*, *H. lanatus*, *Festuca rubra* and *D. glomerata* were taken; in the species-poor plots, *L. perenne* was sampled. The samples were used as substrate for the Hohenheim biogas yield batch test (Helffrich and Oechsner, 2003) in order to determine their substrate-specific methane yield. Methane yield per hectare for the two sward types was calculated based on area-specific harvested dry biomass. The effect of the factors sward type, fertilization, and date of harvest, and of their interactions for specific methane yield, total dry matter yield, and methane yield per hectare were determined calculating a three-way ANOVA. Differences in substrate-specific methane yield of the individual species were determined calculating a Tukey HSD post-hoc test (95% confidence interval).

Results

For the data of multi-species samples of the two swards, results of ANOVA showed that the factors sward type, fertilization, and date of harvest, and their interactions had no significant effect on substrate specific methane yields (Table 1).

Table 1. Substrate-specific methane yield [standard m^3 kg^{-1} organic dry matter (ODM)] of the two sward types, of five of the grass species common in the species-rich sward and of *L. perenne* at three harvest dates and two levels of fertilizer application. Within columns, different superscript letters indicate significant inter-specific differences.

	Harvest date					
	Mid-May		Late May		Early June	
	Fertilization					
	None	80 kg N ha^{-1}	None	80 kg N ha^{-1}	None	80 kg N ha^{-1}
	Standard m^3 kg^{-1} ODM					
Sward type						
Arrhenatherum-dominated	0.305	0.301	0.299	0.273	0.293	0.301
Lolium-dominated	0.327	0.321	0.328	0.319	0.313	0.307
Species						
A. elatius	0.328	0.347	0.329^{b}	0.325^{b}	0.317^{ab}	0.311^{ab}
D. glomerata	NA	0.334	0.322^{b}	0.321^{b}	0.317^{ab}	0.313^{ab}
F. rubra	0.331	NA	0.331^{b}	0.312^{b}	0.325^{b}	0.317^{b}
H. lanatus	0.335	0.326	0.303^{a}	0.308^{a}	0.309^{a}	0.300^{a}
T. flavescens	NA	0.314	0.332^{b}	0.328^{b}	0.315^{ab}	0.309^{ab}
L. perenne	0.334	0.335	0.325^{b}	0.324^{b}	0.325^{b}	0.320^{b}

NA: data not available

Date of harvest ($P<0.001$), fertilization and the sward type × date of harvest interaction ($P<0.05$ in both) had a significant effect on dry matter yield. For methane yield per hectare, the factor date of harvest, and the sward type × date of harvest interaction had a significant (P<0.01) effect.

Dry matter (DM) yield was significantly higher in the *Lolium*-dominated sward: 8.4 and 9.9 t DM ha^{-1} at the late harvest in the non-fertilized and the fertilized sward, respectively, in comparison with 4.5 and 4.8 t DM ha^{-1} in the non-fertilized and the fertilized *Arrhenatherum*-dominated swards, respectively. Hence, methane yield per hectare was significantly higher in the *Lolium*-dominated sward than in the species-rich sward.

ANOVA of the data considering individual species revealed that the factor species had significant effect for substrate specific methane yields. The yield values of *H. lanatus* were significantly lower than those of all the other tested species at the second harvest date, and significantly lower than those of *L. perenne* and *F. rubra* at the third harvest date. The factor harvest date also had significant effect on substrate specific methane yields; fertilization, in contrast, did not significantly determine this parameter.

Discussion

Our results show that management (date of harvest, fertilization), not sward composition, was the prevalent determinant of substrate-specific methane yield of the tested grassland swards. We recognize, however, that the *Lolium*-dominated sward used in this experiment was relatively species-rich (16 species per 9 m^2) in comparison with the swards of typical high-yielding meadows, and we expect differences to be more pronounced when swards of more distinct levels of species number are contrasted because of the substrate-specific methane yield of *L. perenne* being comparatively high even at late harvest dates.

In the present experiment we have determined the substrate-specific methane yield of species common in extensively managed grasslands, but which previously have seldom been considered for biogas production. Our data show that, at the early harvest dates, *A. elatius* and *T. flavescens* yielded values which are comparable to those of *L. perenne*. Merely at later harvest dates did *L. perenne* tend to feature better methane yields.

Even though in both sward types substrate-specific methane yields decreased with later harvest date, methane yield per hectare increased due to the increase in harvestable biomass. Dry matter yield of species-rich stands therefore appears to be the main factor determining the economic viability of the utilization of their herbage for biogas production. It remains to be established whether the accumulation of non-digestible fibre will have a detrimental effect on methane yields in herbage of harvest dates later than those examined in the present experiment.

