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Organic amendment and fungal species in combination can alter collembolan fitness

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ABSTRACT

Organic material of different origin is commonly used as fertiliser in agricultural practices. Clover and wheat straw are here used to determine the importance of organic amendment for population development of fungal feeding collembolans. Two fungal species, *Alternaria infectoria* and *Mucor hiemalis*, were inoculated in three growth substrates, clover amended soil, straw amended soil and non-amended soil, where both amendments and the soil originated from agricultural fields. Food choice as well as growth rate, survival and fecundity of the collembolan, *Folsomia fimetaria*, were measured when fed fungi grown in the three substrates. The type of amendment altered food quality of the two fungi, which was reflected in the collembolan food preference. Growth and fecundity of *F. fimetaria* were enhanced when fed *M. hiemalis* grown in both types of plant amended soils. *F. fimetaria* had a slightly higher fitness when fed *A. infectoria* grown in the straw amended soil, whereas it's fitness decreased when fed with *A. infectoria* grown in clover amended soil. We also examined how the predatory mite, *Hypoaspis aculeifer*, was attracted towards the two fungi as it uses the fungal odour as a potential cue of a prey habitat. *H. aculeifer* was attracted to both fungi when they were grown in clover amended soil where fungal growth also was observed to be massive. Thus, we conclude that amendment applications can cause effects that cascade through several trophic levels.

1. Introduction

Different organic amendments are applied to agricultural fields as fertilizers and within organic farming in particular. Direct incorporation of green manure crops or catch crops is often used but also application of plant residues and farmyard manure when available. When farmers use amendments of organic origin the soil decomposer community is positively affected, as the microbial biomass can increase (Bardgett and Shine, 1999; Lundquist et al., 1999; Tejada et al., 2006; Elfstrand et al., 2007a; Franco-Otero et al., 2012; Scharroba et al., 2012) as well as the abundance of collembolans and mites (Axelsen and Kristensen, 2000; Kautz et al., 2006; Eo et al., 2012). The amendment type (clover, wheat straw, sawdust etc.) and the management of the amendments before application (direct incorporation, green manure, compost etc.) are important to the microbial biomass and community composition (Holland and Coleman, 1987; Elfstrand et al., 2007a, 2007b).

While increased microbial biomass can be expected to cascade up to microbial feeding invertebrates, results from field studies are ambiguous as invertebrate population size can increase or decrease with amendment type (Bardgett and Cook, 1998; Wardle et al., 2001; Eo et al., 2012). Organic amendments can even affect the predator level (Fountain et al., 2008; Chen and Wise, 1999; Wardle et al., 1999), thus adding to the discussion on cascading effects within food webs (Schaefer, 1995; Mikola and Setälä, 1998). The difficulties in predicting effects of organic resource incorporation in field situations may depend on the complex trophic interactions present in soil food webs where a number of direct and indirect interactions occur between species (Bengtsson et al., 1996). When studying direct interactions between a few trophic levels the microbial biomass carbon and nitrogen increase with increasing substrate carbon and nitrogen (Wardle, 1992). Competition about organic material among fungi can determine the species composition of fungal communities, which means that different communities can occupy organic material of different origin (Robinson et al., 1993, 1994). The quality of the organic material (C, N, P and lignin content) will further push the competition among species and thus the given fungal species composition of communities are important factors of the decomposition rates (Griffith and Bardgett,

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2000). On the other hand, fungivorous soil invertebrates such as collembolans are selective in their food choice (Bardgett et al., 1993; Chen et al., 1995; Sabatini and Innocenti, 1995; Hall and Hedlund, 1999; Jørgensen et al., 2003), and generally they prefer fungal species that best support growth, survival and fecundity and avoid fungal hyphae that are lethal to them (Sabatini and Innocenti, 1995, 2000; Klironomos et al., 1999; Jørgensen et al., 2008). Higher trophic levels might indirectly be influenced by amendment applications as for example predatory mites that are able to locate their prey, the fungivorous collembolans, by cues of the fungi (Hall and Hedlund, 1999; Pfeffer and Filser, 2010).

As the identity of the organic material can alter fungal growth and might be able to alter fungal quality as a food resource, we wanted to study how fungi grown in soil amended with different plant material influenced collembolan fitness. Wheat straw and clover were used as amendments to agricultural soil. The fungi *Alternaria infectoria* and *Mucor hiemalis* were offered as food resources. An earlier study showed that these two fungal species grown in pure soil were of different food quality for collembolans (Jørgensen et al., 2008). As fungal biomass C and N change with C and N of the fungal growth substrate (Wardle, 1992) we hypothesised that an addition of organic matter with different C/N ratios to soil would change the fungal food quality for the collembolans, and which would be reflected in the fitness of the collembolans. We expected that fungi grown in clover amended soil would have a more positive effect on collembolan fitness than fungi grown in straw amended soil as food quality for collembolans depend on nitrogen content of the growth substrate (Booth and Anderson, 1979; Lavy and Verhoef, 1996). Firstly we tested whether the quality of amendment (no amendment, straw amended and clover amended) would cascade across the trophic levels. This was determined through the growth, survival and fecundity of the collembolan *Folsomia fimetaria* fed on the two fungal species grown on the different amendment substrates. Secondly we tested the preference of *F. fimetaria* and the predatory mite (*Hypoaspis aculeifer*) of fungi (*A. infectoria* or *M. hiemalis*) grown in the three different soil substrates. All species in the study were chosen as representatives of an agricultural soil community (Domsch et al., 1980; Lagerlof and Andren, 1988; Petersen, 2000)

