Diversity promotes production of ryegrass-clover leys through inclusion of competitive forb species

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Abstract

Highly productive temporary grasslands in Europe are usually composed of only a few plant species, and are typically dominated by perennial ryegrass-clover mixtures. Including additional competitive forb species holds potential for enhancing productivity in temporary grasslands, but requires further demonstration. In a grassland biodiversity experiment, one or all of the three forb species: chicory (*Cichorium intybus* L.), caraway (*Carum carvi* L.) and plantain (*Plantago lanceolata* L.), were grown in different proportions with the perennial ryegrass (*Lolium perenne* L.)-red clover (*Trifolium pratense* L.) mixture under two slurry application levels (0 and 250 kg total N ha^{-1} $year^{-1}$). Dry matter (DM) yield and botanical composition were determined in 2014 and 2015. Results showed that plantain-containing mixtures significantly increased DM yield by on average 9.5% (20% plantain in seed mixture) to 13.6% (60% plantain) compared to the ryegrass-clover mixture, while other mixtures with forb species produced yields similar to that of the ryegrass-clover mixture. These effects were independent of slurry application and consistent over two years. Moreover, plantain-containing mixtures produced higher yield than chicory- and caraway-containing mixtures, through greater biomass of plantain and/or complementary effects on red clover. These findings firstly demonstrate that increasing species diversity through including certain competitive forbs promotes production of ryegrass-clover mixtures.

Keywords: plant diversity, forb, *Plantago lanceolata*, competition, dry matter yield, fertilization

Introduction

Plant productivity is often found to increase with increasing plant species/functional groups in natural ecosystems (Cardinale *et al.*, 2012).Thus, there is growing interest in exploring the role of crop diversity in enhancing agricultural productivity and sustainability (Tilman *et al.*, 2002). In European temporary grasslands, there are usually only a few crop species, which are dominantly by perennial ryegrass, red clover and white clover (*Trifolium repens* L.), since they can complement N use and produce high yield (Rasmussen *et al.*, 2012). Recent studies show that three forb species (chicory, caraway and plantain) are very competitive when grown with ryegrass-clover mixtures, providing the potential of further improving the productivity of the latter (Søegaard *et al.*, 2011). Therefore, this study aimed to experimentally examine the potential of these three forbs, individually and together, when grown with ryegrass-clover mixtures and whether the potential depends upon slurry application level. We hypothesize that inclusion of these competitive forbs will improve or maintain the production of ryegrass-clover leys, depending on the competitiveness of the forb species.

Materials and methods

A field experiment with three replicates was established in spring 2013. In each replicate, two levels of cattle slurry (0, 250 kg total N ha^{-1} $year^{-1}$) and ten seed mixtures were arranged in a two-way factorial design. The seed mixtures consisted of the perennial ryegrass-red clover mixture either alone or grown with different proportions of one or all of the three forb species (Table 1). Seed rates of each species in a mixture were calculated by multiplying their seed rates in pure stand (15, 4 and 12 kg ha^{-1} for perennial ryegrass, red clover and the three forbs, respectively) with proportions of the species in the mixture. Herbage biomass in the whole plot (1.5×8 m) was harvested four times (late May, early July, mid-August and early October) in 2014 and 2015 to determine annual dry matter (DM) yield. Botanical composition

Table 1. Plant species and their proportions in ten seed mixtures.

Plant species	GC	20CI	20CA	20PL	60CI	60CA	60PL	20CCP	60CCP	80CCP
	% of plant species in seed mixtures									
Ryegrass (GR)	50	40	40	40	20	20	20	40	20	10
Red clover (RC)	50	40	40	40	20	20	20	40	20	10
Chicory (CI)		20			60			6.7	20	26.7
Caraway (CA)			20			60		6.7	20	26.7
Plantain (PL)				20			60	6.7	20	26.7

of the mixtures was determined by hand separation of sub-samples into the five sown species and weeds. Annual DM yield over four harvests was analysed statistically using a linear mixed-effects model with replicate and plot as random effects, and with seed mixture, slurry application and experimental year as fixed effects. Differences between factor levels were tested using Tukey's *post hoc* test. All analyses were performed using the R software version 3.2.2.

