Nitrogen fixation in red clover grown in multi-species mixtures with ryegrass, chicory, plantain and caraway

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Abstract

While many studies have investigated effects of species composition and management on N_2-fixation dynamics in simple clover-grass mixtures, there is a lack of knowledge about the performance of legumes and their N_2-fixation in more species-rich grassland including non-legume forbs. This study investigated N_2-fixation and total N yield in grassland mixtures including different combinations of red clover, perennial ryegrass and three deep rooted forbs: chicory, ribwort plantain and caraway, managed without and with slurry application. The percentage of clover N derived from atmosphere (%Ndfa) increased in mixtures, but was unaffected by inclusion of forbs. However, forbs affected the quantity of N_2-fixation and total N yield by affecting the red clover proportion in the mixtures. Mixtures composed of grass, clover and plantain or caraway showed higher N_2-fixation and N yield than with chicory included. Slurry application reduced N_2-fixation due to decreasing clover contents in the sampled biomass. The study highlighted differences among forbs in their influence on legume N_2-fixation and total N yield showing that forbs can be included in grasslands without negative effects on red clover performance.

Keywords: forb, grassland mixture, slurry, N_2-fixation, N yield

Introduction

Nitrogen is often the limiting nutrient for plant production, and the input of N from N_2 fixation in legume-rhizobia symbioses is widely considered as valuable sources of N in agroecosystem. Several studies have reported that plant species diversity of forage legume and non-legume in grasslands increases competition for available soil N, thereby stimulating legumes to increase their dependency on N_2-fixation and reducing the risks of losing N to the environment (Palmborg *et al.*, 2005; Rasmussen *et al.*, 2012). In addition, the introduction of non-legume forb in grasslands has been shown to increase the acquisition of nutrients from deep soil layers (Pirhofer-Walzl *et al.*, 2013) and improve forage quality (Pirhofer-Walzl *et al.*, 2011). The objective of this study was to determine how the inclusion of different forbs in different proportions in multi-species grasslands affects N_2-fixation in red clover and the total N yield of the mixtures.

Materials and methods

The experiment was conducted at Foulumgaard experimental station, Aarhus University, Denmark in a sandy loam soil with a cropping history including both grassland and arable crops. Sixteen seed mixtures (Figure 1) composed of different combinations of perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.) and three forbs: chicory (*Cichorium intybus* L.), ribwort plantain (*Plantago lanceolata* L.) and caraway (*Carum carvi* L.) were sown in 1.5×8 m plots in spring 2013, in a replacement design based on proportions of each species seeding rate in pure stand, which were 15, 4 and 12 kg ha^{-1} for ryegrass, red clover and forbs, respectively. Each mixture was exposed to 0 vs 250 kg N ha^{-1} in the form of cattle slurry applied in 2014 in three replicates. N_2-fixation was quantified using the 15N isotope dilution method (Rasmussen *et al.,* 2012) after labelling a 1×1 m subplot in each experimental plot with ammonium sulphate (0.1 g N m^{-2} and atom fraction 15N = 98%) in early spring 2014. The aboveground biomass was harvested to 5 cm stubble height in each subplot four times during the growing season

(May – October). The samples were sorted to individual species, dried, weighted and analysed for total N concentration and atom fraction 15N. The N_2-fixation was quantified based on excess atom fraction 15N (subtracting the background 15N measured in plant samples from unlabelled plots) in legumes and non-legumes, and total N yield was quantified based on N concentration and aboveground biomass yields of all species in each mixture in the subplot.

Results and discussion

The whole season biomass yield ranged from 4.5 to 15.8 Mg dry matter (DM) ha^{-1} without slurry, and from 7.4 to 16.7 Mg DM ha^{-1} with slurry application (data not shown). Mixtures of ryegrass, red clover and forbs produced almost as high biomass yields as did the pure stand of red clover and standard mixture of ryegrass and red clover. The proportion of red clover in the biomass samples ranged from 30-82% without and 30-61% with slurry application.

