Soil Biology & Biochemistry 41 (2009) 561–567

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated

Nico Eisenhauer [a,*,1,2], Stephan König [b,1], Alexander C.W. Sabais [a], Carsten Renker [b,3], Francois Buscot [b], Stefan Scheu [a,2]

[a] Darmstadt University of Technology, Institute of Zoology, Schnittspahnstrasse 3, 64287 Darmstadt, Germany
[b] UFZ Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Straße 4, 06120 Halle, Germany

ARTICLE INFO

Article history:
Received 20 October 2008
Received in revised form
3 December 2008
Accepted 11 December 2008
Available online 9 January 2009

Keywords:
Aporrectodea caliginosa
$\delta^{15}N$
Grassland
Lumbricus terrestris
Soil organism interactions
The Jena Experiment

ABSTRACT

Earthworms and arbuscular mycorrhizal fungi (AMF) might interactively impact plant productivity; however, previous studies reported inconsistent results. We set up a three-factorial greenhouse experiment to study the effects of earthworms (*Aporrectodea caliginosa* Savigny and *Lumbricus terrestris* L.) and AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) on the performance (productivity and shoot nutrient content) of plant species (*Lolium perenne* L., *Trifolium pratense* L. and *Plantago lanceolata* L.) belonging to the three functional groups grasses, legumes and herbs, respectively. Further, we investigated earthworm performance and plant root mycorrhization as affected by the treatments. Our results accentuate the importance of root derived resources for earthworm performance since earthworm weight (*A. caliginosa* and *L. terrestris*) and survival (*L. terrestris*) were significantly lower in microcosms containing *P. lanceolata* than in those containing *T. pratense*. However, earthworm performance was not affected by AMF, and plant root mycorrhization was not modified by earthworms. Although AMF effectively competed with *T. pratense* for soil N (as indicated by $\delta^{15}N$ analysis), AMF enhanced the productivity of *T. pratense* considerably by improving P availability. Remarkably, we found no evidence for interactive effects of earthworms and AMF on the performance of the plant species studied. This suggests that interactions between earthworms and AMF likely are of minor importance.

1. Introduction

Soil organisms are essential for nutrient cycling and organic matter turnover thereby functioning as key determinants of soil fertility and nutrient uptake by plants (Bradford et al., 2002; Scheu, 2003; Wardle et al., 2004). Nevertheless, studies on belowground communities and their impacts on ecosystem properties are a relatively new field in ecology (Bardgett et al., 2005). Most studies focussed on a particular group of soil organisms and only a limited number of experiments considered belowground interactions and functional diversity (e.g. Bradford et al., 2002; Wurst et al., 2004, 2008; Partsch et al., 2006; Endlweber and Scheu, 2007). Recent studies highlight that interacting effects of functionally dissimilar soil organisms on ecosystem functioning are of particular importance since individual effects of soil organism groups may cancel out each other in combination (Bradford et al., 2002; Wurst et al., 2008). Moreover, impacts of soil organisms on plant productivity likely are plant species specific (Kreuzer et al., 2004; Wurst et al., 2005; Partsch et al., 2006; Eisenhauer and Scheu, 2008a).

Arbuscular mycorrhizal fungi (AMF) is the dominant type of mycorrhiza in grassland ecosystems and most of the herbaceous plants (80%) are colonized (Wang and Qiu, 2006). Fungal symbionts build hyphal networks (mycelia) extending the plant root system and thereby enhancing plant nutrient uptake and growth (Smith and Read, 1997). While P uptake generally is increased in AMF colonized plants (Marschner and Dell, 1994; Tuffen et al., 2002; Wurst et al., 2004), mycorrhization of roots was also reported to enhance plant uptake of N, K, Cu and Zn (Marschner and Dell, 1994; Blanke et al., 2005; Ma et al., 2006). However, mycorrhizal fungi and plant roots may also compete for nutrients; e.g., Wurst et al. (2004) suggested competition for soil N between AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) and roots of *Plantago lanceolata* L. Presumably, the preponderance of mutualistic or competitive mechanisms depends on nutrient availability and likely is AMF and plant species specific.