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The role of additives when ensiling red clover-grass mixture for biogas production

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Abstract

A second cut from a mixed sward of red clover, timothy and meadow fescue was prewilted and harvested with a precision chopper. The grass had a dry matter (DM) concentration of 357 g kg^{-1}, while water soluble carbohydrates were 66, crude protein 122, and D-value 596 g kg^{-1} DM. The grass was ensiled without an additive (control), with *Lactobacillus buchneri,* with *Lactobacillus plantarum* or with AIV®2Plus. Addition of AIV®2Plus restricted silage fermentation as judged by high residual sugar and low lactic acid concentrations. *Plantarum* silages had a homolactic fermentation profile with low pH, high lactic and low acetic acid concentrations. *Buchneri* silages had a heterolactic fermentation profile with high acetic and propionic, and low lactic acid concentrations. There were no statistically significant differences between treatments in biochemical methane potential of the silages. DM losses during ensiling were 27, 35, 10, and 14 g kg^{-1} DM for Control, Buchneri, Plantarum and AIV®2Plus silages, respectively. The losses after silo opening during 259 h aerobic phase were 42, 3, 143 and 9 g kg^{-1} DM, respectively. The high DM loss during the aerobic phase in the *Plantarum* silages was related to high counts of enterobacteria and fungi.

Keywords: aerobic stability, biogas, *Buchneri*, formic acid, losses, silage

Introduction

Grass silage has potential as a raw material for biogas production in areas where maize is not competitive. Further, the role of silage additives to ensure good quality silage is enhanced when the harvest season is short and rainy. An ensiling experiment was conducted to explore the effect of silage additives on fermentation quality, aerobic stability, losses during the fermentation phase and methane production of grass-clover silage.

Materials and methods

A second cut of a sward containing a mixture of red clover (*Trifolium pratense),* timothy (*Phleum pratense)* and meadow fescue (*Festuca pratensis)* was taken on 24 August 2011 in Jokioinen, Finland (61°N), prewilted overnight, and harvested with a precision chopper.
The additive treatments were *Lactobacillus buchneri* (E-75036, 10^6 cfu g^{-1}), *Lactobacillus plantarum* (VTT E-78076 10^6 cfu g^{-1}), AIV®2Plus (formic acid 760, ammoniumformate 55, water 185 g kg^{-1}; 5 ml kg^{-1}) and control without an additive. Three 12 L silos were filled (5.5 kg per silo) for each treatment. The silos were sealed two hours after the filling.
The silos were opened after a 98-day ensiling period. Aerobic stability of the silage samples (157 g dry matter (DM)) was determined as time needed for the silage to heat two degrees above ambient. Biochemical methane potentials of the silages were measured using the automated methane potential test system (Bioprocess Control AB, Lund, Sweden). The treatment effects on dependent variables were tested using variance analysis and Tukey test of SAS GLM-procedure.

Results and discussion

The botanical composition was red clover 410 g kg^{-1}, timothy 290 g kg^{-1} and meadow fescue 240 g kg^{-1}. The proportion of dead grass material was high (60 g kg^{-1}), which is typical for the delayed second cut as well as low digestibility (596 g digestible organic matter kg^{-1} DM). The grass material prior to ensiling had a DM concentration of 375 g kg^{-1}, sugars only 65.9 g kg^{-1} DM and crude protein 122 g kg^{-1} DM, while neutral detergent fibre concentration was 540 g kg^{-1} DM.

Table 1. Fermentation quality of the silages.

Treatment	Dry matter	pH	Sugar	Acetic acid	Propionic acid	Butyric acids[a]	Sum C5-C6[b]	Lactic acid	Ethanol	Ammonium N g kg^{-1} total N[c]
	g kg^{-1}		g kg^{-1} DM							
Control	360^{A}	4.19^{B}	41.1^{B}	25.7^{B}	0.44	0.22^{B}	0.20	79.9^{B}	3.30^{C}	85.3^{A}
Buchneri	333^{C}	4.41^{A}	7.6^{C}	60.4^{A}	6.07	0.57^{A}	0.26	53.3^{C}	8.40^{A}	84.0^{A}
Plantarum	363^{A}	4.04^{C}	48.3^{B}	12.9^{D}	0.22	0.16^{BC}	0.13	101.2^{A}	4.43^{B}	62.4^{C}
AIV® 2 Plus	340^{B}	4.24^{B}	83.0^{A}	16.1^{C}	0.32	0.11^{C}	0.21	52.7^{C}	4.80^{B}	75.2^{B}
SEM[d]	1.6	0.013	1.98	0.31		0.020	0.014	1.75	0.242	1.51

