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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

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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.

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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*.

For staining of mycorrhiza, roots were incubated in 10% KOH at 95 °C for 10–15 min depending on their morphological consistence (*P. lanceolata* 10 min, *T. pratense* 12 min, and *L. perenne* 15 min), rinsed with tap water, and acidified with 3.7% HCl for 5 min (Phillips and Hayman, 1970). Roots were stained with pelican blue ink with the staining solution consisting of 5% ink diluted in 5% acetic acid. For decolorization and short time storage, roots were deposited in tap water (Vierheilig and Piche, 1998). Percentage colonization (total, arbuscules and vesicles) of root length was determined with a Zeiss Axioplan microscope at 100× magnification using the line-intersect method (Ambler and Young, 1977, modified after Schmitz et al., 1991). At least 200 segments of each root sample were counted.

2.3. Chemical analyses

Approximately 3.5 mg of the powdered plant shoot material of *L. perenne* and *T. pratense* and 1.5 mg of body tissue of *A. caliginosa* were weighed into tin capsules. Total N concentrations and ^{15}N signatures were determined by a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Milan) and a gas isotope mass spectrometer (MAT 251, Finngan; Reineking et al., 1993). For ^{15}N atmospheric N_2 served as the primary standard and acetanilide (C_8H_9NO; Merck, Darmstadt, Germany) was used for internal calibration.

Total P concentration of ground plant shoot material was analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Samples were dissolved in concentrated nitric acid and heated in a microwave system at 230 °C for 20 min. Then, total P was measured in a Spectro Ciros ccd (Spectro Analytical Instruments GmbH, Kleve, Germany) at two different wave lengths (177.4 nm and 178.3 nm) according to the EC Guideline EN ISO 11885. The amount of total shoot N and P per microcosm was calculated by multiplying shoot biomass with the N and P concentrations of the corresponding plant species.

2.4. Statistical analyses

Normal distribution and homogeneity of variance were improved by log-transformation, if necessary. Analysis of variance (ANOVA; type III SS) was used to analyze the effects of earthworms (E; with and without *L. terrestris* and *A. caliginosa*) and plant species (S; *L. perenne*, *T. pratense* and *P. lanceolata*) on root mycorrhization (total, arbuscules and vesicles). Further, ANOVA was used to analyze the effects of AMF (M; with and without *G. intraradices*) and plant species on the body fresh weight of *L. terrestris* and *A. caliginosa*, and on N concentration and ^{15}N signatures in body tissue of *A. caliginosa*. Only microcosms containing the respective earthworm species at the end of the experiment were considered. In addition, ANOVA was used to analyze the effects of earthworms, AMF and plant species on plant productivity (shoot biomass per microcosm, root biomass per microcosm, total plant biomass per microcosm and shoot-to-root ratio) and the effects of earthworms and AMF on the number of flower heads per *T. pratense* individual, and on shoot N concentration, ^{15}N signatures in shoots, amount of N per microcosm, shoot P concentration and the amount of P per microcosm (for *L. perenne* and *T. pratense*). Although some earthworm individuals died during the experiment, we did not exclude any replicates from the analyses since at least one earthworm individual survived per microcosm.

ANOVAs and comparisons of means (Tukey's HSD test, $\alpha < 0.05$) were performed using SAS 9.1 (SAS Inst., Cary, North Carolina, USA). Non-transformed means are presented in text and figures (±S.E.).

3. Results

3.1. Soil organisms

Generally, colonization of plant roots by *G. intraradices* in control treatments (without AMF) was negligible (2.00 ± 0.49%) and did not depend on earthworm presence and plant species (not shown). Total mycorrhization of plant roots in the *G. intraradices* treatment was significantly higher in *T. pratense* (×4.0; 85 ± 3%) and *P. lanceolata* (×2.8; 59 ± 13%) than in *L. perenne* (21 ± 5%; Table 1). Similarly, mycorrhization of plant roots by arbuscules and vesicles was significantly higher in *T. pratense* (×5.4; 66 ± 3% and ×5.3; 61 ± 4%) and *P. lanceolata* (×3.9; 48 ± 8% and ×3.8; 44 ± 7) than in *L. perenne* (12 ± 2% and 12 ± 3%; Table 1). However, presence of earthworms did not modify mycorrhization rates of all three plant species (Table 1).