Results and discussion

Annual herbage DM yield varied significantly ($P<0.001$) in the ten seed mixtures, showing a consistent pattern across two years (Mixture × Year: $P=0.13$) and across two slurry levels (Mixture × Slurry: $P=0.15$) (Figure 1). Compared to the ryegrass-red clover mixture (GC), inclusion of 20 or 60% plantain (20PL or 60PL) in seed mixtures increased DM yield by on average 9.5 or 13.6%, respectively, while other mixtures with one forb (chicory or caraway) or with all three forbs, produced similar DM yield. These results indicate that the role of crop diversity in promoting production of ryegrass-red clover mixture depends on forb species. Plantain-containing mixtures enhanced DM yield mainly because of larger biomass of plantain than caraway in both years (Figure 1) and chicory in 2015 (Figure 1C and 1D). Although chicory had comparably large biomass as plantain in 2014 (Figure 1A and 1B), the yield of red clover in chicory-containing mixtures was significantly lower than that *in planta*in-containing mixtures, suggesting that niche complementarity may occur between plantain and red clover.

The positive effect of plantain on red clover may be attributed to rosette leaves of plantain that stay close to the ground, allowing more light to be intercepted by red clover, whereas chicory may have strong competition for light on red clover through its large leaf area (Søegaard *et al.*, 2013). Red clover can in turn facilitate the growth of plantain by providing biologically fixed nitrogen (N) to plantain through rhizodeposition or mycorrhizal networks (Pirhofer-Walzl *et al.*, 2012). Indeed, N was a limiting factor in this study, as evidenced by enhanced annual DM yield (+9%) under slurry application (Figure 1). Slurry application significantly increased DM yield of ryegrass ($P<0.001$), did not or slightly increase yield of all the three forbs, but significantly decreased yield of red clover ($P<0.001$).

Recent studies have shown that forbs (chicory, caraway and plantain) produced higher mineral concentrations (e.g. Zinc) than grasses and legumes (Pirhofer-Walzl *et al.,* 2011), suggesting that including forbs in ryegrass-clover mixtures can not only enhance herbage production but also improve animal nutrition by providing sufficient dietary mineral supply to ruminants.

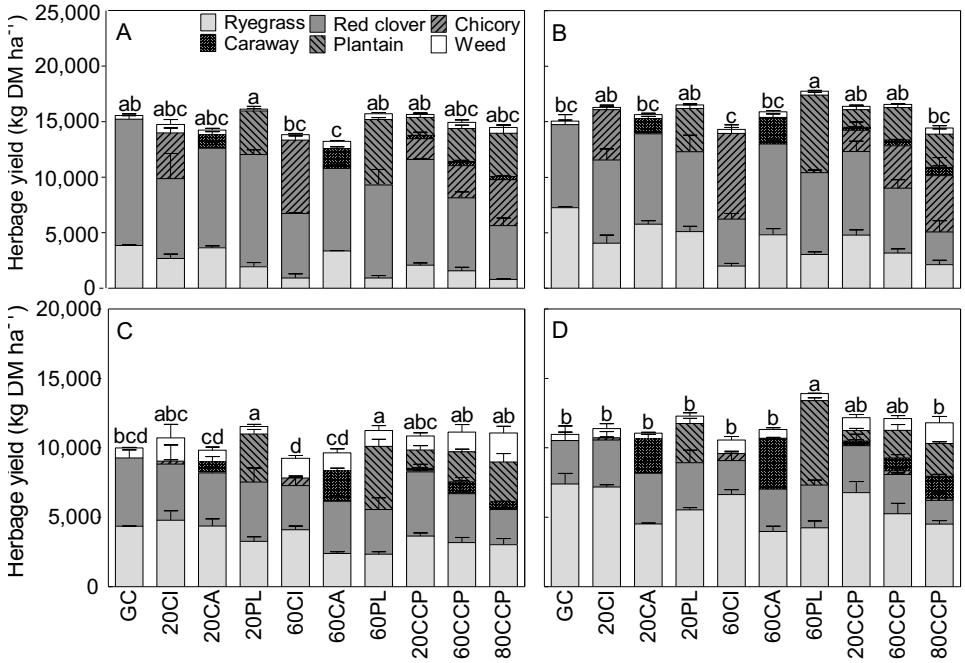

Figure 1. Annual dry matter (DM) yield in ten seed mixtures under two slurry application levels across two years. (A) Without slurry in 2014; (B) with slurry in 2014; (C) without slurry in 2015; (D) with slurry in 2015. Data are means ± standard deviation for each component species (n=3). Abbreviations of ten mixtures in the X-axis refer to Table 1. Means with different lowercase letters are significantly different ($P<0.05$) using Tukey's *post hoc* test.

Conclusions

We firstly demonstrate that including an additional functional group (i.e. forb) in traditional high-producing ryegrass-red clover mixtures improves or maintains productivity, depending on the competitiveness of specific forb species. Plantain-containing mixtures improve productivity, which can be a promising strategy for enhancing agricultural productivity and forage quality in European temporary grasslands.

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