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ORIGINAL PAPER

Exotic earthworms maintain soil biodiversity by altering bottom-up effects of plants on the composition of soil microbial groups and nematode communities

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Abstract

Bottom-up effects of plants on soil communities can be modified by the activity of exotic earthworms, by altering resource availability for soil food webs through feeding, burrowing, and casting activities. The present study explored effects of plants (planting of shrubs) on soil micro-food webs (composition of soil microbial and nematode communities), and whether these effects were altered by the activity of exotic earthworms (exotic earthworms addition). Planted shrubs resulted in a non-significant increase of bacterial biomass and significantly increased the abundance of different nematode trophic groups and total nematode biomass, indicating that planted shrubs had significant bottom-up effects on soil bacteria and nematodes. Planted shrubs decreased nematode diversity, evenness, and richness, but increased nematode dominance in the plots where the abundance of exotic earthworms was not amended. By contrast, these effects of shrub presence on soil biodiversity were not found in the plots that received exotic earthworms. In addition, planted shrubs increased the total energy flux to the nematode community. By contrast, the elevated activity of exotic earthworms mitigated the increase in total energy flux to nematodes in the presence of shrubs, and increased the ratio of fungal to bacterial PLFAs. Both of these changes indicate reduced energy flux in the plots with added exotic earthworms. Nematode diversity decreased, while nematode dominance increased with increasing total energy flux to nematodes, probably because few species benefited from high energy flux. Our study indicates that exotic earthworms can maintain soil biodiversity by reducing the energy flux through soil food webs.

Keywords Soil nematodes · Soil micro-food webs · Exotic earthworms · Bottom-up effects · Energy flux · Microbial PLFAs

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Introduction

Plants play a determining role in fueling soil food webs by providing basal resources (i.e., rhizodeposits, shoot and root litter; Coleman et al. 2004; Pollierer et al. 2007; Scheunemann et al. 2015). The relative abundance of different components of the soil food web can vary tremendously in response to resource inputs, because the quantity and quality of resources can exert significant effects on different groups of decomposer communities (Wardle 2005, 2006). Root-derived resource inputs may induce changes in the belowground community composition by favoring the dominance of a few species being particularly adapted to rhizosphere conditions, such as some bacterial taxa (Kozdrój and van Elsas 2000; Paterson et al. 2008), which may decrease soil bacterial diversity at the small scale.

Such changes in soil community composition and biodiversity may alter predator-prey interactions and correspondingly energy fluxes in soil food webs (Schwarz et al. 2017). In fact, the energy flux and stability of food webs can be closely linked (de Ruiter et al. 1995; Moore et al. 1996; Rooney et al. 2006; Rooney and McCann 2012; Schwarz et al. 2017). Thus, exploring the energy fluxes through soil food webs can help generating a better mechanistic understanding of the regulating forces of soil biodiversity.

Earthworms can stimulate organic matter decomposition as well as nutrient mineralization and modify resource availability for soil food webs through feeding, burrowing, and casting activities (Scheu 2003). Effects of invasive earthworms are exceptionally strong due to their functional dissimilarity to native communities (Wardle et al. 2011). For instance, previous studies showed that European earthworm species introduced into forests in North America could strongly impact soil physico-chemical and biological properties (Eisenhauer et al. 2007; Ferlian et al. 2018). Furthermore, exotic earthworms may alter rhizodeposit C dynamics by shifting microbial community composition in the rhizosphere (Huang et al. 2015), and consequently channeling energy into other compartments of the soil food web (Schwarzmüller et al. 2015). Thus, exotic earthworms may modify the effect of root-derived C inputs on and the energy flux in soil communities as well as their biodiversity.

The accumulated evidence suggests that effects of plants on soil food webs, e.g., providing resources and influencing soil biodiversity through rhizodeposition (Kozdrój and van Elsas 2000; Paterson et al. 2008), can be influenced by earthworms (Huang et al. 2015; Schwarzmüller et al. 2015), but so far it is unknown whether exotic earthworms can change such plant effects. In addition, a previous study showed that plants were one of the key regulators of the effects of earthworms on soil microbes in a subtropical ecosystem (Lv et al. 2016). Taken together, these findings suggest that plants and earthworms may interact with each other in influencing soil food webs. Studying such interactions is particularly important as impacts of exotic earthworms on plant effects on soil food webs may occur at many locations across the globe (Hendrix et al. 2008).

The main primary consumers of detritus in soils are bacteria and fungi, and the associated decomposition pathways are described accordingly as bacterial or fungal energy channels (Moore 1994). As basal components of soil food webs, soil microorganisms are tightly linked to higher trophic level organisms, such as soil nematodes (Fu et al. 2005; Scheu et al. 2005). Nematodes comprise 80% of the total abundance of all multicellular animals (Bongers and Ferris 1999) and are a highly diverse invertebrate group (Hugot et al. 2001). Soil nematodes are ubiquitous and play a major role in decomposition, nutrient transformation, and energy transfer (Bongers and Ferris 1999; Coleman et al. 2004; Freckman 1988; Yeates and Bongers 1999; Zhu et al. 2018). They occupy virtually all trophic levels of soil food webs (Yeates et al. 1993), and studying those can shed light on the whole soil food web structure and functioning (Bongers and Ferris 1999).

