



Effects of species diversity on seasonal variation in herbage yield and nutritive value of seven binary grass-legume mixtures and pure grass under cutting



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ABSTRACT

Intensively managed sown temperate grasslands are generally of low species diversity, although swards based on grass-legume mixtures may have superior productivity and herbage quality than grass-only swards. We conducted a cutting experiment over two years to test the effect of species composition and diversity on herbage yield, contents of N, neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD). Perennial ryegrass (PR, *Lolium perenne*) was sown alone and with each of four forage legumes: red clover (RC, *Trifolium pratense*), lucerne (LU, *Medicago sativa*), birdsfoot trefoil (BT, *Lotus corniculatus*) and white clover (WC, *Trifolium repens*); WC was also sown with hybrid ryegrass (HR, *Lolium × boucheanum*), meadow fescue (MF, *Festuca pratensis*) and timothy (TI, *Phleum pratense*). Herbage productivity was lowest in pure PR followed by PR/BT, and highest in PR/RC; this mixture had the highest legume proportion, N content and N yield. There was less WC in swards with HR and MF than with PR and TI. These differences were reflected in N contents of herbage of the mixtures. Legumes had higher N and lignin and lower NDF contents and IVOMD than grasses. Among legumes, NDF content was highest and IVOMD lowest in LU, followed by BT and the clovers. The highest N content was in WC. Among grasses, PR and HR had lower NDF contents and a higher IVOMD than MF; the highest N content was in PR. The grass component of mixtures had less effect than the legume component on herbage yield and quality. Results are discussed in terms of their potential to contribute to forage resources in farming practice and enhance resource use efficiency and ecosystem services.

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1. Introduction

Grass and forage-based systems allow ruminants to produce high quality proteins for human consumption from home-grown resources. Since the second world war, the use of forage legumes in NW Europe has much declined during a period of ready availability of cheap N fertiliser and realisation that grasses give very large yield responses to N fertiliser application. Farmers and their advisors became more critical of problems associated with legume-based systems, *i.e.*, lower or more variable production from forage legumes, conservation problems and risk to animal health through bloat (Wilkins et al., 2002). The area under mixed grass–legume swards may be increasing in some regions such as Western France

and Switzerland (Peyraud et al., 2009) and decreasing in others such as New Zealand (Woodfield and Clark, 2009).

In view of the need for sustainable intensification of the agricultural production and the importance of crop nutrition in ensuring yields (Royal Society, 2009) agricultural scientists are challenged to search for ways to achieve higher yields without increasing the use of mineral fertilizers. Inclusion of legumes in grasslands could help to achieve this goal (Canfield et al., 2010) as N is a major limiting factor for plant growth. In addition to reduced dependence on fossil energy and industrial N fertilizer, Lüscher et al. (2014) reported other benefits of legumes such as lower quantities of harmful emissions to the environment (greenhouse gases and nitrate), lower production costs, higher productivity, increased protein self-sufficiency and adaptation options to rising atmospheric CO₂ concentrations and climate change.

The ability of mixtures to produce more herbage than their individual components (Kirwan et al., 2007; Connolly et al., 2009; Nyfeler et al., 2011; Sturludóttir et al., 2013) has been called

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transgressive overyielding or niche complementarity and tends to be observed when the species differ substantially in their functional groups (Nyfeler et al., 2009; Sanderson, 2010; Finn et al., 2013; Finn et al., 2013). It occurs with limited N application. A well-known application of such niche complementarity in grassland agriculture is in grass–legume mixtures (Burdon, 1983). Forage legumes grown in a mixture with grass generally receive >80% of their N supply via N₂ fixation (e.g., Heichel and Henjum, 1991; Rasmussen et al., 2012), therefore forage legume productivity determines the amount and proportion of N derived from N₂ fixation. When grown together, total utilisation of atmospherically derived N is higher, as a larger proportion of the N lost to the soil from the legume (through plant tissue decay, death or animal excretion) is recovered by the grass (Høgh-Jensen and Schjørring, 1997; Carlsson and Huss-Danell, 2003; Nyfeler et al., 2011). The transgressive overyielding effects of grass–legume mixtures have been confirmed across a wide range of sites differing in soil type and climate (Kirwan et al., 2007; Sturludóttir et al., 2013). However, apart from yield benefits, for practical farming digestibility and forage quality are of major importance, especially for high producing dairy cows. Herbage intake is related to DM digestibility, structural carbohydrate content and breakdown capacity in the rumen (Armstrong et al., 1986). Legume intake is generally higher than grass intake because legumes have lower cell wall contents, higher crude protein (CP) contents, faster rates of particle size reduction in the rumen, and faster organic matter removal from the rumen (Rook and Yarrow, 2002). Legumes for high productive dairy cows therefore have a dual positive effect: higher intake and animal production plus environmental benefits. In view of animal nutrition, studies on digestive interactions between grasses and legumes have given conflicting results depending on the associated species (e.g., Hunt et al., 1985; Reid et al., 1987).

