Grass and Forage Science
The Journal of the British Grassland Society | The Official Journal of the European Grassland Federation

Complementary effects of red clover inclusion in ryegrass–white clover swards for grazing and cutting

J. Eriksen, M. Askegaard and K. Søegaard
Department of Agroecology, Aarhus University, Tjele, Denmark

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 and crude protein content 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 out-yielded the two-species mixture in years 1 and 2. Across all 4 years, yields were 8–10% higher in cut swards. Inclusion of red clover increased the yields of clover across the 4 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. Future prospects of the inclusion of red clover in sown swards are discussed. These may include higher nitrogen-use efficiency in ruminants, increased soil fertility and improved sward flexibility to cope with changing managements. The findings also suggest positive yield effects of alternating between cutting and grazing within the season or between years.

Keywords: multispecies mixtures, cutting, grazing, grass–clover, *Trifolium pratense*, *Trifolium repens*

Correspondence to: Jørgen Eriksen, Department of Agroecology, Aarhus University, PO Box 50, 8830 Tjele, Denmark. E-mail: Jorgen.Eriksen@agrsci.dk

Received 23 August 2012; revised 26 October 2012

Introduction

Increasing plant species diversity in grasslands has been shown to improve productivity (Kirwan *et al.*, 2007; Frankow-Lindberg *et al.*, 2009a), resilience to environmental stress (Sanderson *et al.*, 2005) and nutrient use (Tilman *et al.*, 2002). Diversity can affect productivity through the effect of either one or a few productive species, or through positive interactions between species due to complementarity (Picasso *et al.*, 2008). Grass–legume mixtures, particularly perennial ryegrass (*Lolium perenne* L.) with white clover (*Trifolium repens* L.), are commonly used for forage production, and clovers are particularly important due to their positive effect on nutritive value (Søegaard, 2009) and N-input to agricultural systems via N_2-fixation (Rasmussen *et al.*, 2012).

In Danish agriculture, white clover has been widely used in mixtures with grasses due to its good persistence, moderate to high yields and its resistance to grazing (Søegaard, 2009). Red clover (*Trifolium pratense* L.) is now also becoming more commonly used because of its high forage yield potential, despite its poor persistence and poor resistance to grazing (Frame, 2005). Grass–red clover mixtures have the additional advantage of improved nitrogen (N)-use efficiency in ruminants due to their content of secondary compounds (Kleen *et al.*, 2011). There are very few reports in the literature on the inclusion of more than one clover species in temperate grass–clover mixtures (Frankow-Lindberg *et al.*, 2009b), but such a strategy could be a solution to achieve a flexible, high-yielding sward suitable for both grazing and cutting in low-input temporary grassland systems. 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, combinations of cutting and grazing, and different fertilizer treatments. The hypotheses were (i) the inclusion of the high-yielding red clover in ryegrass–white clover swards will increase the yield of the mixture under both cutting and grazing regimes and increase the total clover content and (ii) increasing the number of clover species by including red clover in a ryegrass–white clover mixture will increase the stability in terms of yield and clover proportion of the sward through complementarity of the clover species under changing conditions caused by management, sward age or different growth conditions.

Materials and methods

During 2006–2010 we investigated yield, botanical composition and crude protein (CP) content of two- and three-species swards. Five managements represented cutting, grazing and combinations thereof, with different slurry fertilization treatments in 1–4-year-old swards.

Site

The grassland plots in this experiment were part of a mixed crop rotation at Aarhus University Research Farm, Foulumgaard, in the central part of Jutland, Denmark (56°29′N, 9°34′E; elevation 53 m a.s.l.). The soil was classified as Typic Hapludult, according to the USDA Soil Taxonomy System (Møberg and Nielsen, 1986) with 7·4% clay, 10% silt, 81% sand and 1·6% C in the topsoil (0–20 cm). The fields were converted to organic farming in 1987, when a 6-year rotation replacing a conventional cereal rotation (Eriksen *et al.*, 1999, 2004). In 2004 a 6-year crop rotation was introduced (barley with grass–clover undersown – 4 years of grass–clover – barley with catch crop undersown) adjacent to the existing crop rotation (barley with grass–clover undersown – 2 years of grass–clover – barley with catch crop undersown – maize – lupin). The grass–clover was undersown in a spring barley (*Hordeum vulgare* L.) cover crop. The seed mixture (26 kg ha^{-1}) – a Danish commercial mixture – consisted of 15% white clover (11% large-leaved and 4% medium-leaved) and 85% perennial ryegrass (30% medium tetraploid, 27% late diploid and 28% late tetraploid) until 2005, and from 2006 of 4% red clover (cv. Rajah), 14% white clover and 82% perennial ryegrass. Thus, from 2006 to 2010, there was a gradual conversion from a two- to a three-species mixture in the grasslands of the crop rotations (Table 1).