A total of 20 of the 30 *L. terrestris* individuals (67%) and 28 of the 30 *A. caliginosa* individuals (93%) added to the microcosms survived the three month experiment. Survival of *L. terrestris* and *A. caliginosa* was not affected by AMF ($F_{1,24} = 0.73$, $P = 0.40$ and $F_{1,24} < 0.01$, $P = 1.00$, respectively). Survival of *A. caliginosa* was also not affected by plant species ($F_{2,24} = 2.00$, $P = 0.16$), but survival of *L. terrestris* was significantly higher in microcosms with *T. pratense* (100 ± 0%) than in those with *P. lanceolata* (40 ± 16%) but did not differ significantly from survival in microcosms containing *L. perenne* (60 ± 16%; $F_{2,24} = 5.09$, $P = 0.014$). Presence of *G. intraradices* did not affect body fresh weight of *A. caliginosa* (overall mean 96 ± 6% of initial fresh weight) and *L. terrestris* (101 ± 6%; Table 1). By contrast, plant species significantly affected earthworm body weight. Both, *A. caliginosa* and *L. terrestris* lost weight in presence of *P. lanceolata* (74 ± 8% and 71 ± 12% of initial body fresh weight, respectively) compared to microcosms containing *L. perenne* (105 ± 11% and 104 ± 5%, respectively) and *T. pratense* (109 ± 12% and 114 ± 4%, respectively; Table 1). However, N concentration (10.07 ± 0.69%) and ^{15}N signature (0.96 ± 0.04 atom%) in body tissue of *A. caliginosa*

Table 1
ANOVA table of *F*- and *P*-values for the effects of presence of earthworms (E) and plant species (S) on mycorrhizal colonization of plant roots by *Glomus intraradices* ([%]; total colonization, arbuscules, vesicles) and presence of *Glomus intraradices* (M) and S on earthworm biomass [g fresh weight].

	D.f.	*F*-value	*P*-value
Glomus intraradices			
Total colonization			
E	1, 24	0.34	0.564
S	2, 24	**20.07**	**<0.001**
E × S	2, 24	1.00	0.387
Arbuscules			
E	1, 24	0.09	0.773
S	2, 24	**29.66**	**<0.001**
E × S	2, 24	1.15	0.334
Vesicles			
E	1, 24	0.65	0.428
S	2, 24	**24.21**	**<0.001**
E × S	2, 24	0.42	0.657
Aporrectodea caliginosa			
M	1, 22	0.20	0.663
S	2, 22	**6.00**	**0.008**
M × S	2, 22	2.15	0.141
Lumbricus terrestris			
M	1, 14	1.08	0.316
S	2, 14	**6.58**	**0.010**
M × S	2, 14	2.35	0.132

Significant effects are given in bold.
D.f. = degrees of freedom.

neither was affected by AMF ($F_{1,22} = 0.12$, $P = 0.73$ and $F_{1,22} < 0.01$, $P = 0.98$, respectively) nor by plant species ($F_{2,22} = 2.42$, $P = 0.11$ and $F_{2,22} = 0.74$, $P = 0.49$, respectively).