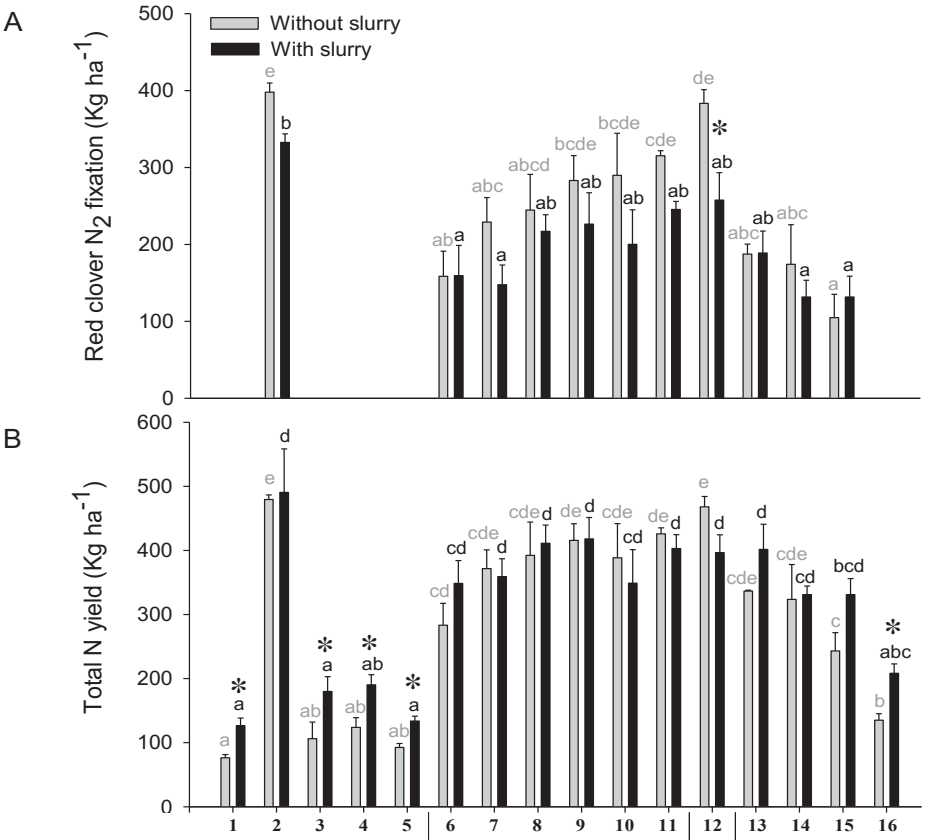

Figure 1. Whole-season N_2-fixation (A) and N yield (B) of above ground biomass without and with slurry application (mean ± standard error; n=3). '*' indicates a significant effect of slurry application, and different letters indicate significant differences within each slurry level ($P<0.05$). '*Std.*' refers to the standard mixture of perennial ryegrass and red clover.

Red clover derived a significantly (P<0.001) higher proportion of its N from N_2-fixation (%Ndfa) in mixtures than in pure stand. There was no significant difference in %Ndfa between mixtures, which was above 90% even in the treatments which received slurry application. On an annual basis, red clover fixed between 104 and 398 kg N ha^{-1} without and from 132 to 333 kg N ha^{-1} with slurry application. The amount of N_2 fixed was highest in the pure stand of red clover followed by the standard mixture of grass and clover (Figure 1A). Red clover in three species mixtures composed of grass, clover and plantain or caraway fixed nearly similar amounts of N_2 as did the red clover pure stand and the standard mixture. The slurry application decreased the amount of N_2 fixed in the majority of the mixtures, and this effect was most pronounced in the standard mixture (P<0.01). The total whole season N yield varied between 76 and 479 kg ha^{-1} without slurry and from 126 to 491 kg N ha^{-1} with slurry application, nearly following the pattern of biomass production. The majority of the mixtures obtained similar N yields as did the red clover pure stand and the standard mixture (Figure 1B). Slurry application increased total N yield in pure stands of non-legumes and mixture of three forb species (P<0.05).

The variations in the amounts of N_2-fixation and N yield in the mixtures were caused mainly by different proportions of red clover, as influenced by the competitiveness of the forbs for biomass production. Chicory and plantain showed better competitiveness than caraway in the mixtures. Without fertilisation, total N yield decreased with high proportions of chicory in the seeded mixture, which showed that the decrease in N_2-fixation was only partly counteracted by the high productivity of chicory. These findings suggested that choice of species and proportion of the forb component are important considerations when designing diversified leys for efficient N use.

Conclusions

Forbs can be included in grassland mixtures without affecting N_2-fixation and total N yield, provided that the mixture does not contain too high a proportion of chicory, as this forb is a strong competitor that might reduce clover yield and N_2-fixation. These findings show promising possibilities for the design and implement of multifunctional, multi-species grassland mixtures, i.e. ones that combine the valuable N inputs from legumes with efficient N uptake in grasses and deep-soil nutrient acquisition and high forage quality of forbs.

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