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# Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies. I. Dry matter yield and dynamics of species composition

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## Abstract

Four-species mixtures and pure stands of perennial ryegrass, tall fescue, white clover and red clover were grown in 3-cut and 5-cut systems at Ås, southern Norway, at a low fertilization rate (100 kg N ha<sup>-1</sup> year<sup>-1</sup>). Over a three-year experiment we found strong positive effects of species diversity on annual DM yield and yield stability under both cutting frequencies. The overyielding in mixtures relative to pure stands was highest in the 5-cut system and in the second year. Among the possible pairwise species-interaction effects contributing to the diversity effect, the grass-grass interaction was the strongest, being significant in both cutting systems and all years. The grass-legume interactions were sometimes significant, but no significant legume-legume interaction could be detected. Competitive relationships between species varied from year to year and between cutting systems. Estimations based on species-identity effects and pair-specific interactions suggested that the optimal proportions of red clover, white clover, perennial ryegrass and tall fescue in seed mixtures would have been around 0.1, 0.2, 0.4 and 0.3 in the 3-cut system, and 0.1, 0.3, 0.3 and 0.3 in the 5-cut system.

**Keywords:** *Festuca arundinacea*, *Lolium perenne*, species diversity, *Trifolium pratense*, *Trifolium repens*, yield stability

## Introduction

Sown mixtures of grassland species are frequently observed to have higher yields than would be expected from the productivity of the individual species grown in pure stand. That is, there is a species diversity effect on yield, or in other words, mixtures are overyielding. The herbage yield of a mixture can exceed that of its best-performing species when grown in a pure stand (transgressive overyielding), making mixtures particularly interesting in an agricultural context (Finn *et al.*, 2013). The effects of biodiversity on ecosystem functioning, including biomass production, and mechanisms behind such effects, were reviewed by Hooper *et al.* (2005). The presence and magnitude of a diversity effect on yield depends on the relative performance of the individual species in pure stand and in mixtures. Positive effects of species diversity on grassland herbage production are due to phenomena such as differentiation in the utilization of resources in time and space (niche differentiation or niche complementarity), positive mutualistic species interactions (facilitation), and selection of the most productive species present in a given environment (sampling effect). While the sampling effect can lead to overyielding, it cannot alone be responsible for transgressive overyielding. Although positive effects of species diversity on yield also occur in mixtures of different grasses (Van Ruijven and Berendse, 2003, 2005; Pontes *et al.*, 2012), the inclusion of legumes in grassland mixtures is particularly useful (Peyraud *et al.*, 2009; Lüscher *et al.*, 2014). Due to N fixation by symbiotic *Rhizobacteria* in legume root nodules (Carlsson and Huss-Danell, 2003) the herbage production of grass-legume mixtures under moderate N fertilizer levels can be similar to that of the best pure stands receiving high inputs of N fertilizer (Nyfeler *et al.*, 2009), thereby providing environmental benefits as well as economic benefits for the farmer. Species mixtures also have the potential to limit the establishment and growth of weeds (Sanderson *et al.*, 2007; Frankow-Lindberg *et al.*, 2009), limit the spread of pests and pathogens (Ratnadass *et al.*, 2011) and improve intra-annual yield stability (Sleugh *et al.*, 2000; Sanderson, 2010). Positive effects of species diversity on the productivity of sown grasslands have been shown to occur under various climatic conditions (Hector *et al.*, 1999; Kirwan *et al.*, 2007; Sturludóttir *et al.*, 2014; Finn *et al.*, 2013). Nevertheless, the underlying phenomena responsible for this vary in nature and strength and they are strongly dependent on the species present, their functional characteristics and on the environment/management (Voltaire *et al.*, 2014).

In Norway, grasslands are mainly sown with mixtures of species rather than a single grass or legume. For example, more than 90% of the perennial grassland seed sold by a major seed provider in

Norway is supplied as mixtures (*pers. comm.* J. A. Repstad, Felleskjøpet A/S, Norway). About 75% of the cultivated grassland area is classified as temporary grassland (Statistics Norway, 2016) and hence resown regularly, sometimes in rotation with other crops. Timothy (*Phleum pratense*) is the most important component in the most commonly used seed mixtures, which also includes species like meadow fescue (*Festuca pratensis*), perennial ryegrass (*Lolium perenne*) and red and white clover (*Trifolium pratense* and *T. repens* respectively). Mixtures are available for a variety of climatic situations and for different purposes: grazing, silage and hay. For example, perennial ryegrass-based mixtures are used in areas with a mild winter climate, and mixtures including grazing-tolerant species like white clover and smooth-stalked meadow grass (*Poa pratensis*) are sown on pastures. In practice, mixtures are composed on the basis of seed availability and knowledge about adaptation, performance and persistence of individual species and cultivars in pure stand. The facilitating effects of N fixation by legumes on grass growth, and the complementary effects of combining high yielding or high quality, but less persistent grass species (e.g. timothy) with more persistent grass species (e.g. meadow fescue), are also utilized to some extent. There is, however, potential for further utilization of other, less well characterized, mechanisms of facilitation and complementarity that could contribute to a diversity effect on yield (Goslee *et al.*, 2013; Volaire *et al.*, 2014), but little information is available on the magnitude and robustness of diversity effects in specific mixtures in specific environments and management systems. Here, we characterized the dry matter (DM) yield, yield stability and species dynamics of a four-species mixture, sown at a range of species ratios, cultivated at a low fertilizer N-input level and at different cutting frequencies over three years. We asked the following questions:

- 1) Is there a species diversity effect on DM yield at both normal and high cutting frequency?
- 2) Which pairwise species interactions contribute to any such diversity effects?
- 3) How does species composition change from year to year, and how is it affected by cutting frequency?
- 4) What effect does species diversity have on intra-annual stability of DM yield?

## Materials and methods

### Field experiment

A field experiment was conducted on a silty loam, on a beach to strand deposits, which had good soil fertility and available soil water capacity at Ås, Norway (59° 40' N, 10° 47' E; 75 m a.s.l.; annual precipitation 785 mm and average temperature 5.3 °C). Weather conditions in the experimental years are available in a supplementary file as Table S1 and Figure S1. The species and varieties sown were perennial ryegrass (*Lolium perenne* L. cv. Fagerlin, Graminor, Norway), tall fescue (*Festuca arundinacea* Schreb. cv. Kora, DLF-Trifolium, Denmark), white clover (*Trifolium repens* L. cv. Milkanova, DLF-Trifolium) and red clover (*T. pratense* L. cv. Lea, Graminor). Pure stands and mixtures were sown at two total seed rates (20 and 10 kg ha<sup>-1</sup>) on 23 June 2010. The sown mixtures consisted of either 0.25 by seed weight of each species (i.e. 5 or 2.5 kg ha<sup>-1</sup> of each species), or 0.67 of one species and 0.11 of each of the other three species. Thus, there were 9 sown sward types: 4 pure stands and 5 mixtures. The mixture with 0.25 of each species is referred to as the centroid. Seeds were mixed and drilled in rows at 0.13 m spacing. Barley was used as a cover crop. The plots were sown in a split plot design with cutting system (3 or 5 cuts a year) as main plots (2 replicates of each), and sward type and seed rate as subplots (18 plots in total per main plot, each with an area of 7.5 m<sup>2</sup>).

The plots were cut once in July and once in September during the establishment year (2010). The cutting treatments were then applied by cutting to a height of 5 cm with a Haldrup plot harvester for three years (2011-2013, see Supplementary File Table S2 for harvest times). After each harvest, the biomass from each plot was mixed and a sample of approximately 1 kg was oven dried at 60 °C for 2-3 days for estimation of dry matter content. Fertilizer (12(N)-4(P)-18(K) and micronutrients) was applied at a rate of 100 kg N ha<sup>-1</sup>year<sup>-1</sup>. In the 3-cut system 40, 36 and 24 % of the fertiliser was applied in spring and after the first and second harvest, respectively. In the 5-cut system 30, 28, 22, 14 and 6% was applied in spring and after the first, second, third and fourth harvest, respectively.

Botanical composition was determined in three ways. First, prior to each harvest, a visual assessment was made of the percentage of biomass contributed by each of the four sown species and weeds in each plot. This assessment was conducted by the same person throughout the experimental period. Secondly, biomass was sampled from four 20 × 25 cm quadrats per plot, sorted into species

fractions, then dried and weighed. This was done for the centroid plots prior to all harvests and for all plots prior to the first and last harvest in all years. Thirdly, species abundance was recorded as subplot frequencies, that is, the frequency of presence in ten 10 cm sections along four 1 m rows in each plot (i.e. 40 subplots per plot). This was done after initial spring growth and 1-2 weeks after the middle and last harvest in 2012 and 2013. Winter damage was recorded after initial spring growth as a visual assessment of the percentage of dead plants in each pure stand plot.

### Statistical analysis

The effect of cutting system ( $cut_i$ ) and the contributions of the different species and species interactions to DM yield were quantified using diversity-interaction modelling (Kirwan *et al.*, 2009). This is a flexible regression-based approach that relates an ecosystem function, e.g. biomass production, to species identity and interaction effects. We also included the interaction of cutting system ( $cut_i, i=3,5$ ) with the species identity and interaction effects. Model 1 was fitted to the response  $Y$  (annual DM yield).

$$Y = \lambda Dens + \beta_{LPij} LP \times cut_i \times year_j + \beta_{FAij} FA \times cut_i \times year_j + \beta_{TRij} TR \times cut_i \times year_j + \beta_{TPij} TP \times cut_i \times year_j + \delta_{ij} E \times cut_i \times year_j \quad [\text{Model 1}]$$

The model was fitted using generalized linear mixed models, including a random split plot effect and a heterogeneous variance covariance matrix that estimated correlations among repeated measures and allowed the error variance to change across years. The identity effect coefficients ( $\beta$ ) represent the annual DM yield of a species grown in pure stand ( $LP$ ,  $FA$ ,  $TR$  and  $TP$  for perennial ryegrass, tall fescue, white clover and red clover, respectively). The identity effects were estimated by including seed weight proportions at sowing. The diversity effect coefficient ( $\delta$ ) represents the average interaction effect between all species pairs. The diversity effect ( $\delta E$ ) is the difference between the actual DM yield of a mixture and that expected from the proportional contribution of the constituent species grown in pure stands (the sum of the identity effects). The average diversity effect across all species pairs was

estimated by including community evenness  $\left( E = \sum_{\substack{i,j=1 \\ i < j}}^s \frac{2s}{s-1} P_i P_j \right)$  (see Kirwan *et al.*, 2007). The model also

includes initial seeding density ( $Dens$ ), with its effect measured by the coefficient  $l$ . Separate  $b$  and  $d$  coefficients were fitted for each year and for the two cutting systems, facilitating a test of the effects of

cutting system on the species identity and interaction effects. We also estimated the strengths of individual pairwise interaction coefficients ( $\delta_{Species\ i \times Species\ j}$ ) using a variation of the separate pairwise interactions model of Kirwan *et al.*, (2009) and Finn *et al.*, (2013). For each pair of species, Model 1 was fitted with an additional term to test the deviation of the pairwise interaction from the average interaction effect of all the other species pairs. The estimated species identity and species interaction coefficients were used to estimate the effect of varying the species composition of the four species-seed mixture on DM yield, keeping the proportion of each species within the 0.1-0.7 range.