3.2. Plant productivity

Shoot biomass per microcosm of *P. lanceolata* (5.96 ± 0.27 g) exceeded that of *T. pratense* (3.47 ± 1.10 g) but did not differ significantly from that of *L. perenne* (4.60 ± 0.23 g; Table 2). Root biomass and total biomass per microcosm of *P. lanceolata* (8.88 ± 0.59 g and 14.97 ± 0.78 g, respectively) and *L. perenne* (9.77 ± 0.88 g and 14.45 ± 1.00 g, respectively) significantly exceeded that of *T. pratense* (2.63 ± 0.67 g and 6.43 ± 1.65 g, respectively). However, shoot-to-root ratio of *T. pratense* (1.55 ± 0.17) significantly exceeded that of *P. lanceolata* (0.70 ± 0.04) and that of *T. pratense* (0.50 ± 0.04). While the presence of AMF significantly reduced shoot (−17%), root (−24%) and total biomass (−22%) of *L. perenne*, shoot (×4.7), root (×2.8) and total biomass of *T. pratense* (×3.7) were increased (Table 2, Fig. 1A). Productivity of *P. lanceolata* was not affected by AMF. Earthworms did not affect plant shoot and total biomass per microcosm, but significantly increased root biomass of *L. perenne* (+74%; Table 2, Fig. 1B). Neither earthworms nor AMF affected plant shoot-to-root ratio. Moreover, none of the plant productivity parameters were affected by the interaction between earthworms and AMF (Table 2).

3.3. Lolium perenne

N concentration in plant shoot tissue (0.73 ± 0.03%), amount of shoot N per microcosm (27.73 ± 5.61 mg), P concentration in plant shoot tissue (0.064 ± 0.026%) and amount of shoot P per microcosm (3.85 ± 0.35 mg) were not affected by earthworms and AMF (Table 3). Also, AMF did not affect ^{15}N signature of plant shoot tissue (0.81 ± 0.03 atom%) but in presence of earthworms it tended to be increased (×3.8; Table 3). Plant performance parameters were not affected by the interaction between earthworms and AMF (Table 3).

3.4. Trifolium pratense

The number of flower heads (1.21 ± 0.30 per legume individual), amount of shoot N per microcosm (50.58 ± 18.60 mg), P concentration in shoot tissue (0.066 ± 0.026%) and amount of shoot P per microcosm (2.92 ± 1.04 mg) were not significantly affected by earthworms, but earthworms tended to increase N concentration (1.06 ± 0.06%) and ^{15}N signature (0.61 ± 0.06 atom%) in shoot tissue (+19% and ×3.1, respectively; Table 3). Further, AMF significantly increased the number of flower heads (×3.8) and the amount of shoot P per microcosm (×4.2), whereas ^{15}N signature in shoot tissue was reduced significantly (−84%) and N concentration tended to be reduced (−16%; Table 3). However, none of the plant performance parameters were affected by the interaction between earthworms and mycorrhiza (Table 3).

4. Discussion

4.1. Soil organisms

Generally, the soil fauna strongly depends on plant-derived C sources entering the belowground system via dead plant material and root exudates. Since the quality and quantity of resources entering the soil differ between plant species, composition and activity of the soil community and of soil processes likely vary with plant species (Wardle et al., 2004). However, earthworms appear to depend on the quantity and quality of litter rather than on plant species composition *per se* (Spehn et al., 2000; Milcu et al., 2008; Eisenhauer and Scheu, 2008b). In the present study both earthworm species performed best in microcosms containing the legume *T. pratense*. Legumes function as key plant functional group by fixing atmospheric N and increasing soil N availability (Temperton et al., 2007; Roscher et al., 2008). Although other studies argued that earthworms are unresponsive to changes in plant species composition (Wardle et al., 1999; Hedlund et al., 2003), results of a recent greenhouse experiment indicate that earthworms also benefit from legumes without legume leaves entering the soil (Milcu et al., 2006). The authors argued that dead roots and root exudates form essential belowground resources for earthworms. Results of the present study underscore these conclusions. Both *A. caliginosa* and *L. terrestris* lost weight in microcosms containing *P. lanceolata* (−26% and −29%, respectively) but not in those containing *T. pratense* or *L. perenne* although aboveground litter material did not differ between the treatments. Further, survival of *L. terrestris* was considerably lower in presence of *P. lanceolata* than in presence of *T. pratense* (−60%). *Plantago lanceolata* appears to detrimentally affect earthworm performance in general as Wurst et al. (2004) also reported a decline in body weight of *A. caliginosa* in presence of this plant species (−34%). *P. lanceolata* most likely provides dead root material and root exudates of low quality and quantity compared to *T. pratense* (N-rich root material) and *L. perenne* (high root biomass). However, the mechanisms responsible for differential performance of earthworms in the rhizosphere of plants need further investigation.

