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Forbs differentially affect soil microbial community composition and functions in unfertilized ryegrass-red clover leys

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ABSTRACT

Increasing plant diversity in agroecosystems is proposed to enhance multiple ecosystem services. Adding selected forbs such as caraway (*Carum carvi* L.) and plantain (*Plantago lanceolata* L.) to productive grass-clover mixtures can further enhance forage yields, root biomass, uptake of mineral nutrients and improve animal performance. Yet, it remains unclear whether and how adding these forbs to grass-clover mixtures can influence soil microbial communities and associated soil carbon (C) and nitrogen (N) cycling. Based on a three-year grassland experiment varying in species diversity and composition with and without fertilizer application, we determined soil microbial community composition and functions related to C and N cycling under laboratory incubations. Results showed that inclusion of caraway modified soil microbial community composition by enhancing fungal-to-bacterial phospholipid fatty acids of the ryegrass-red clover mixture. Adding plantain to the ryegrass-red clover mixture increased the relative decomposition rate of the labile C pool, but not of the recalcitrant C pool. Yet, β-glucosidase activity and net N mineralization were unchanged due to inclusion of either forb. Moreover, fertilization with cattle slurry generally weakened these forb-induced changes in soil microbial properties. These findings demonstrate that adding selected forbs to unfertilized grass-clover leys can modify soil microbial community composition and associated C and N cycling, implying a potential for promoting long-term soil C sequestration through enhanced fungi-to-bacteria ratio, but a limited role in improving soil N fertility.

1. Introduction

Perennial grass-clover mixtures characterized by high productivity, increased resource use, efficient weed suppression and improved soil fertility are increasingly practised in intensively managed grasslands (Finn et al., 2013; Isbell et al., 2017). Agronomists have recently identified several competitive perennial forbs, such as chicory (*Cichorium intybus* L.), caraway (*Carum carvi* L.) and plantain (*Plantago lanceolata* L.) (Elgersma et al., 2014; Hogh-Jensen et al., 2006; Sanderson et al., 2003). Adding these forbs to the productive grass-clover mixtures can further enhance herbage yield and root biomass (Cong et al., 2017), increase uptake of mineral nutrients from deep soil layers (Pirhofer-Walzl et al., 2011) and improve animal performance in terms of meat production and milk quality (Somasiri et al., 2015). Yet, it remains largely unexplored whether including these forbs in grass-clover mixtures can influence soil microbial community composition and associated carbon (C) and nitrogen (N) cycling, which is crucial for assessing their potential for soil C sequestration and soil N fertility.

Soil microbial communities mediate key ecosystem processes that control nutrient cycling and organic matter (OM) decomposition. One of the ways that plant species diversity and composition influence soil microbial communities and associated soil C and N processes is through changes in the total amount of belowground OM input (Bardgett and Wardle, 2010). For example, increasing plant diversity has been found to enhance soil microbial community biomass, microbial respiration, decomposition rate of labile C pool and net N mineralization, which is largely ascribed to increased biomass production associated with greater plant diversity (Dijkstra et al., 2005; Lange et al., 2015; Zak et al., 2003). Another way is through changes in the quality of belowground input that is closely related to the characteristics of added plant species (Bardgett and Wardle, 2010). For example, legumes with high root quality (e.g. low root C/N or lignin/N ratio) are often found to have higher soil microbial biomass, activity of soil enzymes (e.g. β-glucosidase) and soil N mineralization rate than non-leguminous forbs and grasses (Dijkstra et al., 2006; Fornara et al., 2009).

Our recent study has shown that inclusion of plantain enhanced annual herbage yield (by 10–14%) and root biomass (by 20–44%) of ryegrass (*Lolium perenne* L.)-red clover (*Trifolium pratense* L.) mixtures, while inclusion of caraway maintained the similar herbage yield but slightly increased root biomass (by 9–12%) of ryegrass-red clover

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mixtures (Cong et al., 2017). Moreover, Jing et al. (2017) showed that caraway herbage had the highest OM digestibility, followed by white clover and red clover, and plantain with the lowest OM digestibility. Collectively, these results suggest that including caraway and plantain in grass-clover mixtures will differentially affect the quantity and quality of belowground OM input, consequently influencing soil microbial community biomass, composition and functions related to soil C and N cycling.

Apart from plant species diversity and composition, agricultural management practices have a large impact on soil microbial properties. Bünemann et al. (2006) showed that fertilization with organic amendments such as animal manure can generally enhance soil microbial biomass and activity through either direct addition of C and N sources and/or indirect influence on plant residue returns. Several studies found that plant species effect on soil microbial properties significantly differed in soils of different fertility (Bardgett et al., 1999; Innes et al., 2004). Hence, it remains to be elucidated whether the effect of including caraway or plantain in grass-clover mixtures on soil microbial properties will depend on fertilization with animal manure.

In this study, we first explored how inclusion of caraway or plantain influenced soil microbial properties of the ryegrass-red clover reference mixture in both fertilized (applied as cattle slurry) and unfertilized treatments. Then, we examined how these differences, if any, were related to herbage biomass, root biomass and root quality. We hypothesized that (1) adding caraway or plantain to the reference mixture influences soil microbial community biomass and microbial C and N cycling through changes in root C input and quality; and (2) fertilization weakens these forb-induced effects on soil microbial properties.