Experimental treatments

The grass–clover leys were subjected to five different treatments during 2006–2010. These were (i) grazing regime with cattle slurry application in spring (supplying 100 kg total-N ha^{-1}), (ii) grazing regime without slurry application, (iii) one spring cut with cattle slurry application in spring (supplying 100 kg total-N ha^{-1}) followed by grazing, (iv) cutting regime (four cuts) with cattle slurry application (supplying 200 kg total-N ha^{-1}, half in spring and half after first cut) and (v) cutting regime (four cuts) without slurry application. Each of the six fields in the two rotations was divided into two blocks, in which treatments 1–5 were randomly allocated. Grazed plots measured 15 × 18 m and cut plots 15 × 9 m. Thus, 1- and 2-year-old swards were present in four replicates each year, whereas 3- and 4-year-old swards were represented by two replicates.

Table 1 Grass–clover mixtures in the five experimental years. Mixtures were undersown with barley.

	Grassland age, years			
	1	2	3	4
2006	W	W	W	W
2007	R&W	W	W	W
2008	R&W	R&W	W	W
2009	R&W	R&W	R&W	W
2010	R&W	R&W	R&W	R&W

W, white clover in mixture with perennial ryegrass; R&W, red and white clover in mixture with perennial ryegrass.

Slurry was injected into the swards in amounts that provided the required N inputs. The DM content of the slurry was 3–7%, and the NH_4-N content was 0·56–0·66 of the total-N. Cut plots were fenced off, and heifers allowed to graze the rest of the area. Grazing plots were stocked continuously by heifers to a height of approximately 5 cm with on average 1237 grazing d ha^{-1} in grazing-only plots (treatments 1 and 2) and 818 grazing d ha^{-1} in plots with grazing after a spring cut (treatment 3). The grazed area was topped, if necessary, at the same time as cutting the cut plots. The swards were grazed to 5 cm stubble height instead of the cutting height of 7 cm to get homogeneous grazing in the extensively fenced field.

Sampling and analysis

Grass–clover DM yields in the cut plots were measured by harvesting an area of 12–24 m^2 with a Haldrup plot harvester (J. Haldrup a/s, Løgstør, Denmark). Plots were mown four times each year to a stubble height of 7 cm. In the grazed plots, herbage was harvested two times per year corresponding to the first and third cuts under the cutting regime, each time in newly fenced-off, recently topped subplots. In the grazed plots, yield measurements were taken during 2006–

2009 only. Botanical composition, DM yield and N content were determined as described by Søegaard (2009).

Statistical analysis

Dry-matter yields, botanical composition and N content were analysed in a linear mixed model (SAS statistical package; SAS, Cary, NC, USA) which included fixed effects of mixture (two or three species), management (combinations of grazing, cutting and fertilization) and sward age (1–4 years) and random effects of calendar year, block and field.

Results

Weather conditions

Annual mean temperatures ranged from 6·1 to 8·8°C (average 8·1°C), close to the 30-year mean of 7·7°C (Figure 1). Annual precipitation ranged from 599 to 723 mm (average 664 mm), which was lower than the 30-year mean of 770 mm. Spring precipitation is important for forage production, and although not evenly distributed, rainfall from March to May was between 95 and 163 mm (average 120 mm). The June–July precipitation was 70–167 mm, but during this period grasslands were irrigated if necessary. Overall, growth conditions were close to optimum for this site, and conditions were similar during the years of the experiment.

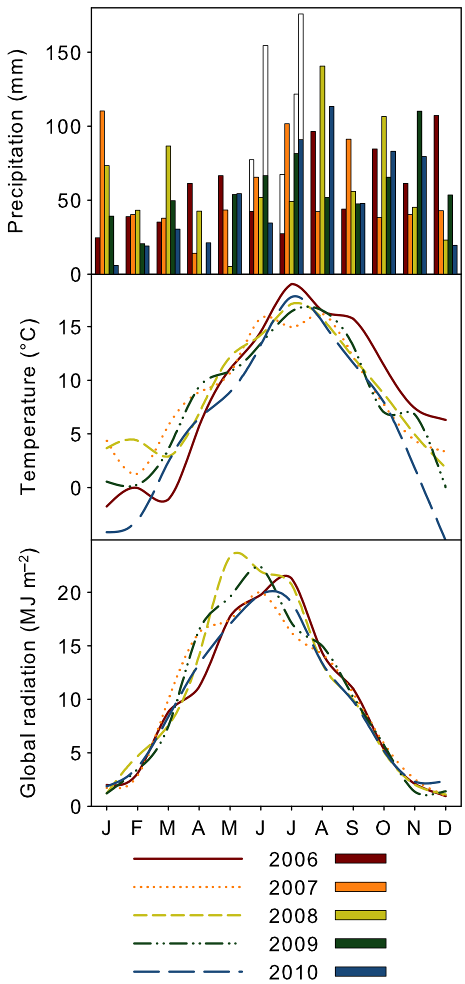

Figure 1 Weather conditions during the five experimental years. Monthly precipitation, mean temperature and mean global radiation. Irrigation shown as white bars.

Herbage production during the season

Dry-matter yields were significantly affected by mixture ($P < 0.05$), management ($P < 0.001$) and age of the sward ($P < 0.01$). There was a significant interaction between mixture and sward age ($P < 0.01$). Thus, 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 4 years old (Figure 2). This was especially pronounced in cuts 1–3, whereas in the autumn cut the three-species mixture never yielded more than the two-species mixture.

