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Species interactions between forbs and grass-clover contribute to yield gains and weed suppression in forage grassland mixtures

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ABSTRACT

Grass-legume mixtures characterized by high productivity, efficient nitrogen (N) use and strong weed suppression were proposed to increase sustainability of grassland production. Adding competitive forbs to grass-clover mixtures can be expected to further enhance the high productivity of grass-clover mixtures. This study investigates whether and to what extent adding forbs to a grass-clover mixture results in yield gains, and estimates the contributions of forb species (identity effect) and interactions with the grass-clover mixture (diversity effect) to these gains. It further examines the effectiveness of weed suppression in forb-containing grass-clover mixtures. Fifteen pure and mixed stands, all comprising the grass *Lolium perenne* L. and the legume *Trifolium pratense* L., and one or three competitive forb species (*Cichorium intybus* L., *Carum carvi* L., *Plantago lanceolata* L.) were established in a two-year field experiment and fertilised at two levels of N application. The diversity effect between *P. lanceolata* and the *L. perenne-T. pratense* mixture and a strong identity effect of *P. lanceolata* jointly contributed to yield gains of 10–21% over the binary L. *perenne-T. pratense* reference mixture for a wide range of *P. lanceolata* proportions across years and N fertilisation levels. In contrast, comparably smaller diversity and/or identity effects of *C. intybus* and *C. carvi* resulted in maximal yield gains of 5% and 7%, respectively, occurring at a narrower proportional range, but also in yield losses at high forb proportion. Including up to 80% forb in the L. *perenne-T. pratense* mixture effectively maintained weed suppression, but the degree of weed suppression decreased with increasing forb proportion in the following year. Including selected forbs, especially *P. lanceolata*, in productive grass-clover mixtures is promising for further enhancing productivity, meanwhile effectively suppressing short-term weed growth in intensively managed grasslands.

1. Introduction

Intensively managed agricultural grasslands play a key role in producing high yields of quality forage. Traditionally, these grasslands are dominated by monocultures of grass species with large inputs of mineral fertilisers, at the expense of high energy consumption and negative environmental impacts (Taube et al., 2014). Selecting species with complementary functional traits for mixtures has recently been promoted as a promising strategy to enhance agricultural productivity and sustainability, while providing other ecosystem services (Isbell et al., 2017; Lüscher et al., 2014). Indeed, a number of studies have demonstrated that combining grass and legume species in intensively managed grasslands can often lead to transgressive overyielding (i.e. higher yield in mixture than in the best-performing monoculture) (Finn et al., 2013; Nyfeler et al., 2009), largely through complementary use of nitrogen (N) derived from the soil and the atmosphere (Nyfeler et al., 2011). However, it remains unclear whether high productivity in intensively managed grass-legume mixtures can be further enhanced by including additional functional groups of species, such as forbs.

Three competitive forb species – *Cichorium intybus* L., *Carum carvi* L. and *Plantago lanceolata*. L. – have been found to establish well in intensively managed grassland mixtures (Elgersma et al., 2014; Li and Kemp, 2005). The three forbs share several common traits such as deep root systems and positive response to N fertilisation, whereas they differ in above- and below-ground architecture. For example, *C. intybus* and *C. carvi* have thick taproots, while *P. lanceolata* has a dense, adventitious root system (Cong et al., 2017; Hoekstra et al., 2015). Architecturally different traits may lead to different interactions of forbs with grass and legume species, consequently influencing the capture of soil resources and biomass production. Indeed, our recent work from a two-year grassland diversity experiment showed that inclusion of *P. lanceolata* enhanced annual herbage yield of *Lolium perenne* L.- *Trifolium pratense*

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L. mixtures, whereas inclusion of *C. intybus* or *C. carvi* produced, on average across all mixture types, similar yields as the *L. perenne-T. pratense* mixture (Cong et al., 2017). Yield differences between grass-clover-forb mixtures *vs.* grass-clover binary mixtures can be attributed to either diversity effects between forbs and grass-clover, and/or identity effects of forbs (i.e. yield in monoculture, see Lüscher et al., 2011); yet, the degree of diversity and/or identity effects that contribute to yield gains remains to be resolved. Moreover, results in Cong et al. (2017) were simply based upon two seed proportions (20% and 60%) of forbs sown in mixtures of *L. perenne* and *T. pratense*. It would be more relevant for designing optimal grassland mixtures if the range of forb proportion sown in grass-clover mixtures over which yield gains are evident could be quantified.

