Legumes reduce fungal biomass in soil

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Abstract

We tested the effect different legumes on soil fungal and bacterial biomass in a field experiment with *Lolium perenne* in monoculture and in binary combinations with the legumes *Trifolium repens*, *Trifolium pratense* and *Medicago sativa*. Fungal biomass was higher in grass monocultures than in grass-legume mixtures, which coincided with lower inorganic N availability and potential N mineralization in grass monocultures. We conclude that grass-legume mixtures induced a shift towards a more bacterial based soil food web by increasing N availability through their higher litter quality.

Introduction

Grassland plant species can select for specific microbial communities by modifying the quantity and quality of organic resources entering the soil (Bardgett et al., 1999; Innes et al., 2004; Wardle et al., 2004). In theory, more fungal dominated microbial communities are associated with slow growing plants that return recalcitrant litter to the soil, whereas bacterial dominated communities are associated with fast growing species that return high quality, easily decomposable litter to the soil (Wardle et al., 2004). In addition to this, it has been repeatedly shown that fungal biomass is reduced by fertilization (Bardgett &McAlister, 1999; De Vries et al., 2006). Thus, legumes, with their high N content and increased ecosystem N inputs because of biological nitrogen fixation, should select for a more bacterial dominated soil microbial community. However, results are ambiguous: in two field studies, we found that fungal biomass was negatively related to white clover (De Vries et al., 2007; De Vries et al., 2006), whereas Smith et al. (2008; 2003) found that fungal biomass was positively correlated to both red and white clover. Here, we test the effects of three legumes in combination with perennial ryegrass on soil fungal and bacterial biomass. We hypothesize that legumes will reduce fungal biomass because of their higher ecosystem N inputs, and we expect that potential N mineralization and inorganic N will be increased in the legume treatments.

Materials and methods

Experimental design

The experiment was situated on a heavy clay soil near Wageningen, the Netherlands (51°58'N, 5°40'E). Plots were sown in 2003 with pure perennial ryegrass (*Lolium perenne* cv. Fennema) and binary mixtures of *L. perenne* with either *Trifolium pratense* (cv. Pirat), *Trifolium repens* (cv. Klondike), or *Medicago sativa* (cv. Daisy). The four treatments were repeated three times in a randomized block design. Plot size was 3 by 8 m. All plots received P and K (60 and 100 kg ha^{-1} y^{-1}, respectively), but no N.

Soil and microbial characteristics

In September 2007 a bulk sample of 30 cores (0-10 cm depth, 3.5 cm diam.) was collected, sieved (5 mm mesh size), and stored at field moisture content overnight at 4 °C. The pH-KCl, bulk density, KCl-extracted NO_3^- and NH_4^+, total soil C and N and root biomass were all determined following standard protocols. Potential nitrogen mineralisation was determined by incubating 200 g soil for six weeks at 50% water holding capacity at 20°C. Using soil suspensions from bulk soil samples, microscopic slides were prepared and fungal and bacterial biomass were measured using epifluorescence microscopy as described by Bloem & Vos (2004).

Results

Both potential N mineralization and inorganic N availability were affected by treatment. Potential N mineralization was lower in *L. perenne* monocultures than in mixtures with *M. sativa* and *T. repens*, and lower in the *T. pratense* mixture than in the *T. repens* mixture (Table 1). As a group, legumes significantly increased potential N mineralization. N availability was lower in *L. perenne* monocultures than in the *T. repens* mixture, and higher in the *T. repens* legume mixture than in the other legume mixtures. Legumes significantly increased soil N availability. Although there were no significant differences between treatments in fungal and bacterial biomass and F/B ratio, fungal biomass was reduced by the presence of legumes (Table 1).

Table 1. Soil and microbial properties. Abbreviations: Lp: grass monoculture; Lp + Ms: grass/lucerne; Lp + Tp: grass/red clover; Lp + Tr: grass/white clover. Values denote means (SE), n=3. *P* treatment: level of significance of treatment effect. *P* legumes: level of significance of monoculture vs. legume mixtures contrast.

	Monoculture	*Legume mixtures*				*P legumes*
	Lp	*Lp + Ms*	*Lp + Tp*	*Lp + Tr*	*P treatment*	
Potential N mineralization (mg kg^{-1} wk^{-1})	-1.4 (1.6)	12.9 (1.8)	4.9 (2.5)	14.0 (4.4)	0.014	0.006
Mineral N (mg kg^{-1})	9.0 (1.5)	11.8 (1.4)	10.9 (1.0)	16.4 (2.0)	0.042	0.048
Total C (g kg^{-1})	19.6 (0.9)	19.9 (0.5)	21.7 (0.8)	20.4 (0.8)	0.314	0.298
Total N (g kg^{-1})	2.02 (0.07)	2.05 (0.06)	2.09 (0.05)	2.11 (0.03)	0.681	0.344
Fungal biomass (μg C g^{-1})	189 (17)	140 (3)	151 (9)	152 (20)	0.142	0.032
Bacterial biomass (μg C g^{-1})	57 (17)	68 (21)	71 (24)	54 (14)	0.902	0.740
F/B ratio	4.5 (2.1)	2.7 (1.2)	3.1 (1.4)	3.0 (0.5)	0.801	0.352

Discussion

The presence of legumes reduced soil fungal biomass and increased inorganic N availability and potential N mineralization, when compared to pure perennial ryegrass treatments. We hypothesized that the reducing effect that legumes would have on soil fungal biomass is caused by the increased N inputs into the soil due to biological N fixation. The negative effects of N inputs on soil fungal biomass are well documented (Bardgett &McAlister, 1999; De Vries et al., 2006). However, although fungal biomass decreased with the presence of legumes, and both N availability and potential N mineralization increased, we did not find a direct relationship between these two parameters and fungal biomass. This suggests that other mechanisms, apart from N inputs, might play a role, like differences in arbuscular mycorrhizal fungal root colonization, which can have considerable impact on ecosystem processes and plant biodiversity.

In summary, we found that legumes reduced fungal biomass, thus favouring a more bacterial dominated soil microbial community. We conclude that legumes affected soil and microbial properties because they influence ecosystem N uptake rates directly through biological N fixation.

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