Grass growth in early spring is very responsive to available soil N and spring applications of fertilizer N or manure (slurry) are common practice. Because forage legumes have much poorer response rates to available soil N and their N₂ fixation is linked with temperature (e.g., Liu et al., 2011), it is more difficult to increase early spring herbage production with forage legumes than it is with grass. Thus in practice, even in mixed grass–legume swards strategic inputs of fertilizer N in spring are generally recommended to increase grass production at that time (e.g., Schils et al., 2000a, b; Humphreys et al., 2009). The spring cut is often used for silage making and is of major importance for farmers to ensure winter feed supply.

A number of forage legume species are widely recognized as being of global commercial importance including *Medicago sativa* L. (lucerne, alfalfa), *Trifolium repens* L. (white clover), *Trifolium pratense* L. (red clover), and *Lotus corniculatus* L. (birdsfoot trefoil) (e.g., Frame et al., 1998; Phelan et al., 2015). In practice, associations of individual legume species with particular farming and ruminant production systems are a result of the different growth habit of the plants, with prostrate stolon/rhizome formers (white clover and birdsfoot trefoil) better suited for grazing than the upright crown-formers (lucerne and red clover) (e.g., Black et al., 2009) that are well-suited for cutting. An optimal combination of suitable grass plus legume companion species is needed to obtain high N use efficiency, high productivity, a desirable seasonal production pattern and high contents of nutritive compounds in grass–legume mixtures. Although the impact of different grass–legume mixtures on the N contribution and effects of different species have been investigated under similar management, environmental and climatic conditions (e.g., Mallarino et al., 1990; Askegaard and Eriksen, 2007; Smit et al., 2008), emphasis has been on legumes rather than grass species. For example, Halling et al. (2002, 2004) published a database on productivity of five legume species based on many trials in Northern Europe carried out from 1997 to 1999, predominantly for pure legume crops and with yield observations without

information on the contribution of unsown species. Gierus et al. (2012) compared binary mixtures of perennial ryegrass (*Lolium perenne* L.) with various legumes grown in Germany, with and without slurry, but only reported data of the first production year. Recent agronomic data on companion grass effects in mixtures in intensively used grasslands are scarce and variation in the nutritional quality of the companion grass in binary mixtures is poorly understood (Gierus et al., 2012). It therefore seemed justified to examine a number of modern varieties of companion grasses and legumes with contrasting attributes. A field experiment was conducted with binary grass–legume mixtures to investigate yield and forage quality of mixtures and companion species during successive harvests.

The aims of this study were (1) to obtain novel information on botanical composition and contents of forage quality parameters in relation to annual and seasonal herbage DM yield in seven binary mixtures with underlying contrasts, i.e., four grass species mixed with white clover and four legume species mixed with perennial ryegrass, and on mixture differences and seasonal patterns across the various harvests in successive years; and (2) to obtain novel information on specific effects of companion grass and legume species on protein and fibre contents and N yield. The legume species studied here, in particular lucerne and red clover, are widely recognized for their ability to produce large annual amounts of herbage (e.g., Frame et al., 1998; Halling et al., 2004) and the four grass species are generally used in temperate and Nordic climate zones.

As competition from the companion grass affects the growth of the forage legume we hypothesised (1) that under a silage cut regime (i.e., 4–5 cuts per year) perennial ryegrass would allow a higher white clover proportion than other, taller grass species. Changes in legume proportion affect yield and also the nutritional quality as a high legume content will increase the CP content and decrease the cell wall content of a mixture. Legume proportion may affect nutritional quality of the companion grass. We hypothesised (2) that individual species (members of a functional group) may not have the same effect on nutritive value variables.

2. Materials and methods

2.1. Experimental site, species and establishment

The experiment was established in spring 2006 at the Research Farm Foulumgaard, Aarhus University, central Jutland, Denmark (56°29'N, 9°34'E; 51 m a.s.l.). The loamy-sand soil is a typical Hapludult, according to the USDA Soil Taxonomy System (Møberg and Nielsen, 1986) with 7.4% clay, 10% silt, 48% fine sand, 33% coarse sand and 1.6% carbon. In the 0–15 cm layer the pH (KCl) was 5.9, exchangeable K was 60 mg kg⁻¹ and exchangeable P was 21 mg kg⁻¹. The site had previously been under cereal cropping for 5 years before the experiment was established, and it was prepared for the experiment using standard ploughing and harrowing.

In spring 2006, seven binary grass–legume mixtures and a perennial ryegrass pure stand (PRpure) were undersown in spring barley (*Hordeum vulgare* L.) sown at 100 plants m⁻² in a randomized block design with four replications. Mixtures were: white clover, red clover, birdsfoot trefoil, or lucerne in mixture with perennial ryegrass, and white clover in mixture with meadow fescue (*Festuca pratensis* Huds.), timothy (*Phleum pratense* L.) or hybrid ryegrass (*Lolium × boucheanum* Kunth). Mixtures are abbreviated as PR/WC, PR/RC, PR/BT, PR/LU, MF/WC, TI/WC and HR/WC, respectively. The cultivar names and seeding rates are given in Table 1. Sowing depth was 0.5–1 cm. Net plot size was 1.5 m × 8 m.

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