Differences between values within the same column without a same superscript are statistically significantly different (P<0.05)
[a]The sum of butyric and isobutyric acids
[b]The sum of valeric, isovaleric and caproic acids. Residual was not normally distributed, test results omitted
[c]In AIV® 2 Plus treatment approximately 10.8 g ammonium N g^{-1} total N is from the additive
[d]Standard error of the mean

The AIV®2Plus restricted silage fermentation, whereas *Plantarum* silages had a typical homolactic fermentation profile and *Buchneri* silages had a typical heterolactic fermentation profile (Table 1). The microbial counts prior to ensiling were high (Table 2). The amount of aerobic heterotrophs declined during ensiling in all treatments. The number of enterobacteria in the *Plantarum* silage was higher than in all other silages. The amounts of yeast and moulds were significantly decreased in AIV® 2 Plus and *Buchneri* silages.

The *Plantarum* silages had poor aerobic stability (64 hours). This was most probably due to the high *Enterobacteria* and yeast counts, both at the silo opening and after the aerobic stability measurement. AIV® 2 Plus and *Buchneri* silages had the best aerobic stabilities as they did not heat during the entire measurement period of 259 hours, which was related to the low yeast and mould counts of these silages after the ensiling period and to the high acetic acid concentration in the *Buchneri* silages. It is remarkable that AIV® 2 Plus silages had good aerobic stability although the yeast and mould number was high (5.9 log cfu g^{-1}) at the end of the aerobic stability test. Control silage had aerobic stability of 201 hours (standard error of means of aerobic stabilities was 13.6 hours, P<0.01 for treatment effect).

Dry matter losses during ensiling reflected the type of fermentation, the losses being smallest for the restrictively fermented AIV® 2 Plus silage and the *Plantarum* silage showing homolactic fermentation (14 and 10 g kg^{-1} DM, respectively). The DM losses of the heterofermentative *Buchneri* and Control silages were 35 and 27 g kg^{-1}, respectively. The vigorous heating of *Plantarum*-treated silage after silo opening was reflected in a remarkable loss in DM (143 g kg^{-1} after 259 hours). Nearly all (92%) of the lactic acid and 73% of the sugars from these silages were lost. Altogether, the sum of DM losses during ensiling and after silo opening resulted in 152 g kg^{-1} for *Plantarum*, 67 g kg^{-1} for control silage, 38 g kg^{-1} for *Buchneri* and 24 g kg^{-1} for AIV® 2 Plus silages.

Although there were clear differences in fermentation profiles of the silages, no differences were detected in biochemical methane potential of the silages (Table 2). This is in line with previous results where no difference (Pieper and Korn, 2010) or only a small difference (Nussbaum, 2009) in methane production between acetic and lactic acids was observed. After the aerobic phase the *Plantarum* silages had lower methane production values than the other silages, reflecting energy losses related to the DM losses during aerobic spoilage.

Table 2. Biochemical methane potential (BMP) and microbial quality (log cfu g^{-1}) of the grass material prior to ensiling and the silages.

	BMP CH_4 m^3 t^{-1} VS	Aerobic heterotrophs	Entero-bacteria	Lactic acid bacteria	Bacterial spores		Yeast + moulds
					Aerobic *Bacillus* spp.	Anaerobic *Clostridium* spp.	
Raw material	323	9.5	7.9	7.1	5.6	< 2	7.6
Control	310	6.6^{B}	< 3	7.9	4.7^{A}	2.8^{B}	4.1^{B}
Buchneri	324	7.0^{AB}	< 2	9.3	3.8^{B}	2.9^{B}	< 2
Plantarum®	320	5.5^{C}	4.2	5.7	4.1^{AB}	3.2^{A}	5.2^{A}
AIV® 2 Plus	296	7.4^{A}	< 2	7.4	4.6^{A}	2.9^{AB}	< 2
SEM[a]	13.8	0.097			0.163	0.075	0.434

Differences between values within the same column without a same superscript are statistically significantly different (P<0.05)
Results of raw material not included to statistical test
[a]Standard error of the mean

Conclusions

The type of silage fermentation or fermentation products did not have a significant effect on the methane production. Reducing organic matter losses during ensiling and during aerobic phase is the most important factor affecting the methane yield per amount of harvested grass material.