Although *A. caliginosa* and *L. terrestris* were shown to selectively feed on fungal mycelia (Bonkowski et al., 2000), the presence of AMF did not impact earthworm performance in the present experiment. This suggests that dead organic matter and root deposits are more important for earthworm nutrition than mycelium of *G. intraradices*. This might have been due to the relatively small extraradical mycelium of *G. intraradices* (De la Providencia et al., 2005).

Colonization of roots by mycorrhiza differed markedly between plant species. The results support previous observations of high

Table 2
ANOVA table of *F*-values for the effects of earthworms (E), mycorrhiza (M) and plant species (S) on plant shoot biomass [g/microcosm], root biomass [g/microcosm], total biomass [g/microcosm] and shoot-to-root ratio.

	D.f.	Shoot biomass			Root biomass			Total biomass			Shoot-to-root ratio	
		F-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value
E	1, 48	0.24	0.625		0.66	0.421		0.17	0.684		0.27	0.607
M	1, 48	**7.48**	**0.008**	↑	**4.34**	**0.043**	↑	**7.17**	**0.010**	↑	0.52	0.473
S	2, 48	**4.27**	**0.020**		**33.88**	**<0.001**		**17.77**	**<0.001**		**26.18**	**<0.001**
E × M	1, 48	0.26	0.610		2.90	0.095		1.86	0.179		1.36	0.249
E × S	2, 48	0.41	0.667		**3.24**	**0.048**		2.38	0.104		0.87	0.424
M × S	2, 48	**11.77**	**<0.001**		**6.72**	**0.003**		**10.80**	**<0.001**		1.15	0.326
E × M × S	2, 48	0.67	0.515		0.83	0.444		0.83	0.442		0.24	0.784

Significant effects are given in bold.
D.f. = degrees of freedom; ↑ = increase.

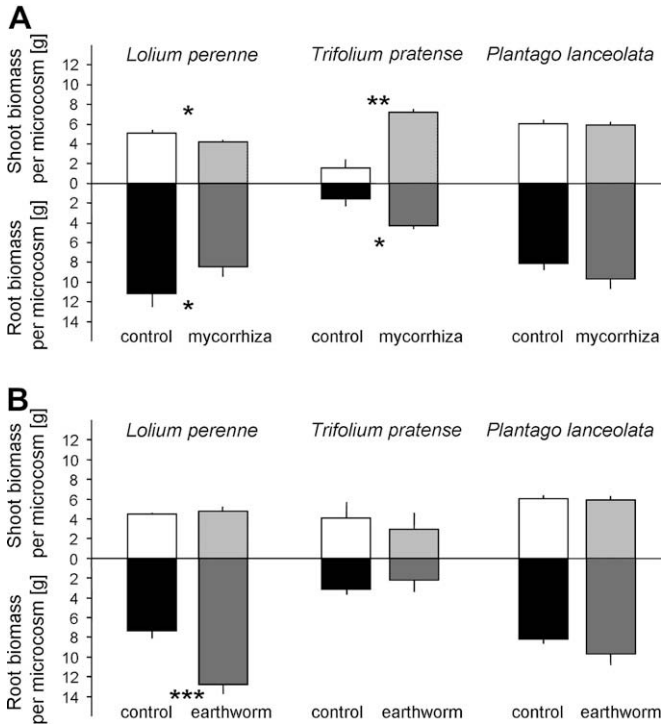

Fig. 1. Variations in shoot and root biomass [g/microcosm] of *Lolium perenne*, *Trifolium pratense* and *Plantago lanceolata* as affected by (A) presence of mycorrhiza (*Glomus intraradices*) and (B) presence of earthworms (*Lumbricus terrestris*). Significant differences are indicated by asterisks (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). Means with S.E.