The intra-annual stability of DM yield was assessed using a mixed models approach to estimate the variability of dry matter yield per harvest ( $y$ ) within a year.

$$y_{ijkl} = \mu + \alpha_{ijkl} (cut_i \times sward_j \times year_k \times harvest_t) + \varepsilon_{ijkl} \quad [\text{Model 2}]$$

Model 2 contains fixed effects means for each sward type ( $sward_j, j=1 \dots 9$ ) in both cutting systems ( $cut_i, i=3,5$ ) for each harvest ( $harvest_t, t=1 \dots 3$  or  $t=1 \dots 5$ ) in each year ( $year_k, k=1,2$ ). The residual matrix  $\varepsilon$  has an expected value of zero and a variance-covariance matrix  $\mathbf{R}$ . A heterogeneity was defined in the covariance structure of  $\mathbf{R}$ . The residuals were grouped by type of sward (pure stand or mixture) and by year, allowing the variability of the function to be estimated for mixtures and pure stands in each year. A likelihood ratio test was constructed to test whether the variance components differ for mixtures and pure stands.

Changes in species composition were assessed using relative growth rate difference (RGRD) analysis (Connolly and Wayne, 2005). If there is no advantage of one species relative to another species, then their proportional representations in the community relative to one another, i.e. their relative dry matter (DM) yield, should remain the same throughout the experiment. The relative growth rate ( $RGR$ ) of a species ( $S$ ) from time  $t$  to time  $t+1$  is measured as the log of the ratio of the DM yield  $Y_{t+1}$  (at time  $t+1$ ) to the initial DM yield  $Y_t$ .

$$RGR_S = Ln \left( \frac{Y_{S,t+1}}{Y_{S,t}} \right)$$

The relative proportional representation of species may change during the year, depending on differences in seasonal patterns of growth and development. We therefore considered the annual DM

yield of each species as  $Y_S$  in our analysis.  $Y_S$  was estimated for each plot and year on the basis of the visual assessments of species biomass proportions and the total DM yield of each harvest:

$$Y_S = \sum_{i=1}^h (\text{proportion}_i \times \text{DM yield}_i),$$

where  $i$  is the harvest number and  $h$  is the total number of harvests per year. The sown species proportions were used to represent the starting point (establishment).

The *RGR* was calculated for each of the four species and weeds and three time intervals. Differences among the *RGRs* were computed, selecting one species as the reference (tall fescue (Fa) in this case), resulting in four *RGRDs*:

$$RGRD_{LpFa} = \mu_{LpFa} + \lambda_{LpFa} \text{ cut} + \varepsilon_{LpFa}$$

$$RGRD_{TrFa} = \mu_{TrFa} + \lambda_{TrFa} \text{ cut} + \varepsilon_{TrFa}$$

$$RGRD_{TpFa} = \mu_{TpFa} + \lambda_{TpFa} \text{ cut} + \varepsilon_{TpFa}$$

$$RGRD_{WeedFa} = \mu_{WeedFa} + \lambda_{WeedFa} \text{ cut} + \varepsilon_{WeedFa}$$

$$\mathbf{RGRD} = \boldsymbol{\mu} + \boldsymbol{\lambda} \text{ cut} + \boldsymbol{\varepsilon} \quad [\text{Model 3}]$$

The matrix of 4 *RGRD* response variables was analysed using multivariate regression techniques, including cutting system as a factor and species DM yield at time  $t$  as covariates. The residual vector  $\boldsymbol{\varepsilon}$  was assumed to follow a multivariate normal distribution. Model selection was performed using Wilks' lambda multivariate test. The vector  $\boldsymbol{\mu}$  represents the relative abilities of the species to increase their DM yield from year to year. For example, consider a simple model comparing the changes in annual DM yield production of species 1 and 2:  $RGRD_{12} = m_{12}$ . If  $m_{12}$  is positive, then species 1 has a higher increase, or a lower decrease, in DM yield relative to species 2. Here, species 1 will gain proportionally in the community at the expense of species 2. Alternatively, if  $m_{12}$  is negative, then species 2 will gain. The vector  $\boldsymbol{\lambda}$  tests whether the cutting frequency alters the competitive relationships among the species. The differences in the change in annual DM yield production among species for a given time period may also depend on their relative abundances at the start of that period. In addition, we included terms to test whether the relationships among species are affected by sward type at time  $t$ . From these multivariate regression parameter estimates, the  $m$  and  $l$  coefficients can be easily inferred for all other *RGRD* combinations (i.e.  $RGRD_{LpTr}$ ,  $RGRD_{LpTp}$ ,  $RGRD_{LpWeed}$ ,  $RGRD_{TrTp}$ ,  $RGRD_{TrWeed}$ ,  $RGRD_{TpWeed}$ ). We analysed the *RGRDs* for three time periods: establishment-year1, year1-year2 and year2-year3.

The effects of cutting system and stand type on overyielding (the increase in DM yield relative to what would be expected from pure stand yields) and transgressive overyielding (the increase in DM yield relative to the best performing pure stand in each cutting system and year) in mixtures as well as winter damage in pure stands, were analysed with analysis of variance.

Models were fitted using the GLM, GENMOD and MIXED procedures in SAS 9.2 (SAS Institute Inc., Cary, NC, USA.).

## Results

### **Effect of species and cutting system on dry matter (DM) yield**

There was no significant effect of seeding density on DM yield ( $P=0.13$ ), and this factor was therefore excluded from further analyses. Species identity effects varied significantly between cutting systems and years (Table 1). In the first year, the grass pure stands produced lower yields in the 5-cut system than in the 3-cut system, while legume pure stands produced similar yields in the two cutting systems (Figure 1). From year 1 to year 3, the pure-stand yields of perennial ryegrass and tall fescue were reduced by approximately 70 and 50%, respectively (in both cutting systems). In the 3-cut system the decrease was gradual, but in the 5-cut system most of the reduction in perennial ryegrass occurred between the two first years, and in tall fescue it occurred between year 2 and 3. The yield obtained from white clover pure stands did not change significantly from the first to the second year, but decreased by approximately 30% in both cutting systems in year 3. It was only in year 3 that the difference between cutting systems in yields of the white clover pure stands was significant (30% lower in the 5-cut system than in the 3-cut system). From year 1 to year 2 the yield of red clover pure stands increased by 20% in the 3-cut system and decreased by 24% in the 5-cut system. From year 2 to year 3 the yield of red clover decreased in both systems, reaching 46 and 67% of the year-1 yields in the 3- and 5-cut system, respectively. The accumulated DM yield over the three years was largest in red clover in both cutting systems (25.7 and 19.5 t ha<sup>-1</sup> in the 3-cut and 5-cut system, respectively).

## Effects of species diversity on DM yield

There was a significant positive effect of species diversity on DM yield for both cutting systems in all years (Table 1, Figure 1). The absolute diversity effect ( $\delta$ ) was highest in year 2 of the 3-cut system. It had a similar and moderate magnitude in year 1 in the 3-cut system and years 1 and 2 in the 5-cut system, and it was lowest in year 3 in both systems. On average across all mixtures, the overyielding (the increase in DM yield relative to what would be expected from pure stand yields) was 42%, 69% and 32% for years 1, 2 and 3 in the 3-cut system, and 50%, 72% and 48% in the 5-cut system, while the transgressive overyielding (the increase in DM yield relative to the best performing pure stand in each cutting system and year) was 24%, 16% and 9% for years 1, 2 and 3 in the 3-cut system, and 21%, 38% and 9% in the 5-cut system. Both overyielding and transgressive overyielding in mixtures, accumulated over the three years, was significantly larger in the 5-cut system than in the 3-cut system ( $P < 0.05$ ). The overyielding peaked in year 2 in all mixtures in both cutting systems (Figure 2). There were significant differences in overyielding among the mixtures in the last two years ( $P < 0.04$ ), with the perennial ryegrass-dominated mixture having the highest overyielding.

Analysis of the contribution of the pairwise species interactions on the diversity effects of the four-species mixture showed that there were significant positive interaction effects between the two grass species, between perennial ryegrass and each of the two legume species, and between tall fescue and white clover, but no significant interactions between tall fescue and red clover or between the two legume species (Table 2). The duration of pairwise species interactions differed depending on their identity and on the cutting frequency. The grass-grass interaction was the most persistent, being significant for both cutting systems in all years. In both cutting systems there were also significant positive interactions of a similar magnitude between perennial ryegrass and white clover in the two first years and between tall fescue and white clover in the first year. The interaction between perennial ryegrass and red clover was significant only in the second year of the 3-cut system and in the third year of the 5-cut system. When we estimated the effect of varying the proportion of one species in the seed mixture from 0.1 to 0.7 while keeping the seed weight ratio between the three other species constant at 1:1:1 (Figure 3A), the maximum accumulated yield over the three years was estimated when the red clover proportion was 0.1 (both cutting systems). Together with the white clover this corresponds to a total legume proportion of 0.4. When we manipulated the white clover proportion, the maximum accumulated yield in the 3-cut system was obtained at a proportion of 0.2. For white clover in the 5-cut

system and tall fescue in both cutting systems the optimal proportion was 0.3, whereas for perennial ryegrass it was 0.4. This corresponds to total legume proportions of 0.4 - 0.5. When we estimated the effect of varying the species seed weight ratios in all combinations, but keeping the minimum proportion of each species at 0.1, we found the maximum accumulated yield in the 3-cut system was obtained at proportions of 0.1 (red clover), 0.2 (white clover), 0.3 (tall fescue) and 0.4 (perennial ryegrass), while the maximum accumulated yield in the 5-cut system was obtained at proportions of 0.1 (red clover), 0.3 (white clover), 0.3 (tall fescue) and 0.3 (perennial ryegrass) (Figure 3B).

### **Intra-annual yield stability**

Intra-annual variability in DM yield in pure stands was more than double that in mixtures across all years ( $\chi^2_{,6df} = 76.3$ ,  $P < 0.001$ , Figure 4). Thus, higher diversity had a positive effect on both DM yield and intra-annual stability. The intra-annual variability in the 3-cut system was about twice as large as that in the 5-cut system ( $\chi^2_{,6df} = 143.4$ ,  $P < 0.001$ ).