Here, we performed a field experiment to explore the effects of the presence of plants (a shrub species, *Evodia lepta*), the activity of exotic earthworms, and their interactions on soil microbial and nematode communities that are important basal representatives of soil food webs (Bongers and Ferris 1999; van der Heijden et al. 2008), and that are often referred to as soil micro-food webs (Shao et al. 2016; Wardle 2002). We hypothesized that (1) planted shrubs have bottom-up effects on soil microorganisms and nematodes. We expected planted shrubs to increase the energy flux in soil micro-food webs as well as to decrease soil biodiversity by favoring the dominance of some soil microbial and nematode groups (Kozdrój and van Elsas 2000; Wardle 2005, 2006). However, (2) exotic earthworms were hypothesized to alter bottom-up effects of plants (Eisenhauer 2010; Schwarzmüller et al. 2015) by decreasing the energy flux in soil micro-food webs and thereby reducing plant effects on soil biodiversity.

Materials and methods

Site description

This study was conducted at the Heshan National Field Research Station of Forest Ecosystem (112° 50′ E, 22° 34′ N), which is located in the middle of Guangdong Province, Southern China. The climate in this region is subtropical monsoon with a hot, humid summer and a cold, dry winter. From 2004 to 2009, mean annual precipitation was 1534 mm, and mean annual temperature was 22.5 °C (Shao et al. 2017). The soil is an Acrisol (FAO 2006), which is characterized by an organic C content of 2.3%, a total N content of 0.2%, and a pH of 3.9. The *Acacia auriculiformis* plantation used in this study was established in 1984 and covered an area of approximately 1 ha. In June 2009, the mean diameter at breast height of the *A. auriculiformis* trees was 17.2 cm. The canopy coverage was about 50%. The main understory plant species in this plantation are *E. lepta*, *Dicranopteris dichotoma*, *Rhodomyrtus tomentosa*, *Litsea cubeba*, and *Ilex asprella*. Based on an earlier investigation of earthworm abundance and biomass in the studied soils from May 2000 to May 2001 (16 sampling times), the average earthworm abundance was 66.5 individuals per square meter, and the abundance of exotic earthworm species *Pontoscolex corethrurus* was 53 individuals per square meter (~ 80% of total earthworms). Furthermore, the average earthworm biomass was 7.8 g per m^2, and the biomass of exotic earthworm species *P. corethrurus* was 5.2 g per m^2 (~ 67% of total earthworms) (Zhang et al. 2005). Compared to tropical forests, this is a relatively low abundance and biomass of earthworms (Lapied and Lavelle 2003), which may be partly explained by the low pH. Notably, *P. corethrurus* is more common and accounts for the vast majority of the total number of earthworms in plantations (~ 80% of total earthworms), but no *P. corethrurus* was found in natural forests in the studied region (personal observation).

Experimental design

Experimental sites were established under the canopy of the *A. auriculiformis* plantation in December 2007. The experiment was designed as a randomized complete block design with four blocks, and plant planting and exotic earthworm addition treatments were assigned randomly to four equally sized plots (1 × 2 m) within each block. This plot size, and even smaller plot size, has been proven useful to study earthworm effects in the field (e.g., Eisenhauer et al. 2008, 2009). Each plot was installed with a spacing of approximately 1–3 m. One block was set up near the hilltop. The plots within other blocks were randomly chosen at a southward hillside with a slope of 5–10°. As a result, the experiment had a two-factorial design (in randomized blocks) with 16 plots. The treatments were (i) earthworm reduction (ER, no earthworm addition, no planted shrubs), (ii) earthworm addition (EA), (iii) earthworm reduction and planted shrubs (ERS), and (iv) earthworm addition and planted shrubs (EAS) (see below for details). An 80-cm-deep trench was dug around each plot to prevent intrusion of roots from the outside. PVC boards (0.5 cm thick, 2 m long, and 1 m wide) were then inserted into the vertical cuts to further isolate the plots; the boards extended to the bottom of the trench and 20 cm above the soil surface to prevent earthworms from moving between plots (Eisenhauer et al. 2009; Liu and Zou 2002). The corners between boards were sealed with cement to reduce the number of earthworms moving among plots.