2. Material and methods

Soil, wheat straw, clover and fungi all originated from organically grown fields. Soil and fungi came from the agricultural research farm Rugballegaard near Horsens, East Jutland, converted into organic farming practices in 1996. The soil is classified as a Glossic Phaeozem (WRB, 1998) with average clay content of 13.2% (Munkholm et al., 2001). The average organic content was 3% and the C/N ratio was 11 (Kristensen et al., 2003). The fungal species used in the experiment were both isolated from the soil of the research farm. The wheat straw came from The Danish Agricultural Museum, Gl. Estrup Manor, Auning and the clover (*Trifolium repens*) from Hanegal farm, Resenbrovej 29, Voel. The collembolan *Folsomia fimetaria* (Linné) and the mite *Hypoaspis aculeifer* (Canestrini) originated from laboratory cultures at the National Environmental Research Institute in Silkeborg.

The fungi *Alternaria infectoria* (E. Simmons) and *Mucor hiemalis* (Wehmer f.) were isolated from soil samples by dilution plating on V8 juice agar (Gams et al., 1998). The two strains were stored in glycerol at −80 °C until use. They were first transferred to SNA (synthetic nutrient-poor agar (Nirenberg, 1981)) and then to MEA (malt extract agar) where they were incubated for six days at 20 °C in darkness followed by eight days in 12/12 h day/night regime to induce sporulation. Spore suspensions in sterile water were made from the cultures of the fungi grown on MEA.

Straw (after harvest) and clover (cut as fresh green, leaves and stems) were dried for 24 h at 50 °C and cut into fine particles with a kitchen blender. The soil was dried, then sieved (2 mm mesh size) and amended with either straw or clover (77 g organic material and 700 g soil). The growth substrates, pure soil, soil amended with straw and soil amended with clover, were sterilised (2 × 25 kGy radiation) and around 0.3 g placed on a sterilised glass filter paper circles (1.4 cm/diameter for preference experiments and 1.9 cm/diameter for feeding experiments) in a sterile glass Petri dishes. Each growth substrate was inoculated with 0.05 ml spore suspension and 0.1 ml sterile water. The inoculated soils were incubated in darkness at 20 °C for seven days after which fungal hyphae were visible on the substrate in all treatments. New inoculations were done once a week to ensure that young hyphae were provided to the collembolans. Seven day old samples were used in the preference experiment and 7–14 day old samples were used as food in the feeding experiment.

2.1. Feeding experiment

At the first day of the experiment, ten 1 to 3 day old collembolans were transferred to a Petri dish (5 cm/diameter, with a layer of moist plaster of Paris/charcoal 8:1). The dishes were kept on ice for 15 min to slow down movements of the collembolans before a digital photo was taken through a binocular microscope of the individuals for length measurements (Software: Image-Pro® Plus. Version 4.0 for WindowsTM. Media Cybernetics (1998)). The two fungal species grown in the three different substrates (a total of 6 treatments) were added to Petri dishes (eight Petri dishes were prepared per treatment). The animals were counted, the length measured and missing individuals registered every other day during 18 days and once a week after that. Fungal inoculated substrates were added twice a week to ensure a surplus of food and once a week the animals were transferred to fresh Petri dishes (with a layer of moist plaster of Paris/charcoal). From day 13 and onwards eggs and fungal remains (eggs could be hidden in the mixture of soil and old fungal hyphae) were collected weekly and stored in Petri dishes for 14 days after which the eggs were hatched, and the young were counted. Fecundity was calculated as the number of young produced per individual per week considering survival and the fact, that no reliable sex determination was possible. The experiment was performed at 20 °C with a day/night regime of 16:8 and lasted for 56 days in total.

2.2. Preference experiment

Both collembolans and mites were tested whether they would discriminate between a fungal species grown in three different substrates. The growth substrates inoculated with one fungal species were transferred to the experimental arenas; Petri dishes (5 cm/diameter) with a moist layer of plaster of Paris/charcoal. The inoculated substrates were presented in pairs and placed alternately on equal distances in a circle (6 inoculated substrates in one Petri dish, 3 mm apart, 10 mm to the centre). These were kept for 24 h in darkness at 20 °C before the start of the experiment to condition the fungus in the experimental arenas. Ten *F. fimetaria* or seven *H. aculeifer* were placed in the centre of the experimental Petri dish and after 90 min, each Petri dish was observed and the position of the animals were recorded. Ten replicates were made of collembolan preference on each of the two fungal species and 2 times 15 replicates of mite preference.

3. Statistics

Collembolan growth rates were estimated by fitting a von Bertalanffy growth curve to the data: $L_t = L_\infty(1 - e^{-k(t-t_0)})$ where