### **Temporal development of species composition in mixtures**

The realized proportions of the different species changed over time and was affected by the cutting system (Figure 5). In the RGRD analysis there were no effects of sward type on changes in species composition. In the first year, the annual DM yield proportion of perennial ryegrass and red clover was higher than the sown proportion, while the DM yield proportion of tall fescue and white clover was lower than the sown proportions in both cutting systems, indicating faster establishment of perennial ryegrass and red clover (Figures 5, 6, and supplementary File Table S3A). From year 1 to year 2, tall fescue increased at the expense of perennial ryegrass and red clover in both cutting systems and also at the expense of white clover in the 3-cut system. In the 3-cut system perennial ryegrass and red clover also increased at the expense of white clover. The advantage of tall fescue relative to perennial ryegrass was higher in the 3-cut system than in the 5-cut system, while the advantage relative to red clover was higher in the 5-cut than in the 3-cut system. Red clover had an advantage relative to perennial ryegrass in the 3-cut system and it was the other way around in the 5-cut system. From year 2 to year 3, both tall fescue and white clover increased at the expense of perennial ryegrass and red clover in both cutting

systems, while perennial ryegrass increased at the expense of red clover. The RGRD analysis was based on total annual yields of each species calculated from species biomass proportions obtained by visual assessments before each harvest. We also did an analysis based on yields of each species in the first and the last harvests each year, calculated from species proportions obtained by botanical sorting. Although the absolute values of  $\mu$  (the relative abilities of two species to increase DM yield) varied somewhat when these two methods were used, there was, in most cases, with the exception of year 1 to 2 for pairs including weeds, an agreement in the sign and significance of  $\mu$  (Supplementary File Table S3). In the first two years weeds made up only a very small proportion of the DM yield. From the second to the third year, the weed yield increased at the expense of all the sown species in both cutting treatments, up to approximately 10 and 20% in the centroid mixture in the 3- and 5-cut systems, respectively (Figure 4).

Visual assessments of winter damage indicated greater damage in the pure stands of legumes than in those of grasses, more damage in legumes in the 5-cut system than in the 3-cut system in the spring of year 2, and an opposite effect of cutting system in the spring of year 3 (Table 3). Most of the reductions in subplot presence of perennial ryegrass, white clover and red clover, during years 2 and 3, occurred during the winter, whereas the presence of tall fescue was more stable (Supplementary File Fig. S2, S3). During the winter prior to year 3, the subplot presence in pure stands of white clover and red clover, respectively, decreased from 100 to 64% and from 88 to 16% in the 3-cut system, while it decreased by only small amounts, from 94 to 91% and from 76 to 49%, in the 5-cut system; i.e. mortality was higher in the 3-cut system than in the 5-cut system (Figure 7). A similar difference between cutting systems was not detected for the grasses. In the 3-cut system there was a similar red clover subplot presence in pure stands and in mixtures in the spring, although the pure stands had a much higher red clover presence in the previous autumn. Thus, there was higher mortality of red clover in pure stands than in mixtures during this winter. Such a difference between pure stands and mixtures was not evident for red clover in the 5-cut system or for white clover in any of the cutting systems.

## Discussion

### Effect of year and cutting system on DM yield in pure stands

Changes in yield in pure stands are the result of intrinsic patterns of growth and survival in the species under the prevailing conditions. Here, the yields of pure stands were all reduced in year 3 relative to year 1, but the magnitude of year-to-year changes depended on the species identity and on cutting system.

Pure stands of grasses had lower annual DM yield in the 5-cut system than in the 3-cut system, while the pure stands of legumes had similar DM yields in the two cutting systems at the beginning of the experiment. Previous studies have shown that higher cutting frequency usually results in lower annual DM yield of grassland swards, e.g. in mixed grass-legume swards (Boswell, 1977); and in perennial ryegrass swards (Wilkins, 1989; Cashman *et al.*, 2016). Several mechanisms may be responsible for this. Firstly, as cutting frequency increases, biomass production gradually becomes limited by the leaf area index. Secondly, root biomass has been found to be down-regulated and, in legumes, nodules are shed and N fixation is reduced in response to defoliation (Wilson, 1942; Evans, 1973; Frame *et al.*, 1998; Erice *et al.*, 2011). Root dieback may lead to lower utilization of nutrients and water. Thirdly, high cutting frequency can limit both regrowth capacity and winter survival ability if reserves are not allowed to accumulate to sufficient amounts between cuts and before winter (Donaghy and Fulkerson, 1998; Belanger *et al.*, 2006). Fourthly, there is a higher proportion of vegetative tillers and leaf tissue in the harvested herbage at higher cutting frequencies. The concentration of N is higher in leaves than in stems, and thus, when N is limiting for growth less shoot biomass can be produced at higher cutting frequencies (Mowat *et al.*, 1965; Wilman *et al.*, 1976; Coblenz *et al.*, 1998; Hoekstra *et al.*, 2007). A fifth factor relevant for certain species, such as red clover, is a limitation in the number of axillary meristems available for regrowth (Van Minnebruggen *et al.*, 2015). We may speculate that during the first harvesting year in our experiment, legumes were less prone to DM yield reduction at higher cutting frequency due to less N limitation on growth. Gierus *et al.* (2012) also observed that DM yield of red and white clover was not significantly reduced in the first harvest year of a 5-cut system as compared to a 3-or 4-cut system. Sheldrick *et al.* (1986), however, found reduced DM yields in both the first and second harvesting year when red clover varieties were cut six times a year rather than three times. Unlike the present experiment, and also that of Gierus *et al.* (2012), Sheldrick *et al.* (1986) did not

apply N fertilizer immediately after cutting, and therefore the plants in their experiment may have been more N-limited during early regrowth. Towards the end of our experiment (year 2 for red clover and year 3 for white clover), DM yields of the legume pure stands were lower in the 5-cut system than in the 3-cut system. This indicates that the 5-cut system was stressful for legume plants over time. One possible mechanism could be that under the 5-cut system plants were not able to accumulate sufficient organic reserves, and therefore there was a higher rate of mortality in winter than for plants in the 3-cut system. Assessments of winter damage in the spring of year 2 indicated that winter mortality of the legumes after the first harvest year was higher in the 5-cut system than in the 3-cut system. After the second harvest year, however, for pure stands of white clover and red clover there was higher winter mortality in the 3-cut system than in the 5 cut system. It thus appears that both cutting frequency and intraspecific competition for light (which is greater in the 3-cut system) can affect winter survival, but at different times in the life of the sward. Both these factors could stimulate allocation of resources to shoots and thereby reduce winter survival ability. Previous studies have shown a detrimental effect of autumn defoliation on the capacity of grassland species to survive winter conditions, and may also affect subsequent regrowth in spring (Vik, 1955; Frankow-Lindberg *et al.*, 1997).

### **Temporal development of species composition in mixtures**

A change in the annual biomass produced by one species relative to another species is the result of intrinsic differences between them in growth and survival under the prevailing conditions (in pure stands), and interspecific interactions due to differences in the ability to compete for resources, facilitation and antagonism (in mixtures).

In this experiment, perennial ryegrass and red clover in mixtures were more dominant than tall fescue and white clover in the first harvesting year, and also less persistent, in both cutting systems. Red clover is a short-lived perennial with limited persistence (Frame *et al.*, 1998; Annicchiarico *et al.*, 2015). Perennial ryegrass can also have limited persistence in cutting-only regimes, and is not very winter hardy (Peeters, 2004).

The relationships between species in mixtures were affected by cutting frequency and this became particularly evident during the second time interval (year 1 to year 2). Perennial ryegrass tended to have an advantage relative to the other species in the 5-cut system, while tall fescue tended to have an advantage in the 3-cut system. At sufficiently high N levels, perennial ryegrass has greater tolerance

to defoliation than tall fescue due to a higher growth rate and a higher number of tillers (Gastal *et al.*, 2010). Our results show that also under low N fertilization, but in mixture with legumes, high cutting frequency confers an advantage to perennial ryegrass, and that low cutting frequency is advantageous to tall fescue. The legume species were affected the most by cutting frequency, with white clover being more competitive in the 5-cut system and red clover more competitive in the 3-cut system.

In the second year, red clover became dominant in the 3-cut system, making up 40-60% of the DM yield of mixtures. This is a typical situation in organic farming systems, in which less nitrogen is available. From the first to the second year of the 3-cut system red clover biomass increased at the expense of perennial ryegrass and white clover, but not tall fescue. Thus, tall fescue may be more compatible with red clover, and a more appropriate grass than perennial ryegrass in this context, particularly in organic farming systems. A Danish experiment (Eriksen *et al.*, 2012) also found that herbage production from perennial ryegrass was reduced in the second year in a 4-cut treatment by the inclusion of red clover in a perennial ryegrass-white clover mixture. This occurred both with and without the application of 200 kg N ha<sup>-1</sup> year<sup>-1</sup>, indicating that the advantage of red clover relative to the other two species at this stage is not only related to its nitrogen fixation. In our experiment, red clover grew very tall with large shoots in the 3-cut system and was probably a stronger competitor for light. We also observed that, unlike perennial ryegrass and white clover, tall fescue grew long leaves in the 3-cut system, indicating that it may be able to intercept more light in a dense red clover canopy than can perennial ryegrass and white clover. This may explain why red clover did not out-compete tall fescue.

During the last time interval (year 2 to year 3) the biomass production of red clover was strongly reduced relative to that of the other species. This was associated with more winter damage in the red clover. White clover is able to compensate for winter kill through vegetative reproduction from stolons, but red clover does not have this ability, and nitrogen released from decaying plant tissue may have facilitated growth of other species in year 3. It is interesting that the higher winter kill in the 3-cut system compared to the 5-cut system, observed in red clover pure stands this winter, was not evident in mixtures. One explanation could be that less intraspecific competition among red clover plants for light and/or nutrients in the 3-cut mixtures may have resulted in a root/shoot allocation of resources that is more favourable for winter survival. Additionally, the presence of other species may have facilitated winter survival: the grasses may have trapped snow or perforated ice cover and thereby protected against freezing or ice cover damage, or a lower density of red clover plants may have reduced the spread and impact of red clover winter pathogens. Such effects, however, do not appear to have played

a large role, as winter survival in mixtures was not better than that in pure stands in the 5-cut system. The functional relationships between plant density, cutting frequency, above-ground biomass production and winter survival deserves further investigation because (i) it has potential to be utilized in the management of red clover-dominated swards, and (ii) it may have implications for breeding aiming at improving both yields and persistence.