The yields for the two-species mixture in the different management regimes were differently influenced by the age of the swards (Figure 2). However, with the two-species mixture, the yields were generally maintained over the years, and there were no significant effects of sward age in any of the cuts. In the three-species mixture, yields decreased with increasing sward age, and all cuts during the season contributed significantly to this.

Fertilizer application affected the two- and three-species mixtures almost similarly with a huge effect in the first cut and no effect in the third cut (Figure 2). One main difference, however, was a large response of the two-species mixture in the second cut of the 3- and 4-year-old swards under the cutting regime.

The effect of grazing was evaluated by harvesting a fenced-off section of the grazed plots in the spring growth and the second regrowth in August. In both the two- and three-species mixtures, there were indications of positive effects on sward production associated with previous grazing of the 2- and 3-year-old swards in fertilized plots (Figure 2). However, the most pronounced effect was in the third cut of the three-species mixture, where yields were increased on plots that had previously been grazed: herbage DM yields increased by 18, 23 and 43% in the 1-, 2- and 3-year-old swards, respectively, based on the average

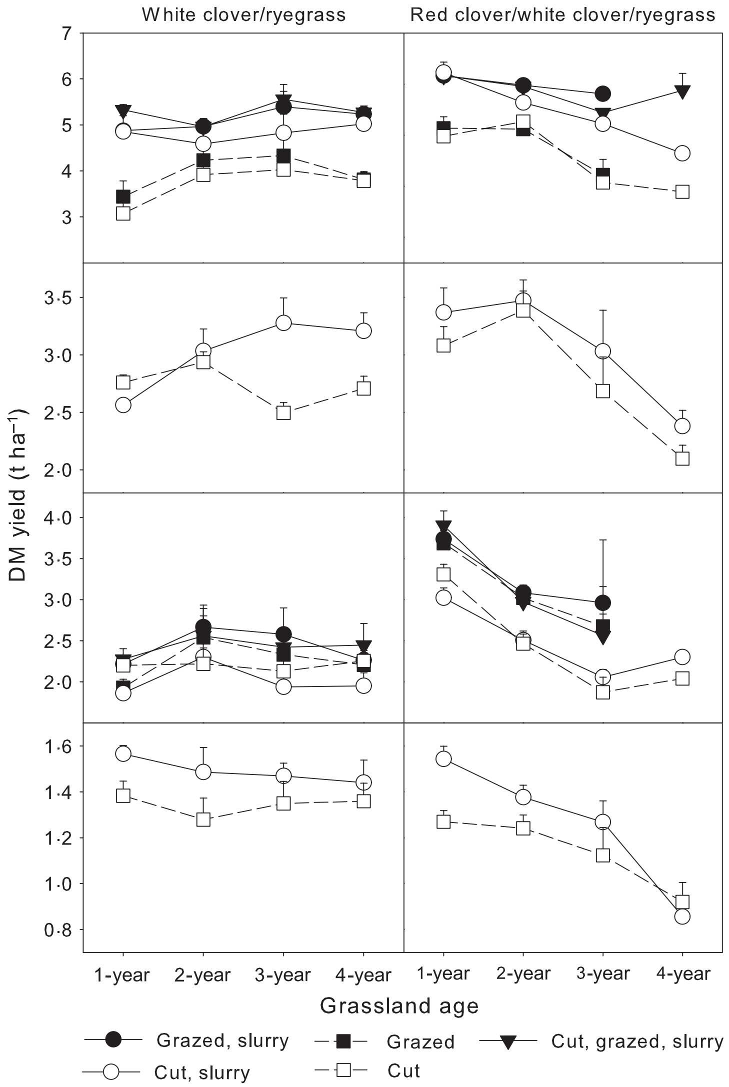

Figure 2 Herbage yields from four annual harvests of 1–4-year-old two-species (perennial ryegrass–white clover) and three-species (perennial ryegrass–red clover–white clover) swards under different managements. In grazed treatments, the cuts were taken from temporarily fenced-off parts. Error bars denote s.e.

of fertilizer levels. In the spring cut, yield response to fertilizer application was significantly ($P < 0{\cdot}001$) affected by sward age under grazing conditions. Thus, the response to fertilizer increased from 13 kg DM kg^{-1} NH_4-N in the 2-year-old sward to 21 kg DM kg^{-1} NH_4-N in the 3-year-old and 25 DM kg^{-1} NH_4-N in the 4-year-old grazed swards as an average of the two mixtures (for the 4-year-old grazed swards, there were data from the two-species mixture only).

Annual yield and botanical composition

The annual yields under the cutting regime for the two mixtures showed very different developments with increasing age of the sward (Figure 3). The two-species mixture gave constant yields over time, whereas the three-species mixture showed a dramatic and linear yield decrease over the 4 years, with the third year being the point of greatest change. For swards <3 years old, the DM yields were higher from the three-species mixture, but for swards <3 years, and also for swards older than 3 years, the two-species mixture gave higher yields. In the 3-year-old sward, however, yields were independent of number of species in the mixture.

