**Inclusion of caraway in the ryegrass-red clover mixture modifies soil microbial community composition**

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# INTRODUCTION

Perennial ryegrass-clover mixtures are widely used in intensively managed grasslands due to their high biomass production. Recent studies have shown that inclusion of competitive forbs such as plantain enhanced above-and below-ground biomass of ryegrass-red clover mixtures (Cong et al., 2016), while inclusion of forbs such as caraway in the grass-clover mixture largely maintained the herbage yield. Meanwhile, inclusion of forbs increases plant litter diversity, which could change root quality of the plant community. Hence, changes in quantity and quality of root litter in forb-containing grass-clover mixtures may potentially affect soil microbial community structure, biomass and associated ecosystem functions, but it is yet to be elucidated. We hypothesized that inclusion of plantain in the grass-clover mixture would enhance soil microbial biomas and functions through its high biomass production and thus belowground carbon (C) input.

# METHODS

A three-year grassland experiment was established in spring 2013 on a loamy sandy soil of 2.0% organic C and 0.17% total nitrogen (N) in Denmark. The experiment was laid out as a completely randomized block design with species composition and fertilisation as the two fixed factors, and with three replicates. The plant species composition consisted of four pure stands (perennial ryegrass, *Lolium perenne* L.; red clover, *Trifolium pratense* L.; caraway, *Carum carvi* L. and plantain, *Plantago lanceolata* L.), the ryegrass-red clover mixture alone or containing either caraway or plantain (Table 1). Two levels of cattle slurry (0 and 250 kg total N ha-1 yr-1) were applied.

In August 2015, eight soil cores (2 mm diameter) per plot were taken up to a depth of 10 cm, pooled and sieved on a 4 mm-size mesh screen. Soil microbial community structure and biomass was examined by phospholipid fatty acids (PLFAs) (Petersen et al., 2002). The bacterial PLFA biomass was represented by the following PLFA’s: i-15:0, a-15:0, 15:0, i-16:0, i-17:0, a-17:0, cy-17:0 and cy-19:0 and the Fungal PLFA biomass by 18:2ω6,9. Microbial activity was assessed by β-glucosidase enzyme activity. The net soil N mineralization rate was determined in a 42-day incubation experiment.

The linear mixed-effects model was employed with blocks as random effects and with species composition and fertilisation as fixed effects. Differences between treatments were tested using Tukey’s *post hoc* test. All analyses were performed using the *R* software version 3.2.2.

# RESULTS and discussion

Rejecting our hypothesis, we found that enhanced biomass by inclusion of plantain in grass-clover mixtures did not significantly affect soil micobial community biomass, structure and functions related to C and N (Table 1 & Fig. 1). In contrast, inclusion of caraway in grass-clover mixtures significantly enhanced fungal-to-bacterial PLFAs (Fig. 1B). Moreover, despite of higher biomass of plantain than caraway in pure stand (Tabel 1), caraway generally had higher microbial biomass (Fig. 1A), enzymatic activty (Fig. 1C) and net soil N mineralization rate (Fig. 1D) than plantain monoculture. These results suggest that root quality instead of root quantity may be the key factor in determining soil microbial properties. Interestingly, higher fungi/bacteria ratio was related to lower root C/N ratio and lignin/N ratio (Table 1), which contrasted with previous findings. This indicates that other mechanisms may function, e.g. specific organic compounds released by caraway roots may foster the growth of fungi.

**Table 1.** **Seed proportions, biomass production and root quality parameters (C/N ratio and lignin/N ratio) of four monocultures, grass-clover mixture and caraway- or plantain-containing grass-clover mixtures.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Monocultures/mixtures | Abbreviation | Seeding proportion† | Biomass (t/ha) ‡ |  | Root C/N |  | Root lignin/N |
| N0 | N250 |  | N0 | N250 |  | N0 | N250 |
| Ryegrass | G | 100 | 23 | 28 |  | 30.0 | 28.0 |  | 12.0 | 5.7 |
| Red clover | C | 100 | 31 | 33 |  | 22.7 | 22.9 |  | 3.5 | 3.5 |
| Caraway | Cc | 100 | 22 | 26 |  | 24.9 | 23.7 |  | 1.6 | 1.8 |
| Plantain | Pl | 100 | 28 | 33 |  | 35.1 | 34.4 |  | 9.5 | 9.6 |
| Grass-clover | G-C | 50-50 | 32 | 32 |  | - | - |  | - | - |
| Grass-clover-caraway | G-C-Cc | 20-20-60 | 29 | 33 |  | - | - |  | - | - |
| Grass-clover-plantain | G-C-Pl | 20-20-60 | 33 | 38 |  | - | - |  | - | - |

† Numbers in this column referes to the proportions (%) of optimal seeding rate of plant species in pure stands.

‡ Cumulative herbage biomass yields from establishment (2013) to the sampling time (the 3rd cut) in 2015.

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**Fig. 1.** **Total PLFAs (A), fungal-to-bacterial PLFAs (B), β-glucosidase activity (C) and net N mineralization rate (D) in soils from different plant species composition. Data are means ± SE (n = 3). Means with different lowercase letters show significant differences (*P* < 0.05) between treatments using Tukey’s *post hoc* test.**

# CONCLUSIONS

Including competitive forbs such as caraway in grass-clover mixtures enhances fungi/bacteria ratio in soil through changes in root quality instead of root quantity.

# REFERENCES

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