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Arbuscular mycorrhizal fungi and collembola non-additively increase soil aggregation

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ABSTRACT

Soil aggregation is a principal ecosystem process mediated by soil biota. Collembola and arbuscular mycorrhizal (AM) fungi are important groups in the soil, and can interact in various ways. Few studies have examined collembola effects on soil aggregation, while many have quantified AM effects. Here, we asked if collembola have any effect on soil aggregation, and if they alter AM fungi-mediated effects on soil aggregation.

We carried out a factorial greenhouse study, manipulating the presence of both collembola and AM fungi, using two different plant species, *Sorghum vulgare* and *Daucus carota*. We measured root length and biomass, AMF (and non-AMF) soil hyphal length, root colonization, and collembolan populations, and quantified water stable soil aggregates (WSA) in four size classes.

Soil exposed to growth of AMF hyphae and collembola individually had higher WSA than control treatments. Moreover, the interaction effects between AMF and collembola were significant, with non-additive increases in the combined application compared to the single treatments.

Our findings show that collembola can play a crucial role in maintaining ecological sustainability through promoting soil aggregation, and point to the importance of considering organism interactions in understanding formation of soil structure.

1. Introduction

Soil structure is defined as the arrangement of primary particles and soil organic compounds into aggregates and corresponding pore spaces, and plays a pivotal role in a wide range of ecosystem processes, including gas and water exchange, nutrient cycling, and resistance to erosion (Tisdall and Oades, 1982; Dexter, 1988; Six et al., 2000; Diaz-Zorita et al., 2002; Rillig and Mummey, 2006). Soil structure itself is affected by a number of soil properties (e.g. texture, soil organic carbon) and the activity of soil biota (Bronick and Lal, 2005). The development of soil aggregates can be viewed in a hierarchical mode starting from primary particles via microaggregates (<0.25 mm) to macroaggregates (>0.25 mm); the latter are formed by biological binding forces, such as plant roots, fungal hyphae, and their exudates (Tisdall and Oades, 1982). One important organism group controlling the formation of soil macroaggregates are arbuscular mycorrhizal fungi (AMF) (Tisdall and Oades, 1982; Tisdall, 1991; Schreiner and Bethlenfalvay, 1995; Miller and Jastrow, 2000; Rillig, 2004; Six et al., 2004; Bronick and Lal, 2005; Rillig and Mummey, 2006).

Generally, AMF are one of the principal functional components in below-ground ecosystems (Smith and Read, 2008), and potentially influence soil aggregation over a spectrum of ecological scales (for a detailed discussion see Rillig and Mummey, 2006). First, AMF are known to affect plant community composition and net primary production, for example by providing differential nutritional benefits to plant species (van der Heijden et al., 1998; Klironomos et al., 2000); thus AMF can indirectly influence soil aggregation at a comparably large scale (Piotrowski et al., 2004; Chaudhary et al., 2009). Second, besides the well-known nutritional advantages a single host plant derives from the symbiosis (Read, 1991; Marschner and Dell, 1994), AMF substantially alter biochemical (Shachar Hill et al., 1995; Jones et al., 2004) and morphological (Berta et al., 1993, 1995) properties of their host plant, including its roots and its rhizosphere, which can convert into effects on soil aggregation. Third, the fungal mycelium itself has a direct effect on soil aggregation (see discussion below). And fourth, AMF can alter soil microbial communities both in their own surrounding and in the host plant rhizosphere (Andrade et al., 1997, 1998; Mansfeld-Giese et al., 2002; Artursson and Jansson, 2003;

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Artursson, 2005; Rillig et al., 2006), which possibly are involved in soil aggregation processes (Caesar-TonThat et al., 2007).

The mechanisms by which the AMF mycelium influences soil aggregation are multifaceted and often strongly interdependent. The AMF mycelium contributes to soil aggregation either directly through the hyphal network, which enmeshes soil particles and forces these together (Tisdall, 1994) or aligns primary particles along its expanding hyphae (Chenu and Stotzky, 2002), or indirectly via exuding compounds into the soil (e.g. glomalin-related soil protein, polysaccharides) that may act like glues and bind soil particles together (Chenu, 1989; Wright and Upadhyaya, 1998). Recently, Rillig et al. (2010) found that even AMF hyphae alone in the absence of any soil biota, such as a plant host or other microorganisms, are sufficient to positively influence soil aggregation. The AMF extramatrical hyphal network is a major component of the soil microbial biomass (Olsson et al., 1999) and can reach lengths of up to 111 m $\times$ cm^{-3} soil (Miller et al., 1995), which suggests that AMF could play an important role in the fungal energy channel of the soil food web, by representing a prey to a variety of soil faunal groups, e.g. collembola (Finlay, 1985; Fitter and Sanders, 1992).

Collembola (or springtails) belong to the soil mesofauna and are one of most abundant soil arthropods often exceeding individual numbers of 100.000 m^{-2} (Petersen and Luxton, 1982). This animal group has a major influence on decomposition processes in soils (e.g. Hopkin, 1997) and is thought to affect soil structure by crawling and digging in the soil. Due to their feeding behavior, collembola incorporate considerable amounts of organic matter into fecal pellets, which increases the surface area and accessibility for bacterial and fungal utilization and thus decomposition (Takeda, 1988; van Amelsvoort et al., 1988; Lee and Foster, 1991; Lussenhop, 1992; Giller et al., 1997; Coleman et al., 2004). Lussenhop (1992) already hypothesized that the feces of soil microarthropods could contribute to soil aggregation by serving as starting nuclei for soil aggregates.