### **Species diversity effects on DM yield and stability in mixtures**

We found a strong and transgressive positive diversity effect on DM yield in both cutting systems and this effect persisted throughout the three harvesting years. Sampling effects among species can lead to overyielding, but transgressive overyielding can only be explained by additive effects on yield via niche complementarity or facilitation among species. Such additive mechanisms may be (i) better utilization of the growing season in mixtures, as grasses produce more at the beginning, and legumes more at the end of the growing season, (ii) better spatial utilization of water and nutrients, as well as light, due to different root and shoot morphology and growth pattern of the involved species, (iii) limitation of pests and pathogens due to lower density of suitable hosts, and (iv) limitation of weed establishment through space occupation. Among potential facilitative mechanisms, the obvious candidates are positive interactions between grasses and legumes on the N fixation in legumes and N uptake in grasses (Nyfeler *et al.*, 2011). Annual DM yields of adjacent pure stands of perennial ryegrass receiving an annual rate of 300 kg N ha<sup>-1</sup>, but otherwise managed exactly like the plots in the experiment, were 1.49, 2.79 and 2.74 times higher than the DM yield of the pure stands of perennial ryegrass in the experiment (receiving 100 kg N ha<sup>-1</sup>) for year 1, 2 and 3, respectively (data from the 3-cut system only). The crude protein concentrations in the perennial ryegrass fractions of mixtures were significantly higher than in pure stands, indicating improved N nutrition of perennial ryegrass in the mixtures (Ergon *et al.*, 2017). Thus, at least part of the diversity effect observed was due to grass utilization of the N fixed in legume root nodules, i.e. facilitation. Indeed, there was transgressive overyielding of crude protein in mixtures (Ergon *et al.*, 2017), implying that species interactions improved the N supply to the above-ground biomass through fixation, mineralization and/or more efficient utilization of available N in the soil. There were also significant positive pairwise interactions detected between perennial ryegrass and both of the clover species, and between tall fescue and white clover on DM yield. Except for the last year in the 5-cut system, there were stronger interactions between grasses and white clover than between grasses and red clover. This is in agreement with the results of Gierus *et al.* (2012) who found higher perennial

ryegrass yields in mixtures with white clover than with red clover. White clover thus appears to be a better facilitator of grass growth than red clover, possibly due to being less competitive for light.

Other, less explored, facilitative mechanisms such as improved soil structure by deep-rooting species, perforation of ice-cover or trapping of a protective snow layer by grasses, may also have played a role. Indeed, the most persistent positive species interaction effects on DM yield were between the two grass species. As there were no binary mixtures in this experiment, we do not know to what extent the interaction between grass species was dependent on the presence of legumes, i.e. through improved N supply. A positive diversity effect on yield has often been ascribed to facilitation of grasses through N fixation, but Van Ruijven and Berendse (2003, 2005) found strong positive and persistent diversity effects on DM yield in mixtures of grass and forb species, with no legumes present. Wilman *et al.* (1998) also observed higher yield in a mixture of perennial ryegrass and tall fescue than in pure stands of the two species, whereas van Eekeren *et al.* (2010) and Cougnon *et al.* (2014) did not. The strong grass species interaction effects that we observed in our experiment may be additive effects due to niche complementarity. Tall fescue can develop deeper roots than perennial ryegrass (Wilman *et al.*, 1998; Van Eekeren *et al.*, 2010; Cougnon *et al.*, 2013), and this could allow the two species to utilize water and nutrients at different depths. Gastal *et al.* (2010) characterized root and shoot traits related to N strategies in perennial ryegrass, tall fescue and a number of other grass species. They found that under high N, perennial ryegrass had a higher number of tillers and higher N uptake and could regrow more quickly after defoliation than tall fescue. On the other hand, tall fescue had longer leaves. This is in agreement with our observations and indicates that the two grasses may have utilized different light niches; perennial ryegrass appears to be able to utilise the early phase after each harvest, while tall fescue with its long leaves presumably intercepts more light in dense canopies. The stronger interactions that we observed between perennial ryegrass and legumes than between tall fescue and legumes may be due to a more efficient N uptake in perennial ryegrass.

Given that, based on morphology, white and red clover appear to occupy different spatial niches both above- and below-ground, it is interesting that there were no interaction effects between them on DM yield. Temperton *et al.* (2007) found a strong reduction in red clover growth when other legumes were present in the community. They found that, unlike non-legumes, red clover did not gain fitness when the tissue N concentration increased. Thus, the legumes do not appear to facilitate each other, and may indirectly inhibit each other through the facilitation of non-legumes. In our experiment, any

positive effects of niche differentiation between the legumes may also have been cancelled out by the competition from facilitated grasses.

We found larger overyielding in the 5-cut system than in the 3-cut system. This is in line with the stress-gradient-hypothesis, which states that facilitation increases, and competition decreases, with increasing environmental stress (He *et al.*, 2013). Increased cutting frequency can be expected to favour species with strong regrowth ability, and reduce the advantage of competitiveness for light. Cutting frequency may also have differential effects on species' resistance to prevailing stresses. Pontes *et al.* (2012) found that in grass mixtures including the high-yielding species *Dactylis glomerata* and *Festuca arundinacea*, the diversity effect on dry matter yield decreased when the number of cuts in a season increased from three to six, probably through less dominance of these species (sampling effect) as the competition for light was reduced. Picasso *et al.* (2008) found no significant difference in the magnitude of the species diversity effect between a 1-cut and a 3-cut system. In both these studies the main driver of the diversity effect was selection of the highest yielding species (sampling effect), while other mechanisms played a lesser role. In our experiment, a higher yield benefit in the 5-cut system appears to be due to a reduction of the proportion of red clover – the species that contributed the least to a positive effect of species diversity on DM yield. The estimation of DM yield as a response to species composition of the four-species seed mixture also showed that, under the prevailing conditions in our experiment, maximum yield would be obtained with a low amount of red clover (10% of the seed weight). Thus, the relatively strong species identity effect of red clover on yield was overridden by the limited net interactions with other species. However, the transgressive overyielding of crude protein in the mixtures (Ergon *et al.*, 2017), suggest that at least a minimum amount of legumes was a prerequisite for the strong positive interaction between the two grass species on DM yield in mixtures. Moreover, red clover may facilitate other species more after its death, as in the third year in the present experiment, probably due to released N and possibly improved soil structure. This, together with the positive effect on nutritive quality and forage intake, could make it advantageous to use more than 10% by weight in seed mixtures.

Higher intra-annual yield stability is desired in practical farming. We found that the intra-annual yield stability was significantly higher in the average mixture than in the average pure stand in both harvesting regimes. This may partly be due to the fact that grasses and legumes utilize growth

conditions differently during the consecutive growths, as they have different seasonal patterns of stem formation and reproductive development.

## Conclusions

Our results suggest that species interactions in grass-legume mixtures have strong positive effects on DM yield and yield stability in low N-input systems at various cutting frequencies. To what extent the diversity effect on yield is affected by cutting frequency may depend on the actual species present, and their contributions to sampling effects and other types of species interactions. Our results indicate that there are positive and stable interactions between perennial ryegrass and tall fescue on DM yield across different cutting frequencies and that it is advantageous to include both these species at more or less equal proportions in seed mixtures with a legume. There was no significant interaction between red and white clover on DM yield. Red clover was the species with the highest accumulated yield over three years in pure stand, and while a minimum proportion of red clover may have been a prerequisite for the positive interaction between grasses, red clover was also the species that contributed the least to the diversity effect, possibly due to its dominance and competitiveness for light. In a 3-cut system, a mixture of the four studied species appeared to obtain the maximum accumulated DM yield over three years when there was a limited proportion of red clover in the seed mixture (about 10% by seed weight) and a moderate proportion of white clover (20%), whereas in a 5-cut system increasing the proportion of white clover up to 30% of the seed mixture was advantageous.

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## Supporting Information

Additional supporting information (tables and figures) may be found in the online version of this article

**Table S1.** Temperature, precipitation and soil moisture conditions during the growth periods of the 3-cut regime.

**Table S2.** Harvest dates

**Table S3.** Predicted  $\mu$  effects (the relative abilities of two species to increase DM yield) from the RGRD model based on A) visual assessment (weighted average of all harvests) and B) dry weights of separated species fractions (weighted average of first and last harvest).

**Figure S1.** Winter (October-March) weather conditions

**Figure S2.** Species abundance recorded as the frequency of presence (%) in forty 10 x 10 cm subplots along the sown rows in year 2 and 3.

**Table 1.** A) Parameter estimates from Model 1 for species identity effects  $\beta$  (LP, *Lolium perenne*; FA, *Festuca arundinacea*; TR, *Trifolium repens*; TP, *T. pratense*) and species diversity effect  $\delta$ , on total annual dry matter yield in mixtures of the four species. All estimates were significant at  $P < 0.001$ . The species identity effects equals the estimate of dry matter yield of pure stands ( $\text{t ha}^{-1}$ ), while the species diversity effect equals the estimate of yield in a centroid mixture minus the average yield of pure stands. B) P-values for tests comparing the parameter estimates between cutting systems. C) P-values for tests comparing the parameter estimates between years.

A)

Parameter	3 cut system			5 cut system		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
$\beta_{LP}$	10.08	5.63	2.45	7.42	2.73	1.58
$\beta_{FA}$	9.41	7.24	4.56	6.66	5.76	2.66
$\beta_{TR}$	6.07	6.93	5.13	6.2	6.46	3.67
$\beta_{TP}$	8.64	10.37	4.37	8.62	6.2	2.35
$\delta$	4.78	6.78	1.81	4.64	4.97	2.35

B)

Parameter	Year 1	Year 2	Year 3
$\beta_{LP}$	<.0001	0.005	0.171
$\beta_{FA}$	<.0001	0.147	0.003
$\beta_{TR}$	0.853	0.648	0.023
$\beta_{TP}$	0.978	<.0001	0.002
$\delta$	0.809	0.045	0.258

C)

Parameter	3 cut system		5 cut system	
	Year 1 v Year 2	Year 2 v Year 3	Year 1 v Year 2	Year 2 v Year 3
$\beta_{LP}$	<.0001	<.0001	<.0001	0.1052
$\beta_{FA}$	<.0001	0.0002	0.0665	<.0001
$\beta_{TR}$	0.078	0.0119	0.5941	0.0001
$\beta_{TP}$	0.0005	<.0001	<.0001	<.0001
$\delta$	<.0001	<.0001	0.4812	0.0002

