The significance of soil collembolans, protozoa and microorganisms and their interactions for soil fertility - A soil mesocosm approach

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Introduction

For sustainable land use there is an urgent need for a better understanding of the interactions between the different groups of organisms inhabiting the soil. Human effects on particular soil organisms may lead to changed conditions for other organisms below- and aboveground, which can then cause changes in both soil community composition and soil functioning. Soil fertility, which is a key concept in this discussion, is often defined vaguely or even ambiguously. Here, we refer to the view presented by Mäder et al. (2002), who suggested that fertile soils provide essential nutrients for crop plant growth, support a diverse and active biotic community, exhibit a typical soil structure and allow for an undisturbed decomposition.

The decomposition of organic matter and subsequent uptake of phosphorus by plants is an important soil function. Plants in phosphorus-deficient soils depend on arbuscular mycorrhiza (AM) fungi to supply them with phosphorus. External hyphae of AM fungi spread away from the colonized plant roots and take up phosphorus from outside the phosphorus depletion zone around the roots. AM fungal hyphae have no direct access to soil organic phosphorus, hence the phosphorus source for plants and AM fungi is mineral phosphate in soil solution or absorbed to soil particles. Thus, mineralization processes will govern AM uptake from organic phosphorus. Soil microorganisms and their predators facilitate this mineralization.

Collembola are a group of microarthropods that participate in the disintegration of particulate organic material, but also feed on various soil organisms depending on the morphology of the collembolan. The collembolan *Folsomia fimetaria* ingests a wide selection of fungi (Jørgensen et al., 2003), but the exact food specificity of this collembolan in soil is unknown. Protozoa feed mainly on bacteria though some also feed on fungal spores, other protozoa, algae or detritus. Predation on fungi has been sparsely investigated, but seems only to be essential for a small fraction of the protozoa (Ekelund, 1998). Saprotrophic bacteria and fungi also participate in the degradation of organic matter.

Fenpropimorph is a widely used sterol synthesis-inhibiting leaf fungicide used for the control of *Erysiphe graminis* (powdery mildew) and *Puccinia recondita* (cereal rust). Some soil protozoa are also sensitive to fenpropimorph at the recommended field dose (Ekelund, 1999), and this effect on protozoan numbers reduced the predation on bacteria at 10× the recommended dose (Thirup et al., 2000). Thus, we expect that the addition of fenpropimorph 10× the recommended dose can be used as a tool to change the soil community responsible for the decomposition of organic matter.

Here, we hypothesize 1) that moderate community composition changes induced by fenpropimorph will result in effects on soil fertility as measured by cycling of phosphorus from mesh bag soil only accessible to AM fungal hyphae and containing ^{33}P-labelled organic matter to phosphorus used for plant growth, and 2) that the presence of the collembola (*Folsomia fimetaria*) will stimulate organic matter mineralization and thus increase plant uptake of phosphorus via AM fungal hyphae from within the mesh bags. We monitored these changes by examining the effects on selected groups of soil organisms and plant growth in soil mesocosms.

Experimental procedures

We grew barley (*Hordeum vulgare*) in pots in a P-deficient, defaunated soil that was divided into a rooting and a root-free compartment, with or without addition of the fungicide fenpropimorph and the collembolan *Folsomia fimetaria*. We harvested eight times. Each treatment was replicated four times. Soil inoculum of the AM fungus *Glomus claroideum* was mixed thoroughly into the soil.

The pots contained mesh bags (25 µm), which exclude roots but allows for passage of AM hyphae. The mesh bags contained soil mixed with 1% dried and ground subclover shoots labeled with ^{33}P. The soil in half of the mesh bags had been amended with the fungicide fenpropimorph. The resulting fenpropimorph concentration in the amended mesh bags was 12.5 mg fenpropimorph kg^{-1} soil, which corresponded approximately to 10× the amount expected in the soil after application at the recommended field application dose (Thirup et al., 2000). We added 100 *Folsomia fimetaria* to half of the amended and unamended mesh bags. We sampled on days 1, 3, 7, 10, 16, 36, 70 and 99. Not all parameters were measured on all sampling days. We extracted ***Folsomia fimetaria*** from the mesh bags in a high gradient heat extractor of the MacFadyen type, collected them on plaster, and counted and measured them using an image analysis system (Krogh et al., 1998). The total number of **culturable bacteria** was obtained on 1/300 tryptic soy agar. **Protozoa** were enumerated using a "most probable number" (MPN) method (Rønn et al., 1995). We used tryptic soy broth (TSB powder 0.1 g l^{-1}) as the growth medium. **Arbuscules and other AM structures** in the roots and **external hyphal** were assessed employing a line intersect method.