Weeds can cause substantial loss of forage yield and quality in intensively managed grasslands. Considerable empirical evidence has demonstrated that increasing species diversity, in particular by mixing grass and legume species, can suppress weed growth better than monocultures (Connolly et al., 2018; Lüscher et al., 2014; Sanderson et al., 2012). Recent studies have shown that growing the deep-rooting *C. intybus* with grass and legume species in four-species mixtures led to stronger weed suppression than averaged monocultures and attributed this diversity effect to increasing functional complementarity and resource capture (Frankow-Lindberg, 2012; Suter et al., 2017). This raises the question of whether including the above-mentioned competitive forbs with differing functional traits in grass-clover mixtures can maintain weed suppression compared to the grass-clover mixture, and whether this effect, if any, persists over years.

In this study, we estimate the contributions of diversity and identity effects to yield gains in forb-containing *L. perenne-T. pratense* mixtures over a reference binary *L. perenne-T. pratense* mixture (hereafter referred to as the "reference mixture"), evaluate suppressive effects on weed growth and test how yield gains and weed suppression are influenced by N fertilisation. Toward this goal, a grassland diversity experiment was established with monocultures and mixtures comprising *L. perenne*, *T. pratense* and the three forbs *C. intybus*, *C. carvi*, and *P. lanceolata*, and was maintained for two years (Cong et al., 2017).

We hypothesised that (1) diversity effects between forb species and the reference *L. perenne-T. pratense* mixture together with forb species' identity effects lead to yield gains in grass-clover-forb mixtures over the reference mixture; (2) the degree of yield gain depends on the forb species added to the reference mixture and the forb species' proportion; (3) N fertilisation weakens the positive effect of adding forbs to the reference mixture and thus reduces yield gains; (4) inclusion of each of the three forb species into the reference mixture maintains suppression of weed growth.

2. Materials and methods

2.1. Experimental site

The experiment was established in spring 2013, in a long-term organic dairy crop rotation at the Foulumgaard Experimental Station, Aarhus University, Denmark (56°29′44 N, 9°34′3 E). The preceding crop in 2012 was winter rye. The experimental site was situated on a loamy sandy soil, classified as a Typic Hapladult with 6.4% clay, 8.5% silt, 44% fine sand and 39% coarse sand. The soil had a pH of 5.9, and contained 2.0% organic carbon (C) and 0.17% total N. In the study years 2014 (year 1) and 2015 (year 2), the annual mean temperature was 9.5 and 8.6 °C, respectively, while annual precipitation was 853 and 904 mm.

2.2. Experimental design and management

A grassland experiment was set up with the experimental factors "species composition" and "N fertilisation" in a two-factorial design. Fifteen species compositions were established following a simplex design (Kirwan et al., 2009), comprising five monocultures (*Lolium perenne* L., cultivar (cv.) Stefani; *Trifolium pratense* L., cv. Rajah; *Cichorium intybus* L., cv. Spadona; *Carum carvi* L., cv. Volhouden; *Plantago lanceolata* L., wide type) and ten mixtures with different species richness and seed proportions (Table S1). Mixtures included a two-species *L. perenne-T. pratense* stand, stands with three species (*L. perenne*, *T. pratense* plus one of the three forbs in two different proportions of 20% and 60%) and stands with five species (*L. perenne*, *T. pratense* plus all three forbs in equal proportions of 6.7%, 20% or 26.7%). Two levels of N fertilisation in the form of cattle slurry, 0 and 250 kg total N ha^{-1} yr^{-1} (52% NH_4-N), were applied to all types of stands (hereafter abbreviated with N0 and N250, respectively). Given that the soils in this experiment were rich in phosphorus (24 mg Olsen-P kg^{-1} soil) and that all plots received 200 kg K ha^{-1} (K_2SO_4) to avoid potassium and sulphur deficiency, application of cattle slurry primarily created a large difference in soil N status between treatments. Cattle slurry was applied four times during the growing season to the N250 treatment in both production years; with 100 kg N ha^{-1} yr^{-1} at the beginning of the growing season (early April) and 50 kg N ha^{-1} yr^{-1} immediately after each of the first three harvests (see below). In dry periods of the growing season, 50–60 mm irrigation was applied to each plot.