The decrease in yield in the three-species mixture from the first to the second year was the net effect of a decrease in ryegrass yield that was larger than the increase in clover production (Figure 3). This was followed by decreasing clover contents in 3- and 4-year-old swards (Figure 3). The maintenance of similar yields over time in the two-species mixture was the outcome of increasing yields of the ryegrass balancing

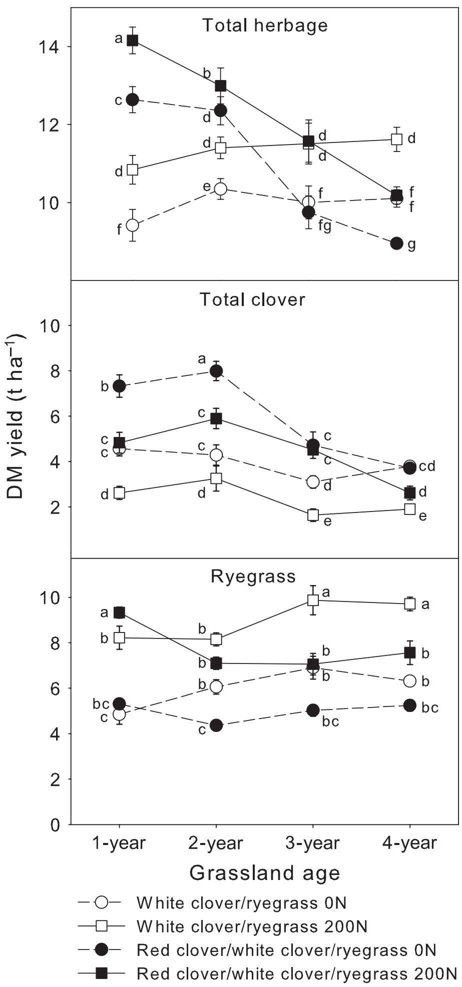

Figure 3 Annual total, clover and ryegrass yields of two-species (perennial ryegrass–white clover) and three-species (ryegrass–red clover–white clover) swards under cutting regime. Error bars denote ±s.e. Symbols with different letters are significantly different at $P < 0{\cdot}05$.

the decreasing clover yields as swards aged from the 1 to 4 years.

Fertilizer responses were similar in both mixtures, and they were also independent of sward age. The overall mean annual yield increase, as a result of slurry application, was 1·34 t DM, corresponding to approximately 11 kg DM kg^{-1} plant-available N.

The dynamics between white and red clover in the three-species mixture (Figure 4) indicate a general shift from red clover dominating in the first 2 years to white clover dominating in years 3 and 4. This development occurred in both the slurry-fertilized and unfertilized treatments, but there was less clover (white clover, $P < 0{\cdot}001$; red clover, $P < 0{\cdot}01$) when fertilized. On average, fertilization reduced the white clover content from 0·27 to 0·19 and red clover from 0·24 to 0·16. White clover content was significantly affected by sward age ($P < 0{\cdot}05$), with the lowest content in 1-year-old swards (0·19) and the highest in 3-year-old swards (0·29). Red clover was more strongly affected by sward age ($P < 0{\cdot}01$), with the highest contents in 1- and 2-year-old swards (0·29) and lowest content in swards aged 3 and 4 years. Thus, total clover content was also affected by sward age ($P < 0{\cdot}001$), with 0·48–0·51 in 1- and 2-year-old swards and a significant drop to 0·34–0·39 in 3- and 4-year-old swards, based on the average of the two fertilizer treatments.

Over the season, there was also a regular pattern, with red clover dominating in herbage harvested at the first and third cuts and white clover dominating in the second and fourth cuts (Figure 4). The effect of cutting time was highly significant ($P < 0{\cdot}001$) for white, red and total clover content. The white clover content was lowest in spring (0·11) and highest in the two summer cuts (0·28–0·29), with a small decline in the autumn cut (0·24). The red clover content increased over the first three cuts (0·26, 0·31 and 0·42 respectively) and then declined in autumn to 0·18. In consequence, the total clover content increased over the season from 0·36, to 0·60 and then to 0·70 in cuts 1, 2 and 3, respectively, and then declined to 0·41 in the autumn cut, as an average of the two fertilizer levels.

In grazed plots, the separation of clover in the three-species mixture was only made in 1–3-year-old swards (Figure 5), and grazed plots were only harvested in spring and August. Compared to the cutting regime, the botanical composition in grazed plots differed in two aspects: (i) where red clover dominated in the first two years under the cutting regime, this dominance was already broken after the first year under grazing and (ii) under grazing, the clover content – especially white clover – was less influenced by fertilization than under the cutting regime, and a significant reduction occurred only for total clover content ($P < 0{\cdot}05$) from 0·44 to 0·36 when applying fertilizer, as slurry, to the grazed plots. 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 2-year-old sward, the red and white clovers were present in almost equal proportions. Thus, the botanical composition in the cutting-and-grazing-combination treatment fell somewhere between that of the compositions of the pure cutting and pure grazing treatments.

