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Additive and interactive effects of functionally dissimilar soil organisms on a grassland plant community

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ABSTRACT

The productivity and diversity of plant communities are affected by soil organisms such as arbuscular mycorrhizal fungi (AMF), root herbivores and decomposers. However, it is unknown how interactions between such functionally dissimilar soil organisms affect plant communities and whether the combined effects are additive or interactive. In a greenhouse experiment we investigated the individual and combined effects of AMF (five *Glomus* species), root herbivores (wireworms and nematodes) and decomposers (collembolans and enchytraeids) on the productivity and nutrient content of a model grassland plant community as well as on soil microbial biomass and community structure. The effects of the soil organisms on productivity (total plant biomass), total root biomass, grass and forb biomass, and nutrient uptake of the plant community were additive. AMF decreased, decomposers increased and root herbivores had no effect on productivity, but in combination the additive effects canceled each other out. AMF reduced total root biomass by 18%, but decomposers increased it by 25%, leading to no net effect on total root biomass in the combined treatments. Total shoot biomass was reduced by 14% by root herbivores and affected by an interaction between AMF and decomposers where decomposers had a positive impact on shoot growth only in presence of AMF. AMF increased the shoot biomass of forbs, but reduced the shoot biomass of grasses, while root herbivores only reduced the shoot biomass of grasses. Interactive effects of the soil organisms were detected on the shoot biomasses of *Lotus corniculatus*, *Plantago lanceolata*, and *Agrostis capillaris*. The C/N ratio of the plant community was affected by AMF.

In soil, AMF promoted abundances of bacterial, actinomycete, saprophytic and AMF fatty acid markers. Decomposers alone decreased bacterial and actinomycete fatty acids abundances but when decomposers were interacting with herbivores those abundances were increased. Our results suggests that at higher resolutions, *i.e.* on the levels of individual plant species and the microbial community, interactive effects are common but do not affect the overall productivity and nutrient uptake of a grassland plant community, which is mainly affected by additive effects of functionally dissimilar soil organisms.

1. Introduction

Plant communities are influenced by abiotic (Ellenberg, 1974: Tilman, 1982) as well as by biotic soil factors (Brown and Gange, 1992; Van der Putten et al., 1993, 2001; De Deyn et al., 2003). Biotic soil factors consist of a wide array of soil organisms that interact with plants in a functionally dissimilar manner. Functionally dissimilar soil

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organism groups such as mycorrhizal fungi (Gange et al., 1993; Van der Heijden et al., 1998a), root herbivores (Brown and Gange, 1992; Schädler et al., 2004) and decomposers (Partsch et al., 2006) influence plant communities in a variety of ways, and their individual impact on productivity ranges from being beneficial to detrimental (Wardle et al., 2004).

Besides the multiple biotic interactions in terrestrial ecosystems, interactions between functionally dissimilar soil organism groups and their impact on plant communities have been very rarely explored. With the exception of Gange and Brown (2002), studies on impacts of soil organism groups on plant communities rather assembled soil organisms according to body size (Bradford et al., 2002), abundance (Wurst et al., 2008a) or origin (De Deyn et al., 2003; Kardol et al., 2006) than functional groups. Gange and Brown (2002) manipulated arbuscular mycorrhizal fungi (AMF) and insect root herbivores by applying soil fungicide and insecticide in the field. They observed that plant cover and plant species richness during early succession were increased when AMF were present, but reduced in the presence of insect root herbivores. However, when both AMF and root herbivores were present together in soil the two opposite effects were additive and thus canceled each other out.

Knowledge on effects of functionally dissimilar soil organism groups on plant communities is crucial to understand and predict the general impact of soil organisms on plant communities, since their individual effects might change in presence of other functionally dissimilar soil organisms that co-occur in nature. For instance, interactions between functionally dissimilar root herbivores and AMF were shown to reduce root damage by the root herbivores (Hol and Cook, 2005; Currie et al., 2006; De la Peña et al., 2006); however, other studies did not find evidence for significant interactions (Wurst et al., 2004; Eisenhauer et al., 2009). Since most of the studies were carried out in pots with only one plant species present or in Petri dishes (*e.g.* feeding assays), it is still unclear whether these interactions are strong enough to change individual impacts of functionally dissimilar soil organism groups on the productivity and structure of a plant community.