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Perspectives of grass biorefining for livestock farms in the Netherlands

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Abstract

The Netherlands has more than one million ha of grassland, which is mainly used for livestock farming. The aim of this study was to provide insight into the perspectives of grass biorefining for livestock farms in the Netherlands. In grass biorefining the harvested grass is pressed, with two products being the result: fibres and nutrient-rich juice. Proteins can be obtained from this juice. The protein, particularly, can be valorised as an alternative to imported protein-rich animal-feed raw materials such as soybeans. A SWOT-analysis was carried out. The analysis showed that grass biorefining is still in its infancy, yet it has prospects. The base product, grass, is amply available and by progressing technology it is expected that increasingly more components of grass can be valorised economically. In operating grass biorefining at a large scale, economic, ecological and social considerations at individual livestock farms as well as considerations at regional and national levels play a part. One can think of, for example, integration into the landscape and a reduction in grazing. A new way of livestock farming may arise, where grass is no longer produced for livestock only, but also for other applications.

Keywords: biorefining, grass, livestock

Introduction

The Netherlands has more than one million ha of grassland, which is mainly used for livestock farming. The question arises whether there are other possibilities for using the available grass. Biorefining may be an option.

Biorefining is the processing of plants and plant residues into several components for different applications. The sum of the added value of these different components is assumed to be greater than the value of the whole plant. In biorefining, the individual components can be extracted and the remainder may be used for energy production. In grass biorefining the harvested grass is usually pressed, with two products being the result: fibres and nutrient-rich juice. From this juice proteins can be obtained. The protein in particular can be valorised as an alternative to using imported of protein-rich raw materials for animal feed, such as soybean extracts. The protein-poor residue can be used as a fodder for dairy cows, while the refined protein can be used in pig and poultry feed. Products of grass biorefining can also be used for energy production (Grass, 2004; Schmer *et al*., 2008). A special feature of grass biorefining is the very wet biomass. The freshly harvested grass product contains 80-90% water. High-speed processing is required to prevent degradation of this wet product.

The idea of biorefining grass is not new. About 35 years ago, possible applications of different grass fractions were examined (e.g. Houseman, 1976). Thus far the technology has not yet been sufficiently well developed to create a profitable activity at a large scale. Recently, however, grass biorefining has received increased attention in the Netherlands. The aim of this study was to provide insight into the perspectives of grass biorefining for livestock farms in the Netherlands.

Materials and methods

A desk study was carried out to assess the strengths, weaknesses, opportunities and threats of grass biorefining in the Netherlands. This SWOT-analysis included grass biorefining at both a small and a large scale.

Results and discussion

When looking at the perspectives of grass biorefining on livestock farms in the Netherlands, several strengths can be seen. Grass is a major crop in the Netherlands with over 50% of the total land area (CBS, 2012). The majority of this grass is on peat soils and heavy clay soils that cannot be used for other crops. Furthermore, many dairy farms have a surplus of grass and there is no market for this surplus. Grass refining can increase the added value in those situations. Another strength is that the dry matter production of the grass can be increased (Prochnow *et al.*, 2009) by improving the grassland management; for example, by using precision harvesting and fertilization. Increases of 20% are possible. A further increase by 20-25% is possible by changing the current mowing/cutting regime (which is commonly practised in the Netherlands) into a regime of cutting only (Handboek Melkveehouderij, 2011). Grass refining may avoid environmental problems. In the Netherlands, the protein in the grass often exceeds the needs of the cow. Grass refining will lead to a feed with less protein for dairy cattle, and thus reduced losses to the environment. Since the protein can be fed, for example, to pigs and poultry, it can also lead to reductions in imports of protein feeds (e.g. soybeans from South America). Other sustainable aspects of grass biorefining are a decreasing use of fossil energy and more C sequestration as a result of an increasing grass area. Finally, grass refining could be carried out at farm scale.

However, there are also weaknesses of grass biorefining. The techniques of grass biorefining are in development, as are the markets to valorise each product of grass biorefining. The seasonality of grass production throughout the year is also a weakness. As a result of changing weather conditions production of grass is not constant throughout the year. The biorefining capacity should be harmonized with the grass supply. During winter only ensiled grass is available. It is not clear yet whether the traditional way of ensiling is also the most optimal way of preserving grass for grass biorefining. The effect of grass ensiling on the quality of the refined products is not yet known. First results in the Netherlands with biorefining of ensiled grass show that the protein-poor fibre can be fed to dairy cows (Klop *et al.*, 2012). A further weakness is that the combination of livestock production and grass biorefining leads to fragmentation of tasks for the farmer. This may lead to lower efficiency and lower profits (this is of course not true in those situations where the farm completely switches from livestock production to grass biorefining). Adjustment of the existing grassland use (mowing / grazing) to mowing-only can be a bottleneck. Currently, approximately 70% of the dairy farms in the Netherlands practise grazing (CBS, 2012). For grass biorefining it is necessary to cut the grass, which means that dairy farms have to adapt to a new situation. Also, in general, cutting grass requires more hours of labour than grazing. A further threat is the logistical implications of grass biorefining. If individual dairy farms refine grass, many small quantities of products would need to be collected in order to have sufficient quantity for commercial use. If grass biorefining is done at a central location, the high moisture content of grass will lead to many transport movements with associated costs and environmental impact. Finally, the farmers' knowledge of grass biorefining is still limited and there are uncertainties about costs and revenues of grass biorefining.