colonization of legume roots by AMF (*T. pratense*; 85%) but low rates in grasses (*L. perenne*; 21%; Hokka et al., 2004; Scheublin et al., 2004, 2007). However, these colonization patterns by AMF were not affected by earthworm presence. Establishing the experiment, we mixed the *G. intraradices* inoculum thoroughly with the upper soil in the microcosms. Therefore, the previously reported positive effects of earthworms on AMF by increasing spore dispersal and plant root colonization (Reddel and Spain, 1991; Gange, 1993; Lee et al., 1996; Gormsen et al., 2004) possibly were eliminated in the present study. However, earthworms may also mechanically damage AMF mycelium and digest fungal spores, thereby, negatively impacting the colonization of plant roots and the mycorrhiza formation (Pattinson et al., 1997; Tuffen et al., 2002; Lawrence et al., 2003; Ortiz-Ceballos et al., 2007). Despite the variety of mechanisms by which earthworms may affect mycorrhiza, we observed neither positive nor negative effects of earthworms on the colonization of plant roots from the three plant species investigated, which is consistent with the results of Wurst et al. (2004). In contrast to Tuffen et al. (2002) who used earthworm densities up to 500 ind./m^2 and similar to the experiment of Wurst et al. (2004), we used earthworm densities resembling those occurring in temperate grasslands (255 ind./m^2). Therefore, we assume that the impact of earthworms on the colonization of plant roots by AMF in temperate grasslands is likely to be negligible.

4.2. Plant productivity

Generally, under given illumination conditions, plant productivity essentially relies on the availability of nutrients. As decomposers, earthworms enhance nutrient mineralization and availability for plants (Edwards and Bohlen, 1996; Scheu, 2003; Eisenhauer and Scheu, 2008a), while AMF form prominent symbioses with roots increasing their potential to mobilize mineralized nutrients, primarily P (Smith and Read, 1997; Tuffen et al., 2002; Wurst et al., 2004). Thus, earthworms and AMF increase plant nutrient supply via very different but congruent mechanisms, and they have been found to additively affect plant nutrient uptake (Tuffen et al., 2002; Yu et al., 2005; Ma et al., 2006). However, in contrast to these findings and our hypothesis (1) earthworms and AMF neither interacted in affecting plant productivity (*L. perenne*, *T. pratense* and *P. lanceolata*) nor plant shoot nutrient concentrations (*L. perenne* and *T. pratense*) in the present study. These results indicate that earthworms and AMF rather independently modified plant performance.

In presence of AMF shoot and root biomass of *L. perenne* were decreased, whereas they were increased in *T. pratense*. In agreement with the hypothesis that AMF predominantly increase plant P supply, the amount of shoot P in *T. pratense* was increased in presence of AMF, and this likely was responsible for the increase in shoot and root biomass and the number of flower heads. In contrast, N concentration and ^{15}N atom% were decreased in *T. pratense* shoots in presence of AMF supporting the conclusion of Wurst et al. (2004) that AMF and plant roots may compete for soil N. Presumably, *G. intraradices* effectively sequestered N mineralized from the litter, allowing to more efficiently exploit soil P and, thereby, increasing plant P supply and plant productivity (shoot,

Table 3
ANOVA table of *F*- and *P*-values for the effects of earthworms (E) and mycorrhiza (M) on plant parameters of *Lolium perenne* (shoot N concentration [%], amount of shoot N per microcosm [mg], ^{15}N atom%, shoot P concentration [%], amount of shoot P per microcosm [mg]) and *Trifolium pratense* (number of flower heads per individual, shoot N concentration [%], amount of shoot N per microcosm [mg], ^{15}N atom%, shoot P concentration [%], amount of shoot P per microcosm [mg]).