2. Materials and methods

2.1. Experimental site and treatments

A three-year grassland experiment was established in spring 2013 in the long-term (since 1987) organic dairy crop rotation at the Foulumgaard Experimental Station, Aarhus University, Denmark (Eriksen et al., 2015). The preceding crop in 2012 was winter rye. The experimental site was situated on a loamy sandy soil, classified as a Typic Hapludult 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 C and 0.17% total N. The annual mean temperature and annual precipitation in 2013–2015 were 7.8, 9.5 and 8.6 °C, and 636, 853 and 904 mm, respectively.

The experiment was laid out as a completely randomized block design with species composition and fertilization 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.), one binary stand (the ryegrass-red clover reference mixture) and two three-species stands (ryegrass-red clover-caraway and ryegrass-red clover-plantain mixtures). Two levels of cattle slurry (0 and 250 kg total N ha^{-1} yr^{-1} with 52% NH_4^+-N) were applied across all stands. In total, we had 42 plots with the plot size of 8 m length × 1.5 m width. In each plot, 10 rows of seeds (0.12 m row distance) were sown by machine in May 2013. Adjacent plots were separated by a 0.3 m buffer.

The sowing proportions of plant species are 50:50 for the binary mixture and 20:20:60 for the three-species mixtures. Optimum seed rates in pure stands were 15, 4, 12 and 12 kg ha^{-1} for perennial ryegrass, red clover, caraway and plantain, respectively. The seed rate of each species in a mixture was calculated by multiplying the seed rate of the species in its pure stand with the sowing proportion of the species in the respective mixture. Cattle slurry was applied four times during the growing season in both 2014 and 2015; with 100 kg N ha^{-1} yr^{-1} at the beginning of the growing season and 50 kg N ha^{-1} yr^{-1} immediately after the first, second and third harvests. All plots received a one-off 200 kg K ha^{-1} (K_2SO_4) application to avoid potassium and sulphur deficiency. In dry periods of the growing seasons, 50-60 mm irrigation was applied.

2.2. Soil sampling and soil chemical properties

Just prior to the third harvest in mid-August 2015, eight soil cores (2 cm inner diameter) per plot were taken to a depth of 10 cm. Soil cores were taken randomly in three mixed stands. Given that 12-41% of biomass yields were occupied by weeds across four pure stands, soil cores were thus taken adjacently to the sown plant species in pure stands meanwhile avoiding weed patches to minimize the side effects from weeds. The samples were pooled, transferred to the lab and sieved on a 4 mm-size mesh screen to remove larger roots, animals and stones to obtain homogenized samples. A subsample was ground in a ball mill and determined by Elemental Vario EL C/N Analyzer (Hanau, Germany) to measure total C and N content. As expected, soil C, N content or soil C/N ratio did not differ between stands after just three years, but fertilization with cattle slurry significantly enhanced soil C and N content (Table S1).

2.3. Soil microbial community biomass and composition

The phospholipid fatty acids (PLFAs) analysis is a rapid and sensitive method to detect broad shifts in soil microbial community that regulate soil C and N cycling (Bardgett et al., 1999; Frostegård et al., 2011). The principle of the PLFAs method is that it creates a fingerprint of the community structure using lipids of microbial membranes as biomarkers for specific microbial groups (Zelles, 1999). The total concentration of PLFAs can also be used as a measure of viable microbial biomass (Zelles et al., 1997). On the same day of soil sampling, about 2 g moist soil was subsampled from each of the treatments. The PLFAs were then extracted using the same procedure described by Petersen et al. (2002). In brief, fatty acids were first extracted using a modified single-phase Bligh-Dyer extraction approach. Phospholipids were then isolated by solid phase extraction, followed by alkaline trans-esterification converting ester-linked fatty acids to the corresponding methyl esters. An internal standard (13:0 Me/19:0 Me) was included for quantification of PLFAs. In total, 19 PLFAs (> 0.3%) were identified and subsequently classified into specific microbial groups: gram-positive bacteria, gram-negative bacteria, actinomycete, fungi and non-specific group (Table 1). Given that many PLFAs are found to be non-specific and common across taxa (Ruess and Chamberlain, 2010; Frostegård et al., 2011), only widely accepted biomarkers are cautiously selected to represent the corresponding microbial groups in this study.

2.4. Soil microbial functions related to C and N cycling

2.4.1. Laboratory soil respiration

Microbial respiration was determined using the procedure described

Table 1
List of 19 identified PLFAs with classification into specific microbial groups.

Group	Name	Reference
Bacteria		
Gram-positive	15:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0, *a*17:0	Frostegård and Bååth, 1996
Gram-negative	*cy*17:0, *cy*19:0	Frostegård and Bååth, 1996
Actinomycete	10Me16:0, 10Me17:0, 10Me18:0	Zelles, 1999
Fungi	18:2ω6*c*	Federle, 1986
Non-specific	14:0, 16:0, 16:1ω5*c*, 16:1ω7*c*, 18:0, 18:1ω9*c*, 18:1ω9t	Ruess and Chamberlain, 2010; Frostegård et al., 2011