When evaluating the perspectives of grass biorefining for livestock farms in the Netherlands, not only strengths and weaknesses should be studied, but also opportunities and threats. Several opportunities can be identified. First of all, through fermentation of residuals from

grass biorefining, green energy can be generated, which is a positive outcome since the supply of fossil fuels is finite. Breeding could lead to higher grass yields per hectare and higher profits, and also to grass varieties with a composition favourable for biorefining. The growing season has extended during recent decades. Further extension of the growing season will contribute to higher grass yields. There is also plenty of grass available from outside the farm at low prices (e.g. roadside grass, grass from nature preserves). Other relatively wet green crops might be similarly refined, e.g. alfalfa, clover, beet leaves, and residues from the food industry. Grass biorefining is also an option for arable farms. Grass fits well into an arable crop rotation and improves soil quality. Products of grass biorefining could be applied in different industries (Chiesa and Gnansounou, 2011), for example, protein in animal husbandry, fibre in the paper industry, and the residual juice could be used as fertilizer. Further technological development could lead to additional added value of grass components, e.g. via innovative industrial applications for fibres such as housing, wind turbine blades or computer equipment. In time, use in the human food chain is also a possibility.
Finally, there are threats. Some of these are rather specific for Dutch circumstances. The current manure legislation in the Netherlands complicates the sale of fertilizer products from grass biorefining. Society does not support technological innovations in general, especially not if refinery plants and storages are very visible in the landscape. Furthermore, society in the Netherlands favours grazing (Van den Pol-van Dasselaar *et al.*, 2008). Grass biorefining will, however, lead to less grazing if it becomes widely practised. And finally, grass biorefining may compete with the grass supply for animal nutrition and/or lead to higher prices for forages.

Conclusion

Grass biorefining is still in its infancy, yet it has prospects. The base product, grass, is amply available and by progressing technology it is expected that increasingly more components of grass can be valorised economically. In applying grass biorefining at a large scale, economic, ecological and social considerations at individual livestock farms as well as considerations at regional and national levels play a part. One can think of, for example, integration into the landscape and a reduction in grazing. A new way of livestock farming may arise, where grass is no longer produced for livestock only, but also for other applications.

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Potassium as a means to increase production and NP-capture from permanent grassland on organic soil

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Abstract

Bioenergy production competes with food production on intensively managed fields. Permanent wet grassland may be an alternative bioenergy source, as marginal land is often abandoned in current farming systems. Biomass production from permanent grassland on organic soils may be increased by addition of K where N and P are in surplus. We examined three different sward types on organic soil to estimate production and nutrient capture, using two cuts per year with and without K addition. On average, 4.0-7.5 t dry matter (DM) per ha per year was harvested from unfertilized permanent grassland swards over a 4-year period. On all three swards addition of K increased DM production. Harvested biomass captured nutrients from environmentally sensitive riverside areas; average amounts captured from unfertilized swards were 62-132 kg N and 6-18 kg P per ha. Addition of K increased the N-capture in two swards and increased P-capture in two different swards. Addition of K seems appropriate where and when there is no need to take account of botanical nature quality.

Keywords: bioenergy, cutting, organic soil, permanent grassland, K vinasse

Introduction

The European Union has a goal of at least 30% of energy from renewable sources by 2020, and biomass for biogas production may provide a substantial part of this. In Denmark a high proportion of existing permanent grasslands can be used for this purpose and, at the same time, N and P nutrients can be removed from soils in environmentally sensitive areas. These nutrients can be used as valuable fertilizer elsewhere following digestion of the meadow grass in a biogas plant. The potential effect of adding potassium to increase NP-capture is of particular interest if the K is supplied as K vinasse, which is a by-product of the sugar industry and its use is allowed in organic farming systems. The aim of this paper is to describe the production and NP-capture from three different types of swards, with and without addition of K vinasse at grassland, on organic soil where N and P are in surplus.