Crude protein content

The CP content (derived from N content) of harvested plant material was not significantly affected by the species mixture. Variation was mainly explained by cutting time ($P < 0{\cdot}001$), as there was a significant increase in CP content from 121 g kg^{-1} DM in spring to 223 g kg^{-1} in autumn (Table 2). There was also an effect of management ($P < 0{\cdot}001$), as unfertilized mixtures under the cutting regime had a significantly

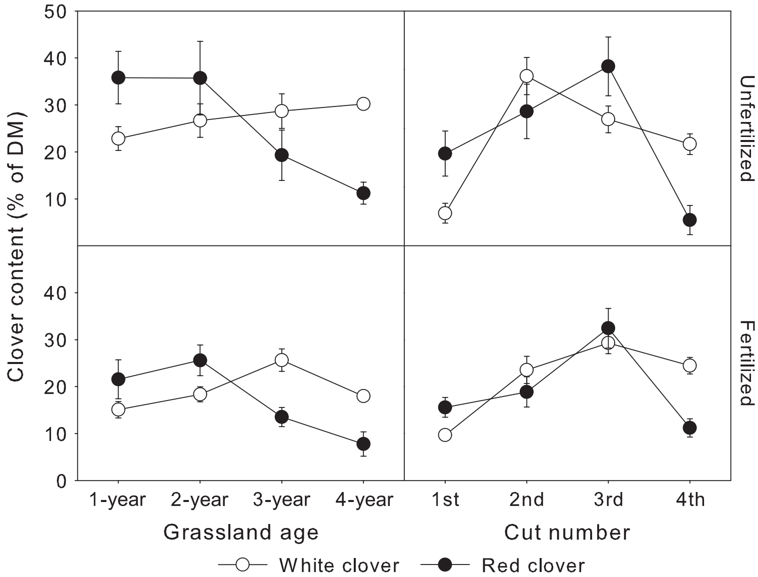

Figure 4 Clover dynamics in ryegrass–red clover–white clover swards under cutting regime. Effect of sward age and season. Error bars denote ±s.e.

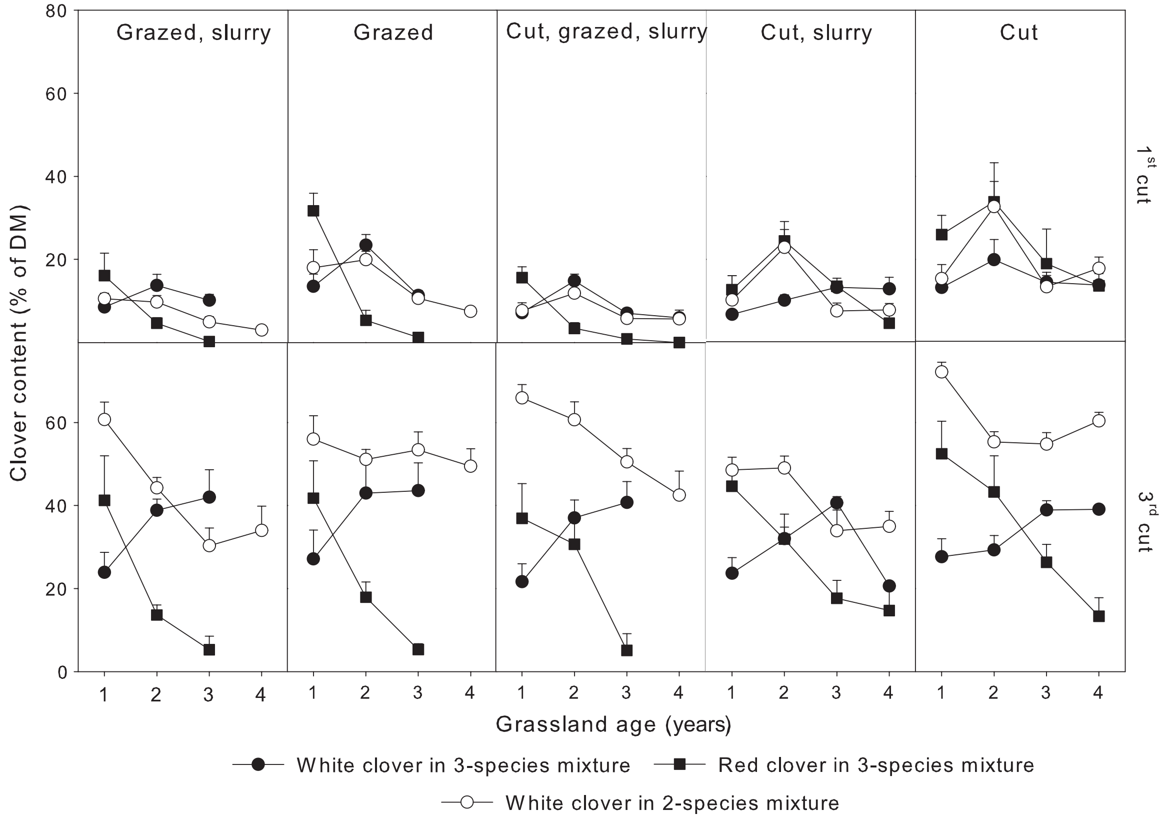

Figure 5 Clover dynamics in two-species (perennial ryegrass–white clover) and three-species (ryegrass–red clover–white clover) swards under different managements. Error bars denote ±s.e.