Results

All parameters measured changed significantly with time. Surprisingly, addition of *Folsomia* had virtually no effect. Except for the effects discussed in more detail below (and a weak effect on juvenile *Folsomia*), fenpropimorph had no clear effects on the systems. During the first ten days, numbers of **protozoa** increased (Figure 1). Numbers of protozoa in amended pots were significantly different from ($p=0.0315$, log-transformed data), and almost consistently lower than those in unamended pots. Numbers in unamended pots stabilized at a level of about 2×10^5 cells g^{-1} soil. The

percentage of barley root length colonized by AM fungi (Figure 2) increased during the experiment, but we saw no treatment effects. Roots in pots without mesh bags were close to significantly less colonized by hyphae than roots in pots with mesh (one-way ANOVA, p=0.098). The results of percent root length colonized by AM fungi with arbuscules were in accordance with the results presented in Figure 2 (data not shown).

Discussion

Our data suggest that the mesh bags acted as sources of phosphorus in a phosphorus-poor environment. We used a phosphorus-deficient soil. All nutrients except for phosphorus were added to this mixture to give a non-limiting supply. During the experiment plants exhibited signs of phosphorus deficiency, and measured shoot P concentrations were well below reported values for severe phosphorus deficiency in barley (Jakobsen et al., 2005). Therefore, we find it justified to consider phosphorus to be the major limiting factor for plant growth in the experiment. A significantly lower shoot biomass production and higher root/shoot ratio in pots without mesh bags (Figure 3) further demonstrate the importance of the mesh bag soil as a phosphorus source. The root/shoot ratio increases when a certain nutrient is deficient.

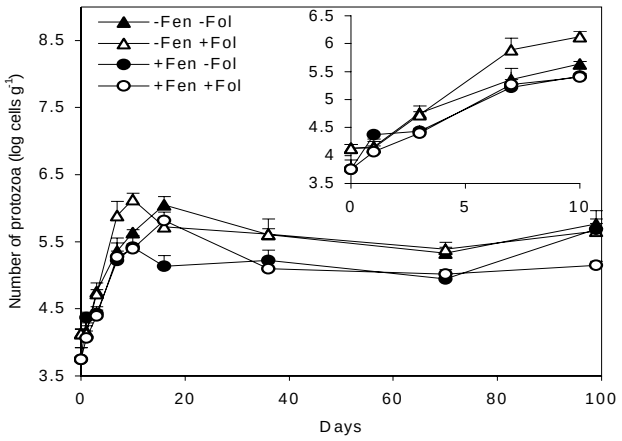

Figure 1. Numbers of protozoa feeding on indigenous soil bacteria in microcosms with defaunated soil during a 99 day period. Microcosms were with or without fenpropimorph treatment (Fen) and addition of *Folsomia fimetaria* (Fol). Points are mean values from four microcosms. Error bars represent 1 SEM.