Materials and methods

Biomass production and nutrient capture were compared on three meadows on organic soil (carbon content of 20-29%). At all sites, randomized-block trials were established with three replications but different plot sizes, adjusted to the possibilities at the sites. At one site the sward was dominated by *Deschampsia cespitosa* (L.) P. Beauv; previous management had been one cut per year in the 4 previous years, without fertilization, and plots of 14×20 m were laid out in 2009. On the second site, the sward was dominated by *Poa trivialis* (L.) and *Elytrigia repens* (L.) Nevski; previous management was cutting, without fertilization, and plots of 12×12 m were laid out in 2010. At the third site the sward was dominated by *Juncus effusus* (L.), management had been abandoned the previous 5 years, and plots of 6×6 m were laid out in 2009. At all three sites different levels of K vinasse were applied in spring as a treatment factor. The highest amount of K vinasse provided 115 kg K ha^{-1} yr^{-1}, but if the K-balance was in surplus the rate was reduced. The levels used are shown in Tables 1-3. In all treatments, production was measured by two yearly cuts, from establishment until 2012.

Results and discussion

The DM production, without K vinasse, varied from an average of 4.0 t ha^{-1} yr^{-1} in the *J. effusus*-dominated sward to 7.5 t ha^{-1} yr^{-1} in a *D. cespitosa*-dominated sward. On all three sites K vinasse increased the DM production compared with no application. Without K vinasse, the DM production in the *D. cespitosa* sward decreased every year, but *ca*. 10 t DM ha^{-1} yr^{-1} was maintained when K vinasse was added. High amounts of N and P were captured with the biomass. By adding K vinasse a significantly higher amount of N was captured from the *D. cespitosa* swards (17 kg more than the 132 kg N ha^{-1} yr^{-1}without K vinasse: Table 1).
A greater amount of N was also captured from swards dominated by *P. trivialis* and *E. repens* (29 kg more at the medium K-vinasse level, in surplus of the 91 kg N ha^{-1} yr^{-1} without K vinasse: Table 2). In this sward a higher amount of P was also captured (5 kg more than the 13 kg P ha^{-1} yr^{-1} without K vinasse). In the *J. effusus* sward, more P was captured by adding K vinasse (2 kg more than the 6 kg P ha^{-1} yr^{-1} without K vinasse: Table 3). The ratio between the concentrations of N and of K in the biomass at the first cut may indicate the potential effect of K vinasse. In the *D. cespitosa* sward this ratio increased from 2.8 in 2009, to 6.0 in 2012 in plots without K vinasse, indicating a higher production potential by adding K. In the *Poa-Elytrigia* sward the N:K ratio increased from 2.5 in 2010 to 3.6 in 2012, but in the *J. effusus* sward the ratio was almost unchanged from 3.0 in 2009 to 3.1 in 2012.
The methane production from different types of Danish meadow grass was found to be 288 L methane per kg VS as an average of 90 samples (Raju *et al*. 2011). The level is higher than both pig and cattle manure on a DM basis (Møller *et al*. 2004), and since the dry matter concentration is 10-20 times higher than liquid animal manure the grass is very valuable for boosting the biogas production on biogas plants with manure as main feedstock.

Table 1. Dry matter production with and without K vinasse fertilization on meadow dominated by *D. cespitosa* shown as average of 2009-2012. Water level from 70 cm below to 10 cm above soil surface.

K vinasse fertilization kg K ha^{-1} yr^{-1}	Dry matter production, sum of two cuts t ha^{-1} yr^{-1}*	Yield of nutrients, sum of cuts kg ha^{-1} yr^{-1}		
		N	P	K*
0	(7.5)	132.2	18.0	(29.5)
90-115	(9.9)	149.1	19.6	(86.7)
LSD, treatment		15.0	ns	
LSD, year		ns	5.0	
LSD, interaction	2.8	ns	ns	27

LSD: least significant difference; ns non-significant at 0.05
*) The average is shown in brackets to indicate the level although a significant interaction was found

Table 2. Dry matter production with three levels of K vinasse fertilization on meadow dominated by *P. trivialis* and *E. repens*, shown as average of 2010-2012. Water level around 20-30 cm below soil surface

K vinasse fertilization kg K ha^{-1} yr^{-1}	Dry matter production, sum of two cuts t ha^{-1} yr^{-1}	Yield of nutrients, sum of cuts kg ha^{-1} yr^{-1}		
		N	P	K
0	4.6	90.7	13.4	28.1
45-58	7.2	120.1	18.1	67.9
90-115	7.3	118.9	18.4	85.0
LSD, treatment	1.1	23.2	3.5	10.1
LSD, year	ns	ns	ns	ns
LSD, interaction	ns	ns	ns	ns

Table 3. Dry matter production with three levels of K vinasse fertilization on meadow dominated by *J. effusus*, shown as average of 2009-2012. Water level from 20-40 cm below to 5 cm above soil surface.