Table 2 Crude protein concentration of sampled herbage (g kg^{-1} DM). Values given are means of two- and three-species mixtures (mixture–treatment differences not significantly different).

		Grassland age, years				Mean	
Time	Treatment	1	2	3	4	Time	Treatment
1st cut	1 Grazed, slurry	110	128	113	109	121^{d}	160^{b}
	2 Grazed	119	122	107	102		159^{b}
	3 Cut, grazed, slurry	110	125	116	118		164^{b}
	4 Cut, slurry	111	147	132	119		177^{b}
	5 Cut	120	158	120	123		185^{a}
2nd cut	4 Cut, slurry	160	185	155	148	167^{c}	
	5 Cut	175	190	164	159		
3rd cut	1 Grazed, slurry	200	210	209	201	207^{b}	
	2 Grazed	196	211	210	208		
	3 Cut, grazed, slurry	195	219	215	216		
	4 Cut, slurry	197	210	206	188		
	5 Cut	206	216	214	210		
4th cut	4 Cut, slurry	220	226	210	218	223^{a}	
	5 Cut	234	227	216	230		
Mean		168^{b}	184^{a}	171^{b}	168^{b}		

Values of main effects with different superscript letters are significant at $P < 0{\cdot}05$.

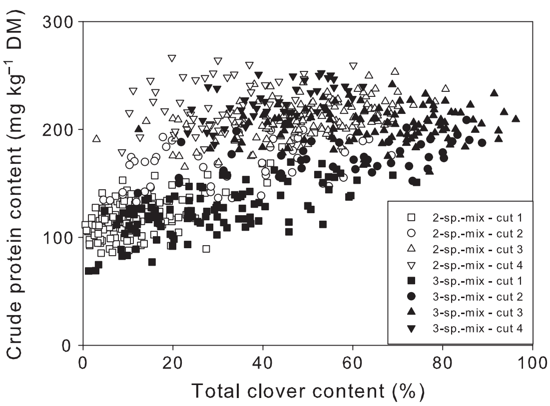

Figure 6 Crude protein content as a function of total clover content in individual samples of two-species (perennial ryegrass–white clover) and three-species (ryegrass–red clover–white clover) swards at different times of cutting.

higher CP content (19 g kg^{-1} DM) than other treatments. Grassland age had a small but significant ($P < 0{\cdot}001$) effect, as 2-year-old swards averaged 184 g kg^{-1} CP in harvested material compared with 169 g kg^{-1} for swards of other ages. There was no linear relationship between CP and clover content, but the clover content set the lower boundary for protein (Figure 6), and generally, a higher clover content was needed in the three-species mixture than in the two-species mixture to obtain the same content of CP in the harvested material.

Discussion

Species mixture composition

The first hypothesis that the inclusion of red clover in ryegrass–white clover mixtures will increase yield and total clover content was confirmed under both the cutting and the grazing regime. Thus, 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.* (2009b) also found a positive yield effect of red clover inclusion in a 3-year study at two Swedish sites in the first production year and also partly in the second year, but not in the third year.

Annual herbage yields were not measured or estimated under the grazing regime, but in temporarily fenced-off parts of the grazed plots harvests were made in spring and August. In the spring harvest of 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 second hypothesis was that the inclusion of red clover in the ryegrass–white clover mixture would increase the yield stability of the sward through complementarity of the species. This was not confirmed under cutting conditions. The yield of the three-species mixture showed an almost linear decrease over the 4-year period, whereas the two-species mixture did not. In the 3-year-old cut swards, the yields were similar for the two mixtures (Figure 3), and in the 4-year-old swards the two-species mixture gave the highest yield. The main explanation for this was decreasing yields in ryegrass as the swards aged. In the three-species mixture, the ryegrass yield had already decreased by the second year (Figure 3) and never recovered. This was in contrast to the fertilized two-species mixture in the third and fourth years, in which the ryegrass yield increased. The drop in yields of both red clover and ryegrass was not exploited by white clover, the species that had the greatest ability of the three species to spread and fill the gaps. The persistence of the depression in ryegrass yield in the three-species mixture suggests that this may have been caused by a decrease in ryegrass plant density due to the vigorous growth of the red clover in spring and, to some extent, in August. Similar reductions of tiller formation of ryegrass in mixtures with tall legumes were found by Roscher *et al.* (2008). The depression of ryegrass occurred despite a very low content of red clover in the seed mixture (1 kg ha^{-1}) compared with more commonly used seed rates of 2–6 kg ha^{-1} (Humphreys *et al.*, 1998; Frame, 2005; Frankow-Lindberg *et al.*, 2009b). 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. Further research is needed to determine appropriate seeding rates of red clover in mixture with perennial ryegrass and white clover.