First objective of our study was to examine the individual and combined effects of three functionally dissimilar groups, *i.e.* AMF (five *Glomus* species), root herbivores (wireworms and nematodes) and decomposers (collembolans and enchytraeids) on key functional properties of a grassland plant community, such as productivity and nutrient content of the plant community. Our main goal was to see whether the combined effects of the functionally dissimilar soil organism groups can be predicted by knowing their individual effects. This is the case when effects of the functionally dissimilar soil organism groups are rather independent leading to additive and thus predictable effects when combined. Our null hypothesis was that functionally dissimilar soil organisms cause independent effects and thus the combined effects of the soil organisms are additive under the assumption that in the absence of significant interactions any observed effect in combined treatments can be decomposed as the sum of the individual treatment effects (Sokal and Rohlf, 1981). For the plant community structure we further expected changes according to the type of functional group of soil organisms present: AMF may promote AMF-depending plant species (Van der Heijden et al., 1998a); root herbivores (nematodes and wireworms) may preferentially feed on some of the plant species (Hemerik et al., 2003) and therefore suppress their competitive ability; decomposers (collembolans and enchytraeids) may increase nutrient availability and promote plants that are strong competitors for nutrients (Partsch et al., 2006).

Second objective of our study was to determine effects of functionally dissimilar soil organisms on soil microbial biomass and community structure using phospholipid fatty acids (PLFA) as microbial markers (Zelles, 1999). We hypothesized that microbial biomass and microbial community structure will change in the presence of AMF since AMF may enhance growth and activity of microorganisms by improving, for example, soil aggregation (Van der Heijden et al., 2006). Root herbivores may positively affect microbial communities by grazing on roots, thus releasing nutrients available for microbial growth (Grayston et al., 2001). Microbial biomass and community structure may be changed through grazing activity of the decomposers (enchytraeids and collembolans) that may selectively feed on fungal hyphae (Cole et al., 2002; Jorgensen, 2002). As null hypothesis we expected to observe additive effects of the functionally dissimilar soil organisms on soil microbial biomass and community structure.

2. Material and methods

2.1. Experimental setup

We established 80 microcosms in pots of 17.7 cm diameter and 19.5 cm height with soil from a grassland in the Netherlands (Van der Putten et al., 2000). The soil was a sandy loam and had on average 21.3 ± 0.5 g/kg of carbon, 1.3 ± 0.2 g/kg of nitrogen, 0.3 ± 0.02 g/kg of phosphorus, and a pH of 6.3 (Hedlund et al., 2003). All pots were filled with 4.94 kg (dry wt) of soil that had been sieved through a 1 cm mesh and autoclaved at 121 °C for 20 min. Then all microcosms were amended with a non-sterile soil solution of the native grassland communities to restore the microbial community of the soil, excluding AMF (Brundrett et al., 1996). This non-sterile soil solution was prepared by suspending 1000 g of the non-autoclaved soil in 4 l of distilled water and filtering it through a 20 μm sieve. This solution contained no meso- or microfauna, which was checked under a stereoscopic microscope (Leica MZ 8).

We selected eight plant species that are common in grasslands of the Netherlands (Lepš et al., 2001). Seeds (obtained from Appels Wilde Samen GmbH, Darmstadt, Germany) of five grasses (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Poa pratensis*, *Festuca rubra*, *Holcus lanatus*), two forbs (*Plantago lanceolata*, *Hypochaeris radicata*), and one legume (*Lotus corniculatus*) were surface sterilized with 5% $CaCl_2O_2$ for 15 min before sowing. About 10–15 seeds of every plant species were sown in each microcosm according to a planting scheme (day 1 of the experiment). The indicated numbers of seeds were used due to the unknown germination rate of the plant species. The planting scheme was related to the position of the plants to assure that each plant species had the same position across the pots. Plants were allowed to establish in the microcosms for 7 weeks, at which time transparent perforated plastic bags, reaching up to 26 cm above the rims of the pots and open at the top, were placed around the pots to prevent potential cross contamination of the soil organism treatments.

We set up a full-factorial design experiment, resulting in 8 treatments [Control (no soil organisms added), AMF (arbuscular mycorrhizal fungi), Dec (decomposers), Herb (herbivores), AMF + Dec, AMF + Herb, Dec + Herb, AMF + Dec + Herb] with 10 replicates per treatment. First we established the AMF treatment in half of the microcosms ($N = 40$) by adding an AMF inoculum (provided by PlantWorks Ltd., Sittingbourne Research Center, Kent, UK) on day 1 of the experiment. This inoculum contained five species of AMF (class Glomeromycota): *Glomus mosseae, G. claroideum*, *Glomus intraradices*, *G. etunicatum* and *G. microaggregatum*. AMF inoculation of the microcosms was carried out according to Brundrett et al. (1996) using 90 g of the inoculum, while the non-AMF microcosms were inoculated with 90 g of autoclaved (1 h at 120 °C) AMF inoculum. Additionally, the non-AMF microcosms were supplemented with 1 ml of a microbial wash of the AMF