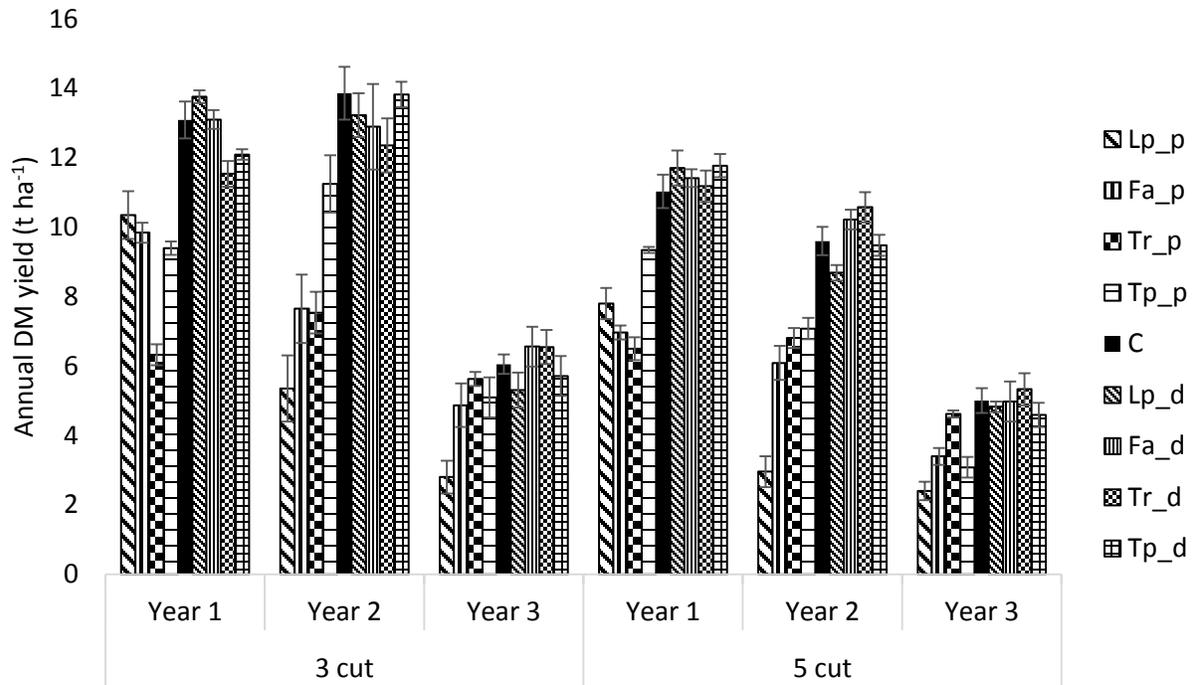
**Table 2.** Parameter estimates for the pairwise species interaction effects from Model 1. Estimates that were significant at  $P < 0.05$  are bolded.

Parameter	3 cut system			5 cut system		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
$\delta_{LP \times FA}$	<b>6.62</b>	<b>14.86</b>	<b>5.13</b>	<b>6.58</b>	<b>8.96</b>	<b>5.65</b>
$\delta_{LP \times TR}$	<b>8.69</b>	<b>12.45</b>	2.92	<b>6.73</b>	<b>8.69</b>	3.56
$\delta_{LP \times TP}$	2.77	<b>9.34</b>	0.20	1.66	2.35	<b>5.50</b>
$\delta_{FA \times TR}$	<b>6.80</b>	4.23	3.42	<b>7.62</b>	7.59	-0.81
$\delta_{FA \times TP}$	0.88	1.12	0.70	2.55	1.25	1.13
$\delta_{TR \times TP}$	2.95	-1.29	-1.51	2.69	0.98	-0.96

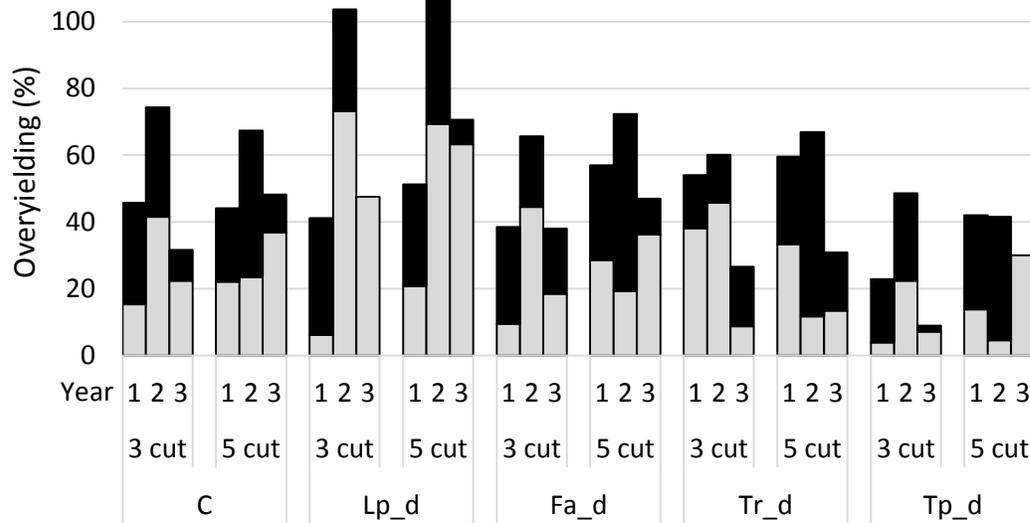
**Table 3.** Winter damage in pure stands recorded as a visual assessment of the percentage of dead plants after initial spring growth. Average  $\pm$  S.E. Lp, *L. perenne*; Fa, *F. arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*; \_p, pure stand.

Year	Cutting system	N	Lp_p	Fa_p	Tr_p	Tp_p
2011	-	8	1 $\pm$ 1	1 $\pm$ 1	33 $\pm$ 8	9 $\pm$ 1
2012	3 cut	4	0 $\pm$ 0	0 $\pm$ 0	8 $\pm$ 1	11 $\pm$ 1
	5 cut	4	0 $\pm$ 0	0 $\pm$ 0	16 $\pm$ 2	33 $\pm$ 7
2013	3 cut	4	21 $\pm$ 10 <sup>1</sup>	1 $\pm$ 1	44 $\pm$ 15 <sup>1</sup>	74 $\pm$ 6
	5 cut	4	15 $\pm$ 4	10 $\pm$ 10 <sup>1</sup>	45 $\pm$ 4	35 $\pm$ 5

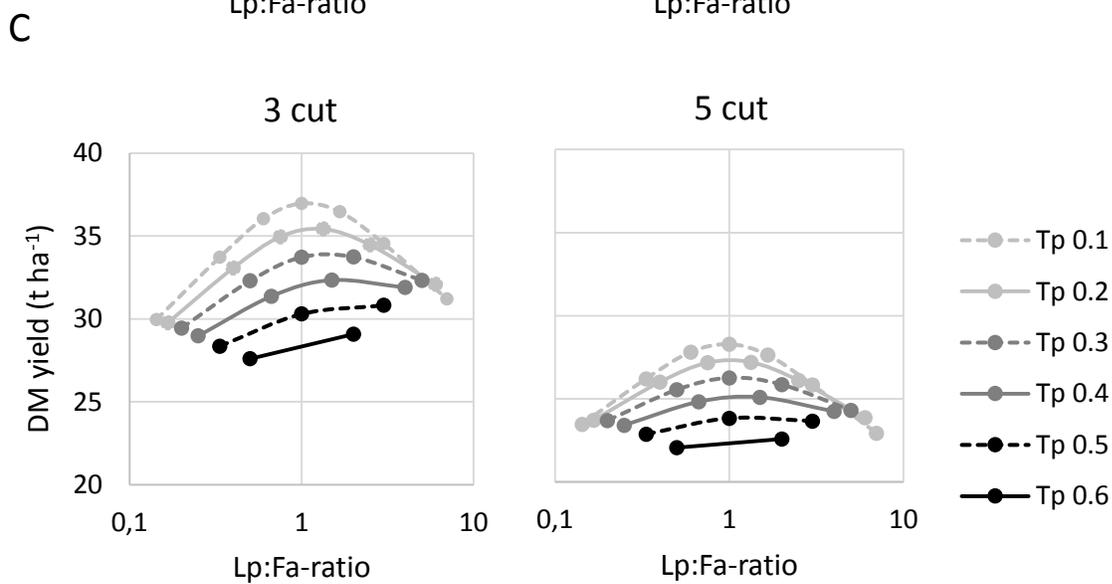
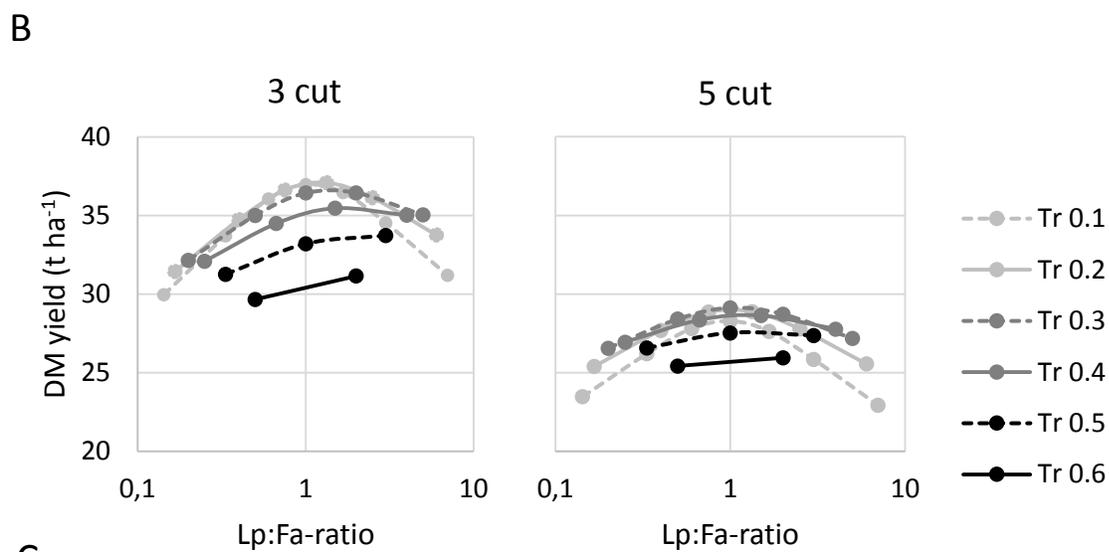
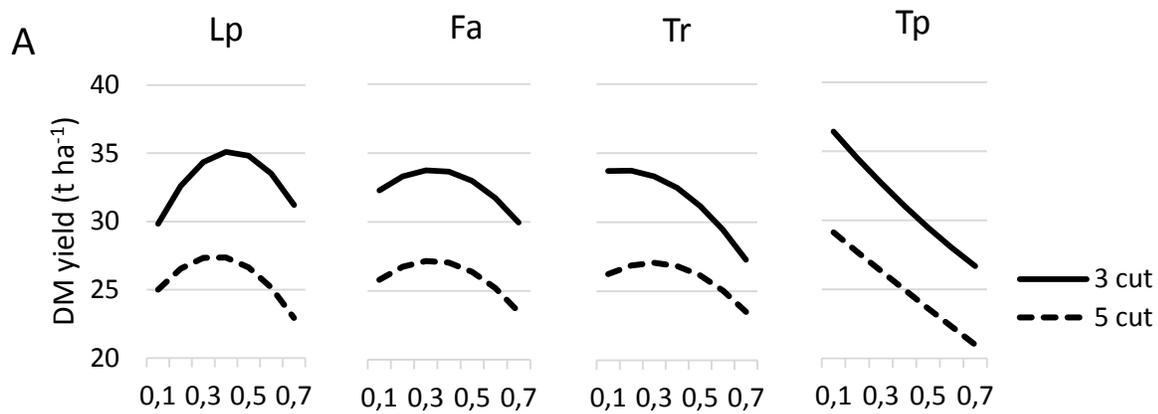
<sup>1</sup> Large variation due to the fact that one of the plots was located in an area with a high level of winter damage apparently due to accumulation of water and ice.