Earthworms are a major component of many terrestrial ecosystems usually dominating the biomass of soil invertebrates in

* Corresponding author. Tel.: +49 6151 164299; fax: +49 6151 166111.
E-mail address: eisenhauer@bio.tu-darmstadt.de (N. Eisenhauer).
[1] Authors contributed equally to this work.
[2] Present address: Georg August University of Göttingen, J.F. Blumenbach Institute of Zoology and Anthropology, Berliner Str. 28, 37073 Göttingen, Germany.
[3] Present address: Natural History Museum Mainz, Reichklarastraße 10, 55116 Mainz, Germany.

0038-0717/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2008.12.017

non-acidic soils (Edwards and Bohlen, 1996). Particularly anecic species, such as *Lumbricus terrestris* L., function as ecosystem engineers by structuring the environment of other soil organisms (Lavelle, 1988; Jones et al., 1994; Scheu and Setälä, 2002). The endogeic species *Aporrectodea caliginosa* Savigny is among the most abundant earthworm species in temperate grasslands (Edwards and Bohlen, 1996) and has been used as model organism in laboratory studies (Tuffen et al., 2002; Wurst et al., 2004; Partsch et al., 2006). Through burrowing, casting and mixing of litter and soil (bioturbation) earthworms influence aggregate stability, soil structure, infiltration of water, aeration of deeper soil layers, microbial biomass and nutrient mineralization (Edwards and Bohlen, 1996; Tiunov and Scheu, 1999; Eisenhauer et al., 2007).

Earthworms may process the upper 10 cm of the soil within a period of 5 years (Edwards and Bohlen, 1996) and *A. caliginosa* feeds predominantly in the upper 7 cm of the soil (Sims and Gerard, 1999). Therefore, earthworms may profoundly impact the symbiosis between AMF and plants directly, e.g. via damaging fungal hyphae, and indirectly, e.g. via modifying nutrient availability. Indeed, earthworms were shown to selectively feed on fungal mycelia (Bonkowski et al., 2000). Beside detrimental effects, AMF may benefit from increased spore dispersal and colonization of plant roots in presence of earthworms (Reddel and Spain, 1991; Gange, 1993; Lee et al., 1996; Gormsen et al., 2004). Recent experiments suggest that earthworms and AMF indeed complement each other in fostering plant nutrient uptake and productivity (Yu et al., 2005; Ma et al., 2006). However, colonization of roots by mycorrhiza has also been shown to be reduced in presence of earthworms (Pattinson et al., 1997; Lawrence et al., 2003; Ortiz-Ceballos et al., 2007). In part the conflicting results may be due to the fact that most previous studies on earthworm–AMF interactions considered different plant species and were each restricted to single plant species. Considering these weaknesses, the present study investigates interactive impacts of earthworms and AMF on the performance (productivity and nutrient uptake) of different plant species from three functional groups. Further, we studied the effects of earthworm presence and plant species identity on plant root mycorrhization and the impacts of AMF presence and plant species identity on earthworm performance to identify the mechanisms responsible for earthworm–AMF interactions. Overall, we hypothesized that (1) earthworms and mycorrhiza interactively impact plant performance and (2) effects vary between different plant species.