K vinasse fertilization kg K ha^{-1} yr^{-1}	Dry matter production, sum of two cuts t ha^{-1} yr^{-1}	Yield of nutrients, sum of cuts kg ha^{-1} yr^{-1}		
		N	P	K*
0	4.0	62.3	5.8	(23.6)
45-58	4.6	66.4	5.6	(49.1)
90-115	5.2	72.0	7.7	(77.5)
LSD, treatment	0.8	ns	1.8	
LSD, year	0.8	11.7	1.8	
LSD, interaction	ns	ns	ns	26.3

Conclusions

Permanent wet grassland seems a relevant biomass source for biogas production. Substantial amounts of N and P can be captured and used as fertilizer. K vinasse can increase yield and nutrient capture in some swards. The data do not show how long NP-capture can last, but it is expected to depend on nutrients supplied from higher-lying fields, floods, etc. and K added should be balanced by K removed. The ratio of N/K at first cut may give an indication of where the addition of K is beneficial. It seems appropriate to consider application of K vinasse at meadows with low botanical nature quality. At meadows with high botanical nature quality, harvest can be a nature management strategy, but in general no K should be added.

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Potential production of biogas of selected grassland species from renovated grasslands

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Abstract

Biogas production is considered an important technology for the sustainable use of grasslands. Knowledge of feedstock and area-specific biogas and methane yields for different grass species and varieties is the most important factor for successful development of biogas production from grasslands. In 2008 a small-plot trial with 4 replications was established to test a total 10 grass species (23 varieties). Dry matter production from the first cut in the second harvest year (2010) and fodder quality was analysed according to standard methods. Specific biogas and methane yields of grasses were determined in batch tests with following processing parameters: temperature 37°C; mixing regime 15 minutes every 2 hours; total digestion period 49 days; reactor volume 3 L. The obtained results of volatile solid-based biogas and methane-specific yields vary in the significant range of 370-480 Nm^3 t^{-1} DM and 200-250 Nm^3 t^{-1} DM respectively. Methane content was detected in the range of 50-56%. In most cases the significant effect of grass species on specific biogas and methane yields was determined.

Keywords: grasses, biomass yields, fodder quality, anaerobic digestion, biogas production, specific methane yield

Introduction

In recent years there has been an increasing interest in alternative use of grasslands, especially as a source of feedstock for renewable energy production. Production of biogas from grasses can maintain or even improve the environmental benefits of grasslands, such as carbon storage, habitat function, preservation of ground and surface water quality, etc. (Prochnow *et al.*, 2009; Rosch, 2009). Therefore, biogas production can be considered to be an important technology for the sustainable use of grasslands.

Materials and methods

A small-plot trial with a range of grass species and varieties was established in August 2008 at Jevíčko by renovation of permanent grassland. The soil type was a gleyic fluvisol, and the plot size 10 m^2 with four replications. The trial was utilized with four cuts: the 1st cut in late May, and cuts 2-4 at 45-day intervals. Nitrogen fertilization consisted of 180 kg N ha^{-1} (distributed as 3 applications of 60-60-60; first in spring and after the 1^{st} and 2^{nd} cuts). Fertilization P and K consisted of 35 kg P ha^{-1} and 100 kg K ha^{-1}, applied in spring. We determined gas yield of the samples of forage conserved by drying at 60°C from the first cut in the second harvest year (2010) in laboratory fermenters. Methane yield was determined for the following species and varieties: Italian ryegrass (´Lubina´); perennial ryegrass (´Algol´, ´Mustang´, ´Jaran´, ´Korok´ and ´Jaspis´); meadow fescue (´Kolumbus´ and ´Pronela´); cocksfoot (´Niva´ and ´Vega´); tall fescue (´Kora´, ´Proba´ and ´Prolate´); Festulololiums (festucoid) (´Felina´, ´Rebab´ (KL-26) and ´Fojtan´); Festulololiums (loloid) (´Hostyn´, ´Lofa´ and ´Perseus´); tall oat grass (´Median´) and yellow oat grass (´Rožnovský´). The