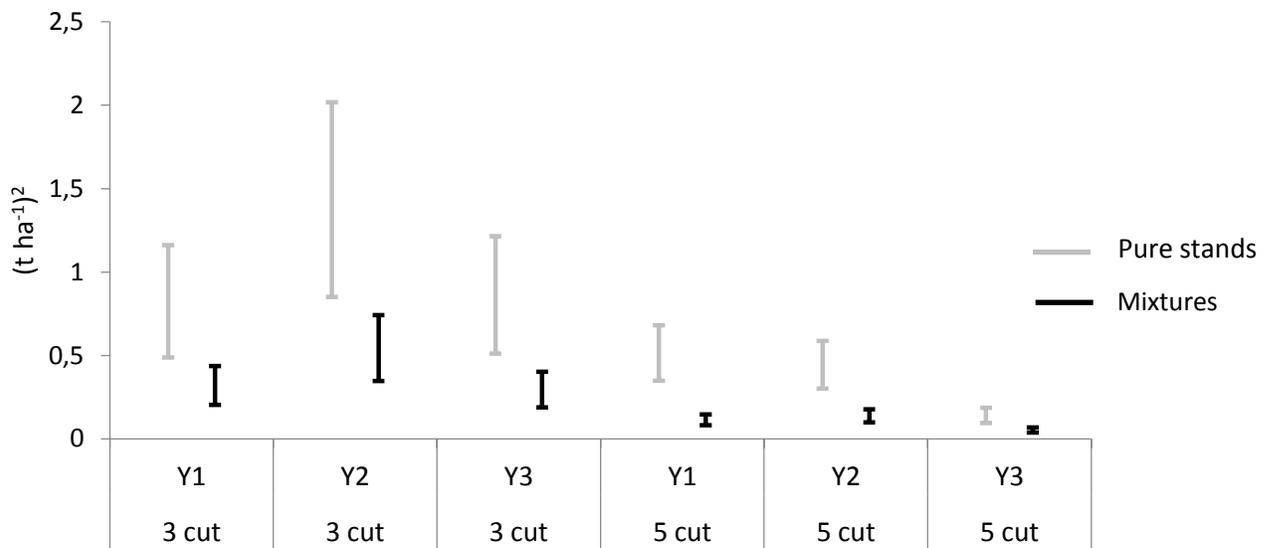
**Figure 1.** Dry matter yield of nine sown species compositions in two cutting systems (3 or 5 cuts per year) during three years. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*; \_p, pure stand; C, centroid (25 % seed weight of each species sown); \_d, dominated (67 % of the indicated species, 11 % of each of the three other species sown). Averages  $\pm$  S.E. are shown (N=4).



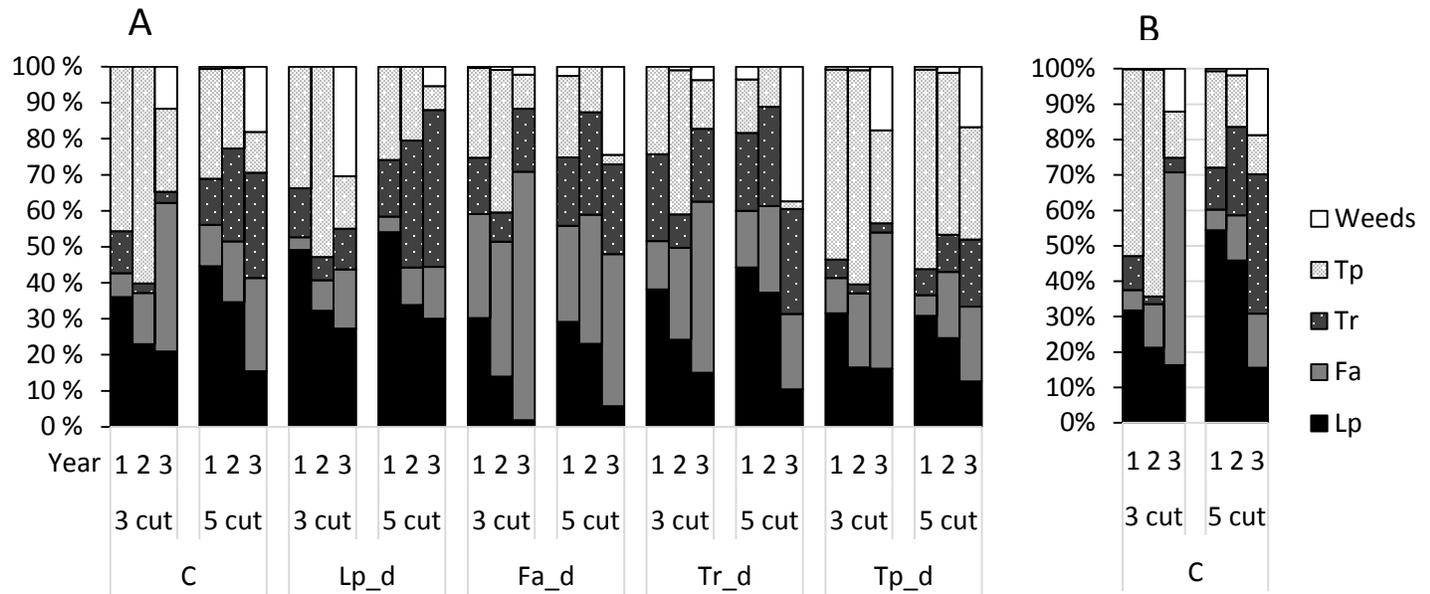
**Figure 2.** Overyielding in mixtures expressed as the percent increase in DM yield relative to what would be expected from the species proportions and pure stand yields in two cutting systems and three years. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*; C, centroid (25 % seed weight of each species sown); d, mixtures with 67 % of the indicated species and 11 % of each of the three other species sown. The portion of the overyielding that is transgressive (higher than the best-performing species in pure stand) is shown in black.



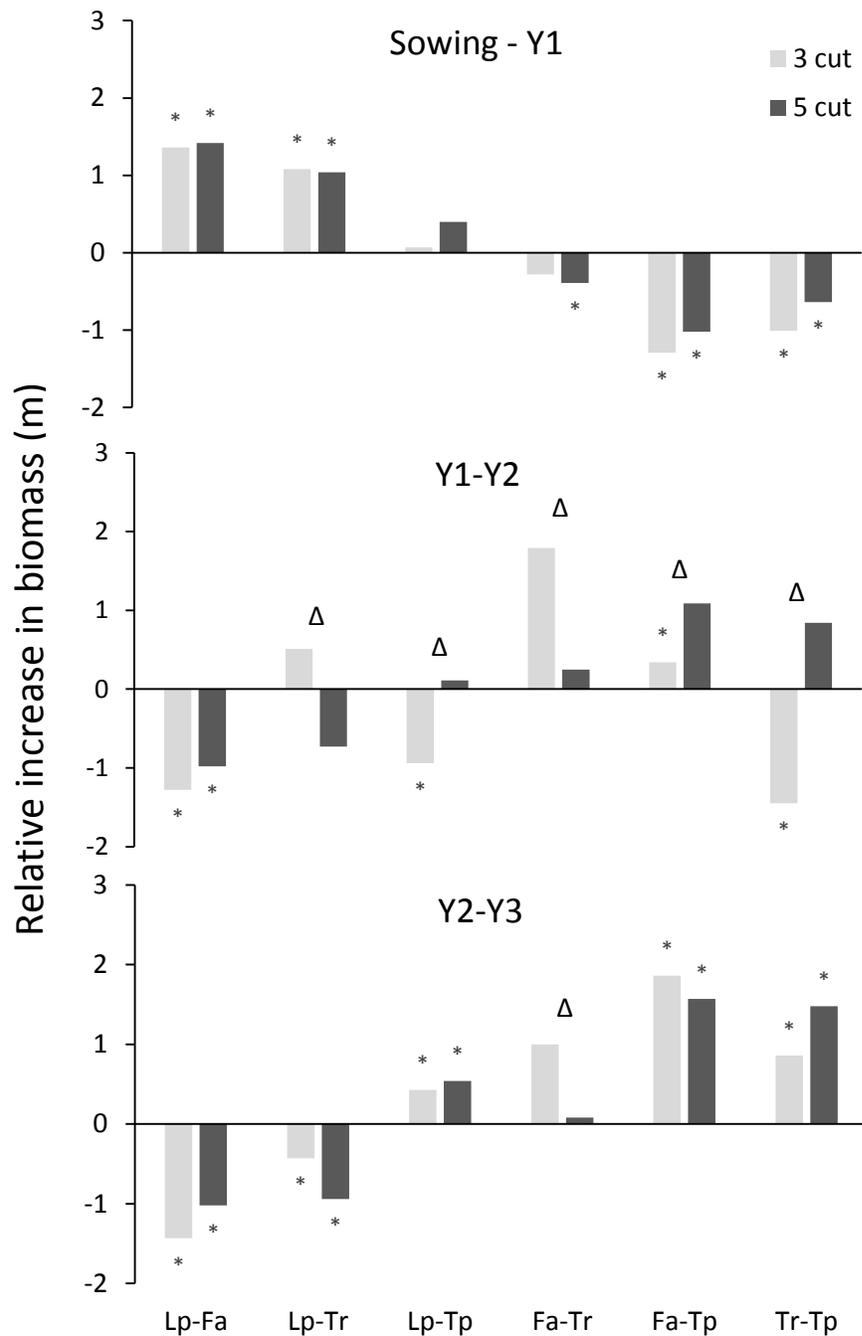
**Figure 3.** Estimation of total dry matter yield accumulated over the three first years in a 3 cut and a 5 cut system as a response to sown species composition (proportions of seed weight), using estimated species identity and species interaction coefficients for each species pair, cutting system and year (see Table 3). Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*. A) The proportion of the indicated species was varied from 0.1 to 0.7, keeping the ratios between the three other species constant at 1:1:1. B) and C) The proportion of Tp or Tr was kept at 0.1 while the proportion of the other legume was varied from 0.1 to 0.6. The ratio between Lp and Fa (x-axis, logarithmic scale) was varied within each level of Tr or Tp proportion.