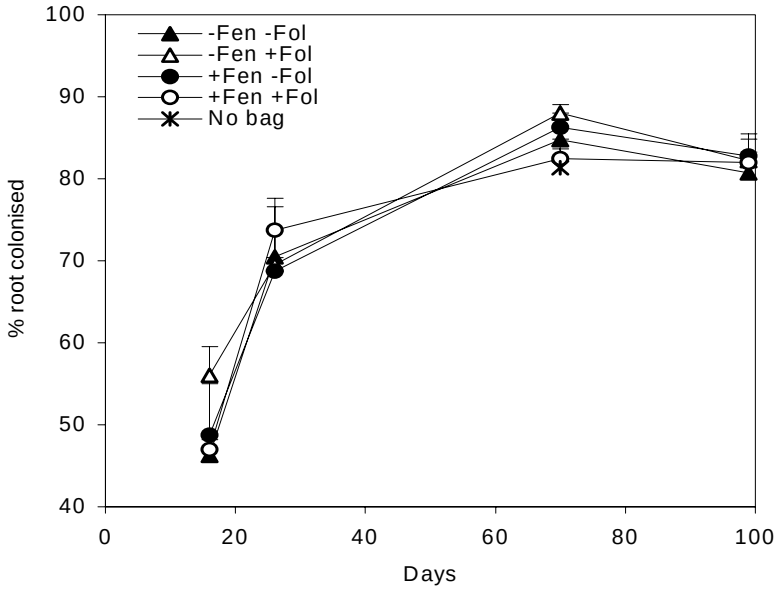

Figure 2. Percent barley root length colonized by AM fungi in microcosms with defaunated soil during a 99 day period. Microcosms were with or without fenpropimorph treatment (Fen) and addition of *Folsomia fimetaria* (Fol). Three microcosms without mesh bags with organic matter were sampled on day 70 (no bag). Points are mean values from four microcosms. Error bars represent 1 SEM.

As expected, fenpropimorph altered the soil community composition. We observed effects on protozoa (Fig 1). Still, the effects of fenpropimorph were moderate, and it did not significantly affect the numbers of adult *Folsomia*, bacteria or FDA-active hyphal length. This moderate effect only on some key organisms in the system allows us to draw some general conclusions about undisturbed soil systems from the experiment.

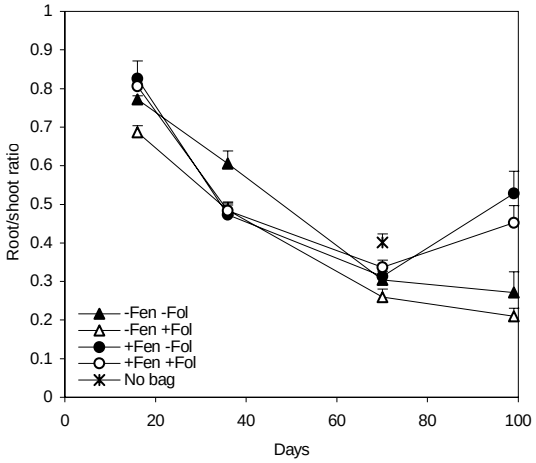

Figure. 3. Root/shoot ratio of barley plants (dw) in microcosms with defaunated soil during a 99 day period. Microcosms were with or without fenpropimorph treatment (Fen) and addition of *Folsomia fimetaria* (Fol). Three microcosms without mesh bags with organic matter were sampled on day 70 (no bag). Points are mean values from four microcosms. Error bars represent 1 SEM.

There was a striking effect of fenpropimorph on the root/shoot ratio. Root/shoot ratio of herbaceous plants generally decreases with age/size, which we observed for all treatments until day 70. However, after 99 days fenpropimorph-amended pots showed a marked increase to values below 0.4 characteristic of nutrient limitation, most likely of phosphorus. Probably due to some nutrient limitation in these pots, the root/shoot ratio in pots without fenpropimorph continued to decrease after day 70, albeit at a lower rate than previously. This confirms our hypothesis that moderate community composition changes induced by fenpropimorph would result in effects on soil fertility as indicated by transport of phosphorus from organic matter to plant growth.

Protozoa were the biotic component in the system that fenpropimorph affected most severely, in accordance with Ekelund (1999), who found that fenpropimorph had detrimental effects on the soil protozoan community even in very small concentrations. Protozoa generally stimulate plant uptake of mineral nutrients from soil organic matter (Bonkowski, 2004), including phosphorus (Cole et al., 1978). Hence, we suggest that reduced protozoan activity was a key factor responsible for the increased root/shoot ratio in the pots with fenpropimorph.

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Soil animals and plant-soil interactions

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Nitrogen availability limits primary production in most terrestrial ecosystems and plants are under strong selection for developing features that enhance their nitrogen acquisition ability. Among these features, one conceivable option for plants is to try to manipulate soil decomposers in a way that leads to higher net nitrogen mineralization