measured results were statistically evaluated and differences between averages were tested with the Tukey test ($DT_{0.05}$, $DT_{0.01}$).
Laboratory experiments with gasification were carried out. The apparatus consisted of forty-eight 3-litre glass anaerobic fermenters (reactors) heated to 37 ± 1°C and stirred for 15 minutes every two hours. Testing of potential biogas and methane production was carried out in accordance with the guideline VDI VDI 4630 (Anonymous, 2006). The ratio of the input of sample organic dry matter to inoculant was about 3:10. The inoculant was the digestate of a full-scale farm biogas plant which processes animal excreta, maize silage and forage haylage in proportions of about 40:40:20. The experimental data were mainly recorded once a day, but at the time of the highest intensity of biogas production recording was made several times a day. Qualitative biogas analysis was carried out with a specialized biogas analyser (BioGas; Geotechnical Instruments, Leamington Spa, UK) and measurement precision was checked with a gas chromatograph with a TCD detector. The total time of fermentation experiment was uniformly set to 49 days. This is a sufficient time for the intensive stage of biogas production to cease in all tested substrates. However, in many cases biogas production did not fully stop. This was connected with gradual fermentation of components such as celluloses and hemicelluloses, which are difficult to ferment. The intensive stage of biogas production usually lasted about 2-4 weeks after deduction of a lag stage during the experiment. The lag stage usually lasted about 2-8 days.

Results and discussion

The results of the production potential of selected grass species used for grassland renovation, from the viewpoint of above ground herbage mass and corresponding methane production as the main biogas component, are summarized in Table 1 and are presented in terms of the first cut and the total annual production for four cuts. These values are the dry matter (DM) production for 2010, which was a year in which vegetation growth was slow due to the cold spring and wet weather at the beginning of harvest affected the first cuts significantly. The first-cut yields of grass species ranged from 2.61 t ha^{-1} DM (perennial ryegrass) up to 6.38 t ha^{-1} DM (yellow oat grass).

Table 1. Dry matter yields, methane yield and corresponding values of potential methane yield by particular tested grass species.

Grass species	No. of varieties	DM production from 1st cut (t ha^{-1})	Specific methane yield (Nm^3 t^{-1} DM)	Methane yield from 1st cut (Nm^3 ha^{-1})	Total annual DM production (t ha^{-1})	Potential methane yield (Nm^3 ha^{-1})
Italian ryegrass	1	2.96	237	702	8.82	2090
Perennial ryegrass	5	2.61	233	608	8.47	1974
Meadow fescue	2	3.74	214	800	9.59	2052
Timothy	2	5.47	227	1 242	12.11	2749
Cocksfoot	2	4.76	230	1 095	11.11	2555
Tall fescue	3	4.01	239	958	11.08	2648
Festucololium (festucoid)	3	3.62	228	825	10.75	2451
Festucololium (loloid)	3	4.61	222	1 023	10.62	2358
Tall oat grass	1	5.78	247	1 428	11.66	2880
Yellow oat grass	1	6.38	200	1 276	10.70	2140
$D_{T0.05}$		2.18	19	225	2.74	551

The results demonstrated high specific methane yield as the main component of biogas from grass species, which ranged from 200 to 247 Nm^3 t^{-1} DM. Tall oat grass had the highest methane yield (247 Nm^3 t^{-1} DM). Conversely, the lowest methane yield was from yellow oat grass (200 $Nm^3 t^{-1}$), which reached an extraordinary DM yield in the 1st cut in 2010. The main grass species in the conditions of the CR reached a relatively even methane production in the range 225-235 $Nm^3 t^{-1}$ DM. We can observe a tendency that grass species with higher DM yield usually showed lower gas yield. For instance, the lowest methane yield (200 $Nm^3 t^{-1}$ DM) and at the same time the highest first-cut yield were found with yellow oat grass.

The calculation of overall potential methane yield per ha per year was based on the results of methane yield from the first-cut samples. This result can be expected to be higher after accurate determination of methane yield from other cuts, as forage from the second and subsequent cuts usually has better quality than the first cut, and therefore methane yield per unit of biomass DM is usually higher. Potential annual methane yield from one ha of grassland ranges from 1974 $Nm^3 ha^{-1}$ (perennial ryegrass) to 2880 $Nm^3 ha^{-1}$ (tall oat grass). The second and third places (from the viewpoint of potential methane production) were timothy (2749 $Nm^3 ha^{-1}$) and tall fescue (2648 $Nm^3 ha^{-1}$). Minimum conclusive differentiation ($P<0.05$) of annual dry matter production was 2.74 t DM from 1 ha, and of annual methane production it was 551 $Nm^3 ha^{-1}$ methane. This means that DM yields and corresponding methane production are highly statistically significant with a number of evaluated species of grasses ($P<0.05$). Apart from above-mentioned species, especially cocksfoot, tall fescue and Festulolium are important from the viewpoint of production and methane yield per ha because they reached 1.5-2 times higher yield in the first cut in comparison with ryegrasses, which significantly influences methane production per ha.

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