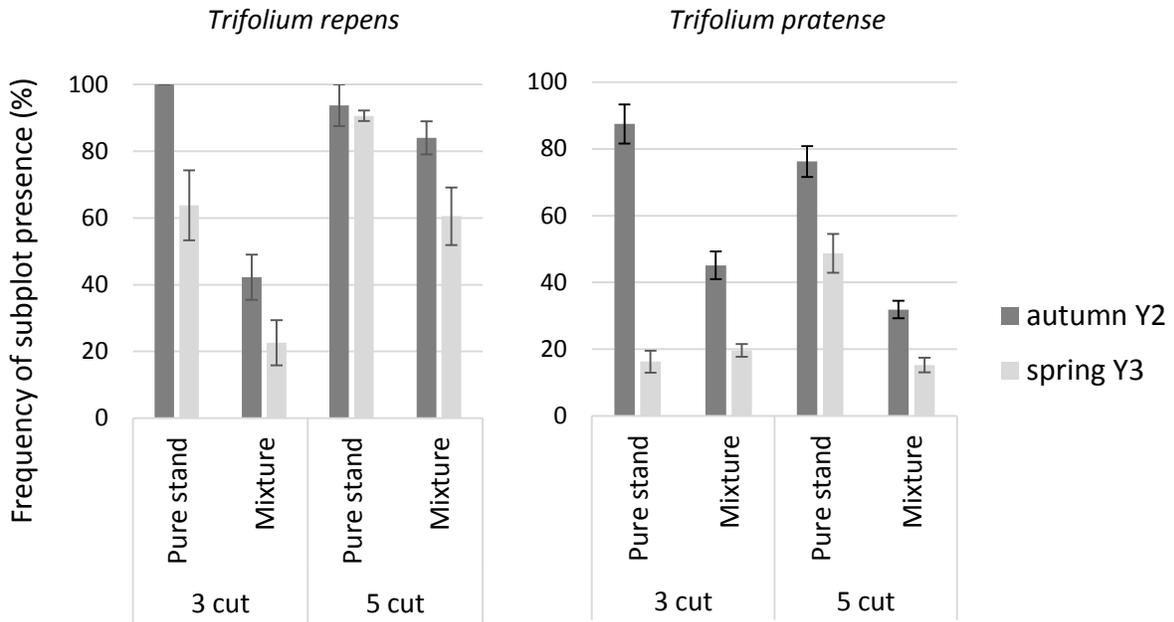
**Figure 4.** Confidence intervals of the mean (95 %) for the intra-annual variability in dry matter yield between harvests within years in two cutting systems during three years. The intra-annual variability was estimated as variance components were estimated from Model 3.



**Figure 5.** Realised species proportions in annual dry matter yield in three harvesting years and two cutting systems. The data shown are weighed averages of all harvests within a year. A, species proportions were assessed visually just prior to harvest. B, samples were separated into species fractions which were dried and weighed. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*; C, centroid (25 % seed weight of each species sown); \_d, mixtures with 67 % of the indicated species and 11 % of each of the three other species sown. N=4.



**Figure 6.** The relative abilities of the sown species to increase their annual dry matter yield from one year to the next (coefficient  $m$  in Model 3), based on visual assessment of botanical composition prior to harvesting. Each annual time interval and cutting system were analysed separately. Positive values indicate that the former species had an advantage over the latter species, negative values indicate the opposite. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*; \*, significant effects ( $P < 0.05$ );  $\Delta$ , significant difference between cutting systems ( $P < 0.05$ ).



**Figure 7.** Frequency of subplot presence of *Trifolium repens* and *T. pratense* before and after the third winter after establishment in two cutting systems in pure stands (N=4 plots) and species mixtures of *Lolium prene*, *Festuca arundinacea*, *Trifolium repens* and *T. pratense* (N=20 plots). Averages  $\pm$  S.E. are shown.

## Supplementary information

**Table S1.** Temperature, precipitation and soil moisture conditions during the growth periods of the 3 cut regime. The first growth was assumed to start at the first passage after April 1<sup>st</sup> of the seven day running mean temperature of 5 °C. The temperature index (TI) for plant growth was calculated according to Torssell and Kornher (1983). The soil moisture index (SMI) is the ratio between actual and potential evapotranspiration from plants calculated on a daily basis according to Skjelvåg (1982). The temperature levels were about 10 °C for the first growth, mostly somewhat lower than 15 °C for the second and the third growths; though with 12.5 during the drier period of the second growth in 2012, and with 16.0 during the drier period of the third growth in 2013. Otherwise the soil moisture supply has been sufficient, that is SMI = 1.00, of this Retic Stagnasol, with 137 mm plant available water down to 65 cm depth, except for the first growth in 2011. From the day of the third cut until growth cessation, defined as the day of first passage of seven day running mean temperature of 5 °C in autumn, air temperature varied from 8.1 to 9.4 °C, the TI index from 0.46 to 0.58, and the soil moisture index SMI = 1.00 during three seasons.

Year	First growth				Second growth				Third growth			
	°C	TI	mm	SMI	°C	TI	mm	SMI	°C	TI	mm	SMI
2011	9.7	0.61	95	0.93	15.1	0.94	143	1.00	14.5	0.91	468	1.00
2012	10.5	0.59	92	1.00	12.5	0.83	76	0.71	14.8	0.94	209	1.00
2013	10.9	0.65	153	1.00	14.9	0.95	124	1.00	16.0	0.97	82	0.77

**Table S2.** Harvest dates

Cutting system	Harvest	Year		
		2011	2012	2013
3 cut	1	30.05	29.05	11.06
	2	06.07	03.07	16.07
	3	22.09	10.09	09.09
5 cut	1	10.05	21.05	31.05
	2	08.06	11.06	18.06
	3	28.06	05.07	18.07
	4	03.08	07.08	14.08
	5	15.09	17.09	12.09

**Table S3.** Predicted  $\mu$  effects (the relative abilities of two species to increase DM yield) from the RGRD model based on A) visual assessment (weighted average of all harvests) and B) dry weights of separated species fractions (weighted average of first and last harvest). A positive value indicates that the former species has a competitive advantage over the latter species. A negative value indicates the opposite. Significant effects ( $P < 0.05$ ) are in bold. An asterisk indicates that the coefficient differs significantly between the two cutting systems. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens*; Tp, *T. pratense*.

A)

	Sowing - Year1		Year 1 - Year 2			Year 2 - Year 3	
	3 cut	5 cut	3 cut	5 cut		3 cut	5 cut
Lp-Fa	<b>1.36</b>	<b>1.42</b>	<b>-1.28</b>	<b>-0.98</b>		<b>-1.43</b>	<b>-1.02</b>
Tr-Fa	0.28	<b>0.39</b>	<b>-1.79</b>	-0.25	*	-1.00	-0.08
Tp-Fa	<b>1.29</b>	<b>1.02</b>	<b>-0.34</b>	<b>-1.09</b>	*	<b>-1.86</b>	<b>-1.57</b>
Weed-Fa			<b>0.29</b>	-1.65	*	<b>3.06</b>	<b>4.42</b>
Tr-Lp	<b>-1.08</b>	<b>-1.04</b>	<b>-0.51</b>	0.73	*	<b>0.43</b>	<b>0.94</b>
Tp-Lp	-0.07	-0.40	<b>0.94</b>	<b>-0.11</b>	*	<b>-0.43</b>	<b>-0.54</b>
Weed-Lp			1.57	<b>-0.67</b>	*	<b>4.49</b>	<b>5.45</b>
Tp-Tr	<b>1.01</b>	<b>0.64</b>	<b>1.45</b>	<b>-0.84</b>	*	<b>-0.86</b>	<b>-1.48</b>
Weed-Tr			<b>2.08</b>	-1.40	*	<b>4.06</b>	<b>4.51</b>
Weed-Tp			0.63	<b>-0.56</b>		<b>4.92</b>	<b>5.99</b>

B)

	Sowing - Year1		Year 1 - Year 2			Year 2 - Year 3	
	3 cut	5 cut	3 cut	5 cut		3 cut	5 cut
Lp-Fa	<b>1.43</b>	<b>1.82</b>	<b>-1.14</b>	<b>-0.58</b>	*	<b>-2.02</b>	<b>-2.36</b>
Lp-Fa	0.00	<b>0.84</b>	<b>-2.02</b>	-0.47	*	-0.58	-0.36
Tp-Fa	<b>1.73</b>	<b>1.64</b>	<b>-0.46</b>	<b>-1.63</b>	*	<b>-2.80</b>	<b>-1.36</b>
Weed-Fa			<b>-0.76</b>	0.29	*	<b>1.34</b>	<b>1.92</b>
Tr-Lp	<b>-1.43</b>	<b>-0.98</b>	<b>-0.87</b>	0.11	*	<b>1.44</b>	<b>1.99</b>
Tp-Lp	0.31	-0.18	<b>0.68</b>	<b>-1.05</b>	*	<b>-0.78</b>	<b>1.00</b>
Weed-Lp			0.38	<b>0.88</b>		<b>3.36</b>	<b>4.27</b>
Tp-Tr	<b>1.73</b>	<b>0.81</b>	<b>1.55</b>	<b>-1.16</b>	*	<b>-2.22</b>	<b>-1.00</b>
Weed-Tr			<b>1.25</b>	0.77		<b>1.92</b>	<b>2.28</b>
Weed-Tp			-0.30	<b>1.93</b>	*	<b>4.14</b>	<b>3.28</b>