Collembola feed on a great variety of different food sources, such as bacteria, debris, roots or nematodes (Crossley et al., 1992), but almost all feed on fungal hyphae and show strong preferences for specific fungal species (Moore et al., 1985; Fountain and Hopkin, 2005). Even if among the fungi the AMF are not the preferred food source of collembola (Klironomos and Kendrick, 1996; Klironomos and Ursic, 1998), they can significantly affect AMF. The activities of collembola were reported to have varying effects on AM mycelium and the AM-plant symbiosis with respect to plant growth, ranging from stimulative to repressive, (Warnock et al., 1982; Kaiser and Lussenhop, 1991; Klironomos and Kendrick, 1995; Larsen and Jakobsen, 1996a; Johnson et al., 2005; Steinaker and Wilson, 2008). Whereas in some experiments collembola reduced the numbers of AMF spores (Bakonyi et al., 2002), or decreased AM colonization and soil hyphal length (Boerner and Harris, 1991; Larsen and Jakobsen, 1996b), other studies revealed that they can positively influence the dispersal of AM inoculum (Klironomos and Moutoglis, 1999), and enhance plant growth, root biomass, or plant N uptake (Harris and Boerner, 1990; Lussenhop, 1996). Based on these effects, Gange (2000) hypothesized that the response of an AM-plant association influenced by an increasing collembolan density is likely bell-shaped, with intermediate animal densities stimulating AMF and their functioning.

Considering the important role of collembola in the decomposer food web, and their interactions with a major player of soil aggregation, the population of AMF, it is important to examine the effects of collembola on soil aggregation, directly and indirectly via AMF. With the exception of our recent paper (Caruso et al., 2011) we are not aware of any literature that experimentally investigated the role of collembola (or soil microarthropods in general) on soil aggregation. Previously, Davidson and Grieve (2006) have employed a size fraction approach in which at least collembola, mites and enchytreids were jointly examined in their effects on soil structure.

Therefore, in this study we asked the following main questions: (i) Do Collembola have an effect on soil aggregation? (ii) And, do Collembola alter AMF-mediated effects on soil aggregation? Our hypotheses were that collembola would enhance soil aggregation in a hierarchically structured soil, but that they would reduce the positive influence of AMF on soil aggregation, because of consumption of AMF hyphae or severing considerable parts of the AMF hyphal network from its plant host. In order to test these ideas we carried out a factorial experiment in the greenhouse, manipulating the presence of both AMF and collembola.

2. Materials and methods

2.1. Experimental design and greenhouse experiment

We tested the effects of, and interactions between, plant species, presence/absence of collembola, and presence/absence of AMF in a 2 $\times$ 2 $\times$ 2 factorial greenhouse experiment in which seven replicates were set up for each treatment combination for a total of 56 experimental units (pots).

We used a sandy soil (Albic Luvisol) collected from the experimental field of Freie Universität Berlin. The soil properties were: sand = 74%, silt = 18% and clay = 8%; 6.9 mg/100 g P (calcium-acetate-lactate); 5.0 mg/100 g K (calcium-acetate-lactate); 0.12% N (total); 1.87% C (total) and soil pH was 7.1 (analyses conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany; and on a Euro EA C/N analyzer, HEKAtech GmbH, Wegberg, Germany). The soil was chosen due to its high mycorrhizal inoculum potential (Rillig et al., 2010). Soil was sieved (10 mm) prior to use to remove stones and root materials. In order to reduce soil fertility, the soil was thoroughly mixed with sand (70% soil with 30% sand). Following that, the soil was steamed at 90 °C (4 h) to eliminate AMF and collembola, and then filled into 4 L pots (3.0 kg soil per pot). For the AMF treatment soil was thoroughly mixed with an AMF spore inoculum. Soil for inoculum was also collected from the same experimental field, and this inoculum was produced according to the method of Klironomos (2002). Inoculum from 300 g of soil was added to each pot. At the same time, we collected microbial wash through a 20 μm sieve; the filtrate was added to pots not receiving AMF inoculum in an attempt to equilibrate the microbial communities between the treatments. The plant treatment consisted of one of two species: *Sorghum vulgare* or *Daucus carota;* the plants were chosen to represent different root characteristics. We initially added two seedlings per pot, but after 1 week we thinned to one plant (per pot) which was left to grow for a period of 16 weeks. We added collembola on the 2nd week of our experiment in order to permit initial development of AMF mycelium. The collembola treatment consisted of 80 *Proisotoma minuta* (Family- Isotomidae; Order- Collembola) (laboratory culture since 2005; was isolated from northern Germany) individuals per pot which we reared in our lab before starting the greenhouse experiment. The average temperature of the greenhouse was 22 °C, and pots were watered as needed to avoid water stress (about every two days). The position of pots was re-randomized once a week.

2.2. Plant and fungal measurements

After harvesting, plant shoots and roots were dried at 40 °C for 72 h and then weighed. We then measured the root length using a scanner-based method and Win Rhizo software (Scanner: Epson Perfection V700 PHOTO; Software: Win RHIZO, Pro, 2007d; Regents Instruments, Quebec, Canada). We confirmed the presence of AMF