	Earthworms			Mycorrhiza			E × M	
	F-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value
Lolium perenne								
N concentration	2.13	0.164		1.13	0.304		1.64	0.219
N amount	0.69	0.419		0.06	0.813		1.05	0.321
^{15}N atom%	3.93	0.065	(↑)	0.27	0.607		1.70	0.210
P concentration	0.91	0.355		0.87	0.365		0.64	0.436
P amount	0.99	0.333		0.25	0.622		0.55	0.469
Trifolium pratense								
Flower heads	0.33	0.573		**15.52**	**0.001**	↑	0.85	0.371
N concentration	3.64	0.074	(↑)	3.99	0.062	(↓)	1.25	0.280
N amount	0.13	0.721		2.78	0.114		0.82	0.376
^{15}N atom%	3.12	0.096	(↑)	**9.62**	**0.006**	↓	0.57	0.462
P concentration	1.01	0.329		0.62	0.440		0.30	0.591
P amount	0.01	0.952		**6.77**	**0.019**	↑	0.23	0.638

Significant effects ($P < 0.05$) are given in bold.
Degrees of freedom (D.f.) factor = 1; D.f. error = 16; ↑ = increase; ↓ = decrease.

root and total biomass and number of flower heads). Therefore, *T. pratense* likely was limited by P and benefited from the symbiosis with AMF despite lower N capture from the litter. By contrast, negative AMF effects on *L. perenne* cannot be attributed to competition for soil nutrients. Rather, decreased shoot and root biomass of *L. perenne* in presence of AMF without changes in plant N and P concentrations indicate that the plants suffered from allocation of C to the symbiont. Mycorrhizal associations were shown to range between mutualism and parasitism (Johnson et al., 1997) depending on soil fertility and the species involved (Klironomos, 2003). Presumably, *G. intraradices* functioned as a parasite of *L. perenne* in the present study by receiving C-rich root exudates but providing little N or P to the plants.

Surprisingly, earthworm effects on plant productivity were of minor importance; only root biomass of *L. perenne* was increased. In contrast to the majority of studies (79%) reviewed by Scheu (2003) reporting earthworms to beneficially affect plant growth, shoot and total biomass were not affected by earthworms in the present study. This lack of earthworm effects might have been due to the pre-treatment of the soil. Autoclaving of soil, as done in the present study to eliminate mycorrhiza and to kill seeds before experimental setup, is known to mobilize nutrients (Alphei and Scheu, 1993). Despite the leaching of nutrients two weeks before experimental start, nutrients, particularly N, therefore may not have limited plant growth. As the effect of earthworms is known to largely rely on the mobilization of nutrients, the effects of earthworms on plant performance might have been less pronounced than in non-autoclaved soil. However, root biomass of *L. perenne* was increased considerably in presence of earthworms supporting the conclusion of Eisenhauer and Scheu (2008a) that earthworms stimulate root growth by the formation of nutrient-rich patches (casts and burrows) thereby stimulating root foraging (Hutchings et al., 2000). Although interactive effects of earthworms and AMF on plant productivity did not occur, we confirmed our hypothesis (2) by showing that single soil organism effects varied with plant species.

5. Conclusions

Results of the present experiment underline the importance of dead roots and root exudates for earthworm performance. Further, we highlighted that although AMF effectively competed with *T. pratense* for soil N (as indicated by $\delta^{15}N$ analysis), they enhanced the productivity of *T. pratense* considerably by improving P availability. However, we found no evidence for interactive effects of earthworms and AMF on the productivity and shoot nutrient content in different plant species and suggest that they likely are of minor importance.

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