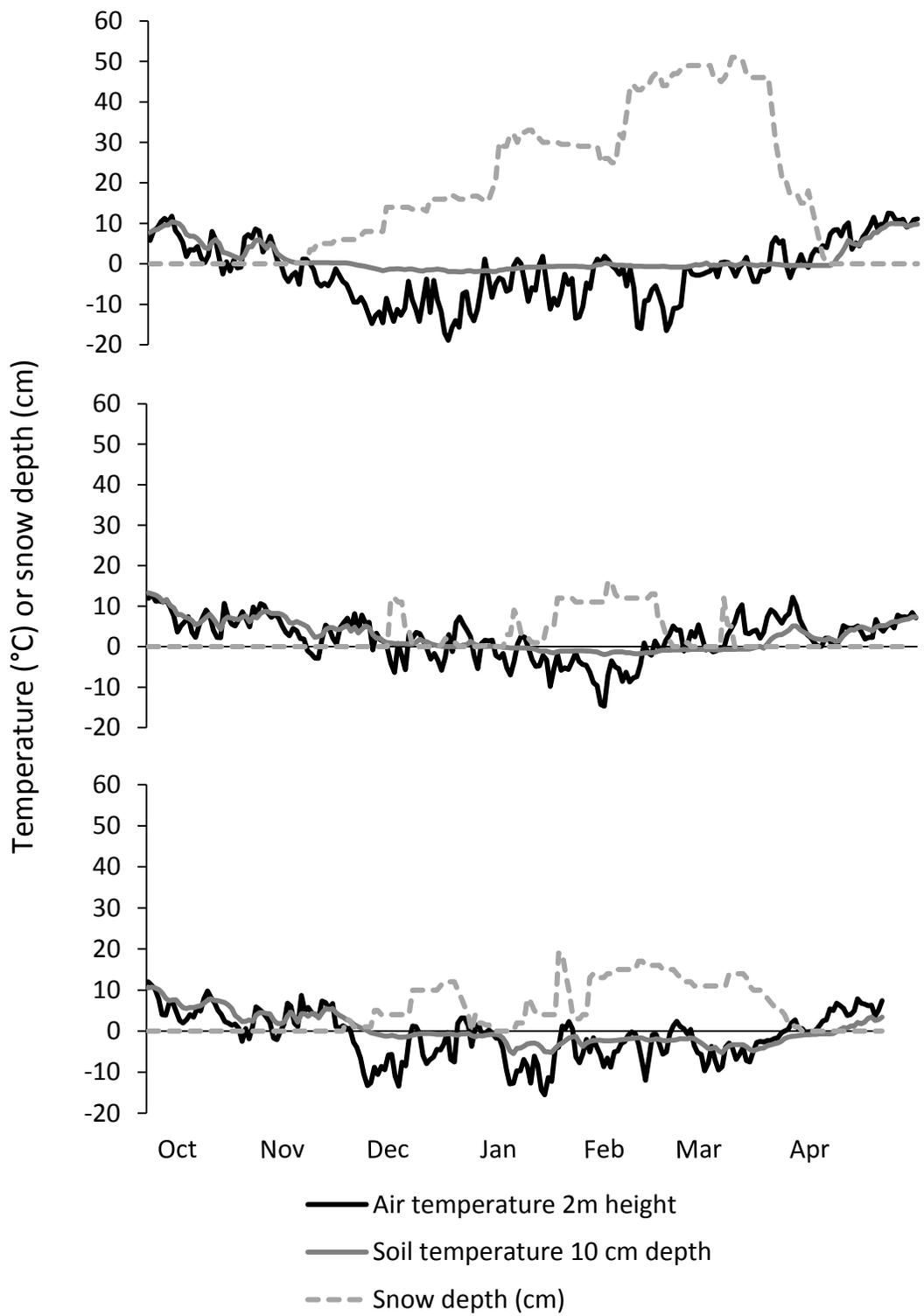
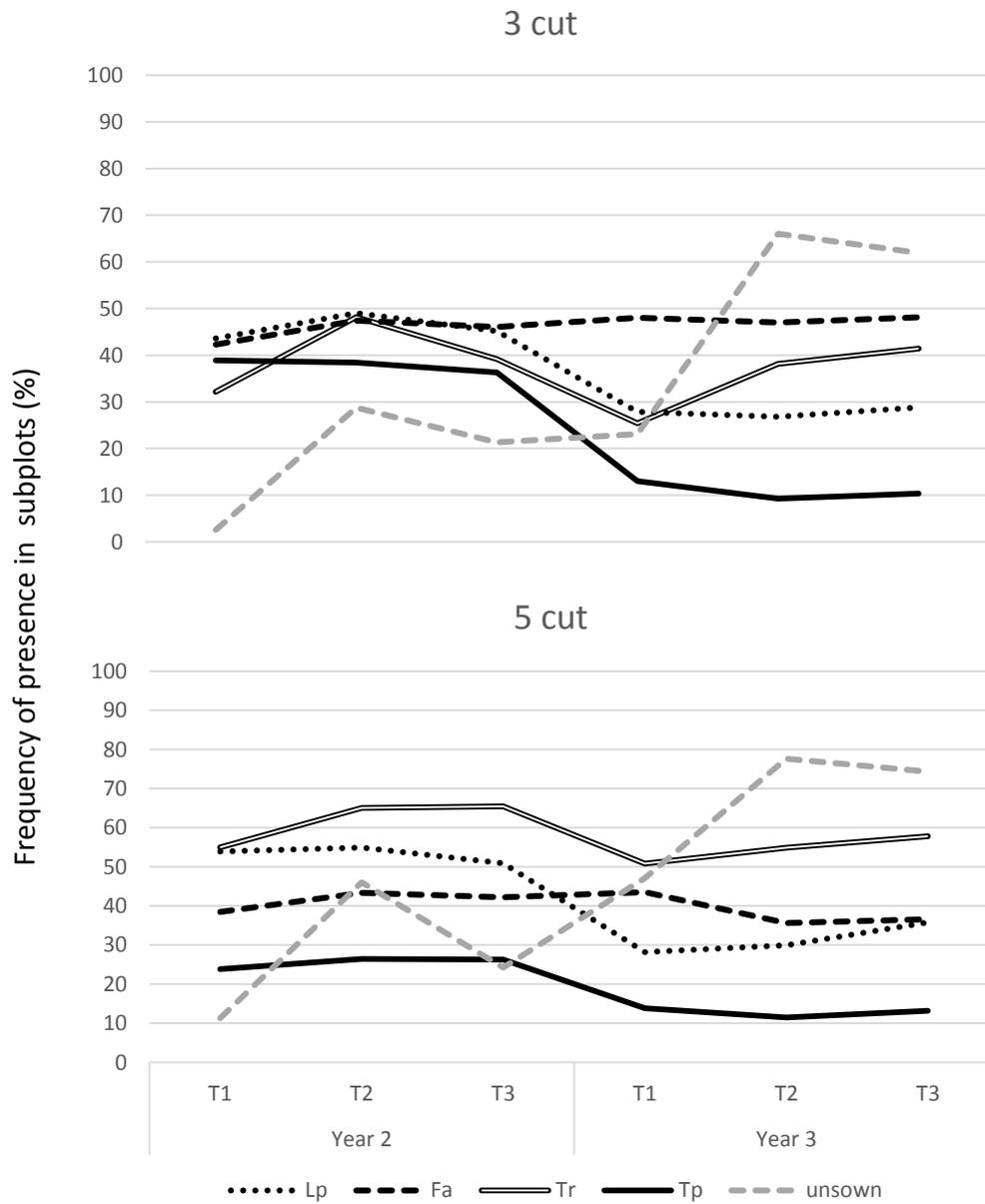
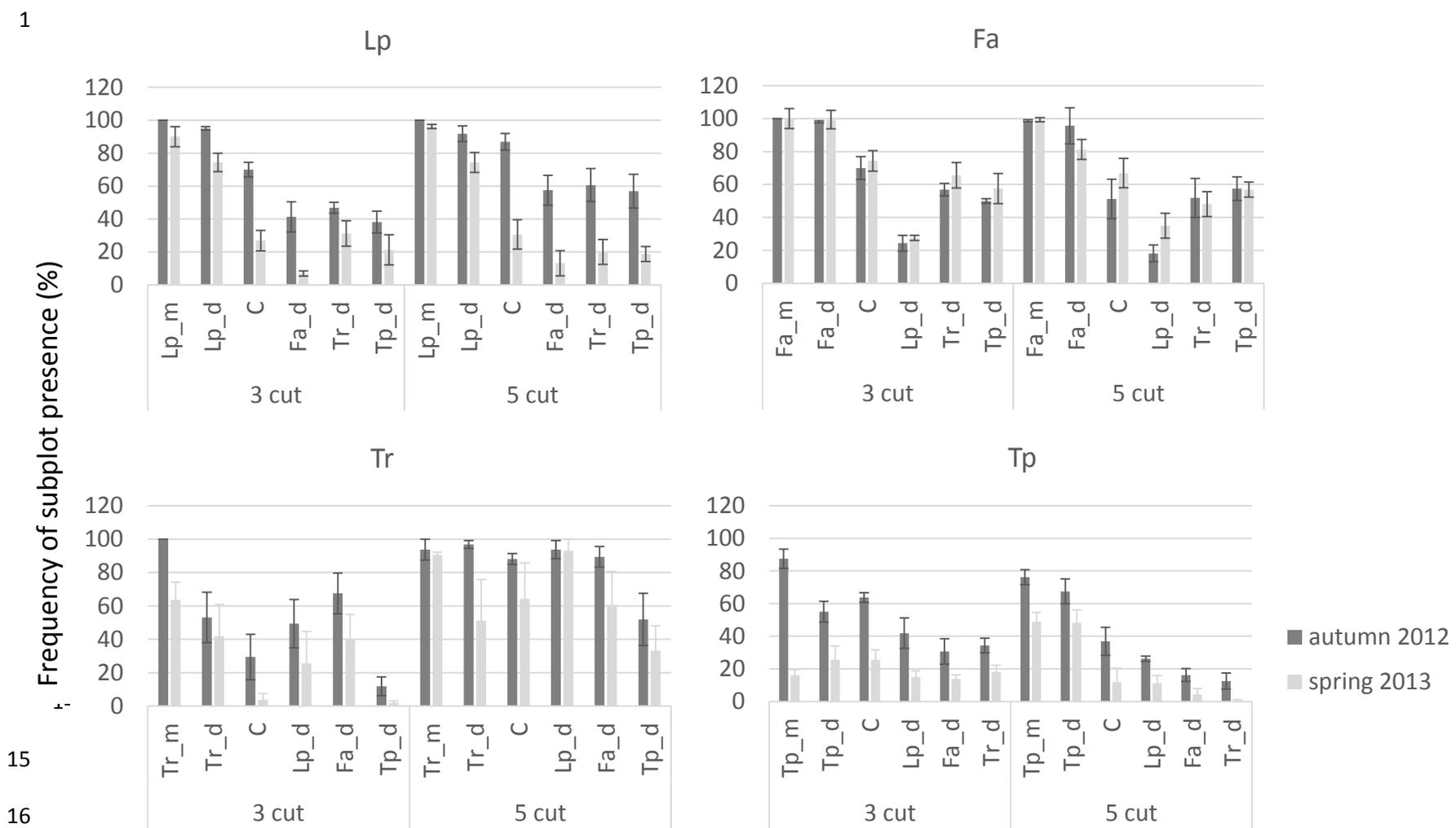


Fig. S1. Winter climate.



**Fig. S2.** Species abundance recorded as the frequency of presence (%) in forty 10x10 cm subplots along the sown rows in year 2 and 3. Averages across all plots in each cutting system (N=36). T1, in spring; T2, 1-2 weeks after the middle harvest; T3, 1-3 weeks after the last harvest. Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Tr, *Trifolium repens* and Tp, *T. pratense*.



17 **Fig. S2.** Frequency of subplot presence of *Lolium perenne* (Lp), *Festuca arundinacea* (Fa), *Trifolium repens* (Tr) and *T. pratense* (Tp) before and  
 18 after the third winter after establishment in pure stands and mixtures in a 3 cut system (dark grey) and a 5 cut system (light grey). \_p, pure  
 19 stand; C, centroid (25 % seed weight of each species sown); \_d, dominated (67 % of the indicated species, 11 % of each of the three other  
 20 species sown). Averages of four plots  $\pm$  S.E. are shown.

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