Plots (8 m × 1.5 m) were established in a randomized complete block design with three replicates. In total, there were 90 plots (2 N levels × 15 species compositions × 3 replicates). Adjacent plots were separated by a 0.3 m buffer. In each plot, 10 rows of seeds (0.12 m row distance) were sown by machine. The seed rates in pure stands were 15, 4 and 12 kg ha^{-1} for *L. perenne*, *T. pratense* and each of the three forbs, respectively. The seed rate of each species in a mixture was calculated by multiplying the monocultures' seed rate with the seed proportion in the respective mixture (Table S1).

2.3. Measurements

A plot harvester (Haldrup C-85, Denmark) was used to cut the herbage at 7 cm stubble height. The harvests were performed two times (early July and early October) in the establishment year 2013 and four times (late May, early July, mid-August and early October) in the production years 2014 (year 1) and 2015 (year 2). This paper presents data from the two production years. In each harvest, ca. 500 g subsample was dried at 80 °C to constant weight to calculate herbage dry matter yield. Botanical composition of the mixtures was determined by separating ca. 300 g subsample into the five sown species and pooled unsown species (i.e. weeds).

2.4. Data analysis

The analysis of simplex designs is based upon multiple linear regression. Kirwan et al. (2009) have developed the diversity-interaction modelling framework to quantify both the effects of interspecific interactions and species identity on ecosystem function. Following this approach, we tested the effects of species composition and N fertilisation on total annual dry matter yield (y) using a linear model as follows:

$$
\begin{aligned}
y = {} & \beta_1 \mathrm{P}_{\mathrm{Lp}} + \beta_2 \mathrm{P}_{\mathrm{Tp}} + \beta_3 \mathrm{P}_{\mathrm{Ci}} + \beta_4 \mathrm{P}_{\mathrm{Cc}} + \beta_5 \mathrm{P}_{\mathrm{Pl}} + \delta_1 \mathrm{D}_{\mathrm{Lp.Tp}} + \delta_2 \mathrm{D}_{\mathrm{GL.Ci}} \\
& + \delta_3 \mathrm{D}_{\mathrm{GL.Cc}} + \delta_4 \mathrm{D}_{\mathrm{GL.Pl}} + \delta_5 \mathrm{D}_{\mathrm{FO.FO}} + \gamma_1 \mathrm{P}_{\mathrm{Lp}} \mathrm{N}_{\mathrm{Treat}} + \gamma_2 \mathrm{P}_{\mathrm{Tp}} \mathrm{N}_{\mathrm{Treat}} \\
& + \gamma_3 \mathrm{P}_{\mathrm{Ci}} \mathrm{N}_{\mathrm{Treat}} + \gamma_4 \mathrm{P}_{\mathrm{Cc}} \mathrm{N}_{\mathrm{Treat}} + \gamma_5 \mathrm{P}_{\mathrm{Pl}} \mathrm{N}_{\mathrm{Treat}} + \varepsilon \qquad (1)
\end{aligned}
$$

where coefficients β_i estimate the identity effect of plant species i (Lp: *L. perenne*, Tp: *T. pratense*, Ci: *C. intybus*, Cc: *C. carvi*, Pl: *P. lanceolata*). P_i is the proportion (relative abundance) of species i in a stand; consequently, if $\mathrm{P}_i = 1$, β_i measures the species' yield in monoculture. Coefficients δ_i estimate the strength of single or pooled pairwise interspecific interactions, termed diversity effects (D). The diversity effects D were defined to reveal specifically the pairwise interaction between Lp and Tp ($\mathrm{D}_{\mathrm{Lp.Tp}}$), the pooled pairwise interaction between either Lp or Tp and the forb Ci ($\mathrm{D}_{\mathrm{GL.Ci}}$), the pooled pairwise interactions between Lp or