Red clover has been found to decrease the CP content of grass–clover mixtures relative to that of grass–white clover (Søegaard and Nielsen, 2012). In our study, there were no significant differences between the two mixtures in the CP content of harvested herbage. This was probably a compensatory effect of the generally higher clover content, as there was a relationship between clover content and the minimum CP content. Clearly, higher clover content was needed in mixtures with red clover to achieve the same overall CP content as in grass–white clover herbage.

Grassland management

Herbage production from grass–clover swards is affected by grazing animals, partly through the recycling of N in animal excreta. This immediately affects the growth and N_2-fixation of the clover (Rotz *et al.*, 2005), but may also have some effects on pasture production in the longer term. Grazing also imposes animal trampling and more frequent defoliation, and different clover species respond differently under grazing conditions (Brummer and Moore, 2000; Frame, 2005). This was very much the case in this experiment, where 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 multipurpose mixture in grasslands used for grazing, cutting and combinations thereof. Also, new varieties of red clover are being developed which will provide better persistence, particularly under grazing, than current commercially available varieties (Marshall *et al.*, 2012).

In our study, grazing was found to increase considerably the fertilizer response in the spring cut of 3- and 4-year-old swards. Similar results have not been found by others. However, the N response under grazing of pure grass (Lantinga *et al.*, 1999) and younger ryegrass–white clover swards (Søegaard, 2009) has been found to be lower than under a cutting regime, probably due to the effects of N excretion by cattle. Also, a considerable residual effect of grazing increased the yield in the August cuts, especially in swards of the three-species mixtures, when following grazing.

The response to fertilizer application for both mixtures of an additional 11 kg DM kg^{-1} N per year under a cutting regime was close to the 8–10 kg DM kg^{-1} N typically found for ryegrass–white clover swards (Frame, 2005). Others have found a decreasing N response with sward age (Laidlaw, 1980; Søegaard, 2009), the suggested cause of which was mineralization of organic N accumulated in grassland soils. In this experiment, the N response under cutting conditions was not related to sward age. Under grazing, however, the N response in the spring cut increased dramatically from the 2-year-old swards (13 kg DM kg^{-1} N) to the 3–4-year-old swards (21–25 kg DM kg^{-1} N), which may simply be an effect of the decreasing clover content in the third and fourth year.

Sward age

The motivation for grassland cultivation on farmland is usually herbage yield loss due to sward deterioration caused by, for example, the effects of damage from wheel traffic and invasion of less-productive unsown species (Søegaard *et al.*, 2007), but utilization of the accumulation of soil fertility also plays a role (Eriksen *et al.*, 2008). The European Commission defines permanent pasture as 'land used to grow grasses or other herbaceous forage naturally (self seeded) or through cultivation (sown) and that has not been included in the crop rotation of the holding for 5 years or longer' [Commission Regulation (EC) No 796/2004]. Thus, by this definition, the 4-year-old swards in this experiment would have reached the maximum age limit for temporary grasslands. In Denmark, there is a tradition for keeping grasslands for even shorter periods (2–3 years) based on a comprehensive experimentation in the 1960s/1970s, which showed average yield reductions in ryegrass–white clover swards of 36 and 40%, in years 4 and 5, respectively, compared with first-year yields (Gregersen, 1980). Our study suggests that grasslands of longer duration are an economically viable option in situations where the yield increase cannot pay for the costs of resowing of the sward. Increasing the duration of grasslands will also have other beneficial environmental effects such as increased carbon sequestration (Acharya *et al.*, 2012).

Within the EU context of temporary (<5-year-old) grasslands, the three-species mixture performed significantly better than the two-species mixture. Although the three-species mixture produced high but decreasing yields over time due to the poorer persistence of red clover, it still gave higher yields than the two-species mixture, which showed good persistence but nevertheless lower yields.

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 4 years the yield was still 8–10% higher. More information is needed on appropriate red clover seed rates in the mixture to minimize the competitive reduction in the perennial ryegrass component responsible for the yield decline in years 2–4. More spectacularly, under the cutting regime, the effect of red clover inclusion on total clover yield across the 4 years led to a 51% increase in the absence of slurry fertilizer and a 90% increase when fertilized with slurry. The complementary effect was evident across the season, with red clover dominating the herbage at the first and third cuts, and white clover at the second and fourth cuts, and also across managements with red clover dominating under cutting and white clover under grazing conditions. The practical prospects of this may be for a higher N-use efficiency in ruminants, increased soil fertility from N fixation and a lower fertilizer requirement in the arable phase of the mixed crop rotation and finally a more flexible sward that is able to perform well under changing managements. The study also suggests positive yield effects of alternating between cutting and grazing within the season or between years.

Acknowledgments

The technical assistance of the staff at Foulumgaard Experimental Station is gratefully acknowledged. This study was supported financially by the International Centre for Research in Organic Food Systems (Org-Grass project).

References

Acharya B.S., Rasmussen J. and Eriksen J. (2012) Grassland carbon sequestration and emissions following cultivation in a mixed crop rotation. *Agriculture, Ecosystems and Environment*, **153**, 33–39.