2. Materials and methods

2.1. Experimental setup

We set up microcosms consisting of PVC tubes (inner diameter 10 cm, height 25 cm) covered by a 1 mm mesh at the bottom to prevent earthworms (*L. terrestris* and *A. caliginosa*) from escaping but allowing water drainage. Furthermore, a plastic barrier (10 cm height) prevented earthworms from escaping from experimental containers. The soil (pH 8.1, C concentration 4.6%, N concentration 0.3%, C-to-N ratio 15.7, and gravimetric water content 17%) was taken from the field site of the Jena Experiment (Jena, Germany; Roscher et al., 2004). The Jena Experiment is a long-term grassland study investigating interactions between plant diversity and ecosystem processes and focussing on element cycling and trophic interactions (Roscher et al., 2004). The site was formerly used as typical Central European mesophilic grassland and the soil is a Eutric Fluvisol (FAO-UNESCO, 1997). A total of 60 microcosms each filled with 1.5 kg (fresh weight; height of soil core 20 cm) of sieved (2 cm), defaunated (autoclaved twice, each 20 min at 120 °C) and homogenized soil were placed in a temperature controlled greenhouse at a day/night regime of 16/8 h and 20/16 ± 2 °C. Before starting the experiment the microcosms were watered regularly for two weeks (50 ml of deionized water every second day) to leach nutrients released as a result of the defaunation procedure. We added 10 g AMF inoculum to half of the microcosms (treatments with AMF) consisting of culture substrate mixed with *G. intraradices* hyphae and spores (Sybio-m s.r.o., Lanskroun, Czech Republic) and mixed the inoculum with the upper 5 cm of the soil core. Then, 25 ml soil suspension was added to each microcosm to inoculate the autoclaved soil with microorganisms. For preparing the suspension, 500 g fresh soil from the field site of the Jena Experiment was dispensed in 1.5 l deionized water and filtered through a 25 μm mesh for eliminating AMF spores (Schroeder and Janos, 2004).

Six pre-germinated plant individuals (three weeks old, height 4–8 cm, grown up in autoclaved Jena soil) belonging to one of three plant species each representing one functional group, i.e. *Lolium perenne* L. [grass], *Trifolium pratense* L. [legume] and *P. lanceolata* L. [herb], selected from the species pool of the Jena Experiment (Roscher et al., 2004), were transplanted separately into microcosms creating three plant species treatments (~200 ind./m^2). Dried ^{15}N labeled *L. perenne* litter (800 mg, 40 atom% ^{15}N, C concentration 35.8%, N concentration 1.5%, C-to-N ratio 24.7, cut into pieces about 2 cm in length) was placed on top of the soil of all microcosms prior to the addition of the earthworms to simulate field soil surface conditions. Earthworms were extracted at the field site of the Jena Experiment using the octet method (Thielemann, 1986) three weeks before experimental setup. One subadult *L. terrestris* (average fresh weight with gut content 1.42 ± 0.05 g) and one subadult *A. caliginosa* (0.45 ± 0.01 g) were added to half of the microcosms establishing two treatments (with and without earthworms). We set up five replicates of each of the treatments (plant species [3] × earthworms [2] × AMF [2]).

The experiment lasted for three months and light intensity varied between 450 and 650 μE/ms depending on weather conditions. The water regime was gradually increased by irrigating four times a week with 35 ml (weeks 1–3) to 50 ml (weeks 4–6) and 100 ml (weeks 7–12) deionized water. Thereby, all microcosms received the same amount of water to avoid effects of different water availability. Microcosms were randomized every two weeks.

2.2. Sampling

Before harvesting the plants, we counted the number of flower heads per *T. pratense* individual. Then, plant shoots were harvested by cutting them at soil surface level and pooled per microcosm. Roots were washed out of the soil using a 1 mm mesh. A subsample of roots (2.20 ± 0.11 g dry weight) was fixed in formaldehyde–acetic acid (FAA; 6.0% formaldehyde, 2.3% glacial acetic acid, 45.9% H_2O, and 45.8% ethanol (v/v)) to analyze the colonization of roots by mycorrhiza. Shoot and the rest of the root materials were dried at 60 °C for three days. To follow the flux of N from the labeled litter material and the flux of P from the soil to the plants we ground the shoot material of *L. perenne* and *T. pratense* harvested from each microcosm separately. The analyses were restricted to these two plant species since only the productivity of these species was affected by the treatments.

Earthworms were collected by hand and weighed individually (fresh weight with gut content). Then, earthworms were killed by freezing (−20 °C) and dried at 60 °C for three days. The anterior end of *A. caliginosa* (without gut content) was used to analyze N concentration and ^{15}N signatures in earthworm tissue. The analyses were restricted to this species due to the similar effect of plant species on both earthworm species and the higher number of replicates for *A. caliginosa*.