Brummer E.C. and Moore K.J. (2000) Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. *Agronomy Journal*, **92**, 466–471.

Eriksen J., Askegaard M. and Kristensen K. (1999) Nitrate leaching in an organic dairy/crop rotation as affected by organic manure type, livestock density and crop. *Soil Use and Management*, **15**, 176–182.

Eriksen J., Askegaard M. and Kristensen K. (2004) Nitrate leaching from an organic dairy crop rotation: the effects of manure type, nitrogen input and improved crop rotation. *Soil Use and Management*, **20**, 48–54.

Eriksen J., Askegaard M. and Søegaard K. (2008) Residual effect and nitrate leaching in grass-arable rotations: effect of grassland proportion, sward type and fertilizer history. *Soil Use and Management*, **24**, 373–382.

Frame J. (2005) *Forage legumes for temperate grasslands*. Plymouth, UK: Science Publishers, Inc.

Frankow-Lindberg B.E., Brophy C., Collins R.P. and Conolly J. (2009a) Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Annals of Botany*, **103**, 913–921.

Frankow-Lindberg B.E., Halling M., Höglind M. and Forkman J. (2009b) Yield and stability of yield of single- and multi-clover swards in two contrasting temperate environments. *Grass and Forage Science*, **64**, 236–245.

Gregersen A.K. (1980) Vand og kvælstofgødning til flerårigt græs og kløvergræs [Water and nitrogen supply for grass and grass-clover of longer duration]. *Tidskrift for Planteavl*, **84**, 191–208.

Humphreys J., Jansen T., Culleton N. and MacNaeidhe F.S. (1998) Comparison of annual herbage yield, botanical composition and mineral content of swards of perennial ryegrass sown with white clover and red clover. *Irish Journal of Agricultural and Food Research*, **37**, 159–172.

Kirwan L., Lüscher A., Sebastià M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadóttir A., Baadshaug O.H., Brophy C., Coran C., Dalmansdottir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Golinski P., Grieu P., Gustavsson A.-M., Höglind M., Hugenin-Elie O., Iliadis C., Jørgensen M., Kadžiuliene Z., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykänen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology*, **95**, 530–539.

Kleen J., Taube F. and Gierus M. (2011) Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. *Journal of Agricultural Science, Cambridge*, **149**, 73–84.

Laidlaw A.S. (1980) The effects of nitrogen fertilizer applied in spring on swards of ryegrass sown with four cultivars of white clover. *Grass and Forage Science*, **35**, 295–299.

Lantinga E.A., Deenen P.J.A.G. and van Keulen H. (1999) Herbage and animal production responses to fertilizer nitrogen in perennial ryegrass swards. II. Rotational grazing and cutting. *Netherlands Journal of Agricultural Sciences*, **47**, 243–261.

Marshall A.H., Lowe M. and Vale J. (2012) Improved persistence of red clover (*Trifolium pratense* L.) varieties in mixed swards. *Grassland Science in Europe*, **17**, 73–75.

Møberg J.P. and Nielsen J.D. (1986) The constituent composition of soils from Danish State Agricultural Research Stations. Tidsskrift for Planteavl no. S1870. Danish Institute of Plant and Soil Science.

Picasso V.D., Brummer E.C., Liebman M., Dixon P.M. and Wilsey B.J. (2008) Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. *Crop Science*, **48**, 331–342.

Rasmussen J., Søegaard K. and Eriksen J. (2012) N_2-fixation and residual effect of four legume species and four companion grass species. *European Journal of Agronomy*, **36**, 66–74.

Roscher C., Schumacher J., Weisser W.W. and Schulze E.-D. (2008) Genetic identity affects performance of species in grasslands of different plant diversity: an experiment with Lolium perenne cultivars. *Annals of Botany*, **102**, 113–125.

Rotz C.A., Taube F., Russelle M.P., Oenema J., Sanderson M.A. and Wachendorf M. (2005) Whole-farm perspectives of nutrient flows in grassland agriculture. *Crop Science*, **45**, 2139–2159.

Sanderson M.A., Soder K.J., Muller L.D., Klement K.D., Skinner R.H. and Goslee S.C. (2005) Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. *Agronomy Journal*, **97**, 1465–1471.

Søegaard K. (2009) Nitrogen fertilization of grass/clover swards under cutting or grazing by dairy cows. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science*, **59**, 139–150.

Søegaard K. and Nielsen K.A. (2012) White and red clover in highly productive short-lasting grassland mixtures. *Grassland Science in Europe*, **17**, 147–149.

Søegaard K., Gierus M., Hopkins A. and Halling M. (2007) Temporary grassland – challenges in the future. *Grassland Science in Europe*, **12**, 27–38.

Tilman D., Knops J., Wedin D. and Reich P. (2002) Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. In: Loreau M., Naeem S. and Inchausti P. (eds) *Biodiversity and ecosystem functioning: synthesis and perspectives*, 1st edn, pp. 21–35. Oxford: Oxford University Press.