Plant treatments and their implications

Understory plants with roots attached were removed by hand in all plots before treatments were applied. Re-growing understory plants were removed from all plots throughout the course of the experiment, because nematode abundances can be strongly controlled by the understory plant community (De Long et al. 2016; Shao et al. 2016). In May 2008, we planted seven saplings of *E. lepta* per shrub treatment plot, which is common in South China, grows on hills, plains, and forest margins, and is the most common shrub species with medicinal value in the local plantation. In May 2009, the average diameter at the sapling base was 1.6 cm, the average height was 1.0 m, and the average canopy of saplings was 0.7 m × 0.9 m. The saplings covered about 75% of the plot area. Notably, a recent study conducted at the site found that the litter dry weight of *A. auriculiformis* was similar across the plots (Shao et al. 2018). However, the dry weight of *A. auriculiformis* litter was substantially greater than that of *E. lepta* that represented the planted shrubs treatment based on the comparison of litter quantities of *A. auriculiformis* and *E. lepta* (collected in June 2014) (Shao et al. 2018). Moreover, the fine root biomass of *E. lepta* (diameter less than 1 mm) in “ERS” and “EAS” treatments was 13.38 ± 5.09 and 9.27 ± 5.07 g dry weight m^{-2} (Table 1), respectively. Planted shrubs did not affect soil organic C (SOC), total soil N (TN), total soil P (TP), pH, soil microbial biomass C (MBC), and soil microbial biomass N (MBN) (Table 1). These results together with the similar dry weight of litter across the plots indicate that differences in the rhizodeposition (quantity and quality) of planted shrubs may have a greater impact on the soil food web than the plant-induced changes in soil nutrient status.

Earthworm treatments and their implications

The manipulations performed for each treatment, timing of manipulation, and timing of measurement are shown with the experimental design as Supplementary Information (Fig. S1). From January 2008 to May 2009, the electroshocking method was used to reduce earthworm numbers once per month in all plots (Liu and Zou 2002; Szlavecz et al. 2013), because this method caused little disturbance to the soil. A previous study showed that there are no indications that it causes negative long-term effects on nematodes, enchytraeids, other microarthropods, plant roots, or microbial populations (Čoja et al. 2008). Specifically, electrodes were inserted into the soil down to a depth of 30 cm and supplied with 110 Volts (AC) for about 30 min in conditions when soil moisture was greater than 15%. When soil moisture was lower than 15%, however, the voltage was applied for 1 h to increase extraction efficiency. Normally, epigeic earthworms occur at the soil surface within 5 min. However, the earthworm species

Table 1 Fine root biomass of *E. lepta* (diameter less than 1 mm), soil organic C (SOC), Total N (TN), Total P (TP), pH, soil microbial biomass C (MBC). and soil microbial biomass N (MBN) in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Data are means ± SE ($n = 4$). Different letters indicate significant differences among treatments ($P = 0.05$) for mean values of each variable

	Fine root biomass (g dry weight m^{-2})	SOC (%)	TN (g kg^{-1})	TP (g kg^{-1})	pH	MBC (mg kg^{-1})	MBN (mg kg^{-1})
ER		1.42 ± 0.40a	1.51 ± 0.22a	0.19 ± 0.02a	4.33 ± 0.05a	243.70 ± 82.03a	46.54 ± 10.88a
EA		1.70 ± 0.13a	1.70 ± 0.16a	0.18 ± 0.01a	4.14 ± 0.10ab	251.21 ± 21.79a	52.02 ± 5.45a
ERS	13.38 ± 5.09	1.68 ± 0.12a	1.66 ± 0.06a	0.20 ± 0.02a	4.24 ± 0.06ab	380.29 ± 59.63a	70.45 ± 8.39a
EAS	9.27 ± 5.07	1.84 ± 0.10a	1.76 ± 0.16a	0.19 ± 0.01a	4.00 ± 0.10b	350.18 ± 75.42a	57.07 ± 12.34a

P. corethrurus appears at the soil surface after more than 30 min. All earthworms that appeared at the soil surface were picked and removed from the plots. We found two major earthworm groups, i.e., native pheretimoid species (including the genera *Amynthas*, an epigeic species, and *Metaphire*, an anecic species) and *P. corethrurus* (endogeic species, the only exotic species), at our study site (Zhang et al. 2005). Notably, *P. corethrurus* is widespread in tropical and subtropical regions in China (Zhang et al. 2005).

Pheretimoid earthworms were more sensitive to electroshocking compared to *P. corethrurus* (Shao et al. 2017), and this result was consistent with the findings of a previous study by Rhea-Fournier (2012), which observed that extraction efficiencies were greater for epigeic *Amynthas* sp. in terms of both abundance and biomass (82 and 94%, respectively), while extraction efficiencies were lower for *P. corethrurus* (abundance 60%, biomass 83%). Thus, most pheretimoid earthworms were more efficiently reduced by electroshocking. In contrast, *P. corethrurus* respond slowly to electroshocking, and thus some of the individuals may have remained in the soil (Shao et al. 2017). As a result, the electroshocking treatment reduced the population abundance of earthworms in the "earthworm reduction (ER)" and "earthworm reduction and planted shrubs (ERS)" plots. In May 2009, 2012, 2013, and 2014, we collected specimens of the exotic earthworm species *P. corethrurus* by digging soils around those plots. The collected earthworms were washed in tap water and then added to the surface of the soil at the rate of 100 individuals per square meter at each earthworm addition event to compensate for a potential decrease in abundance over time and to standardize earthworm abundance across treatments (Shao et al. 2017); this is about the average earthworm abundance in tropical forests (Lapied and Lavelle 2003) and a relatively high abundance compared to the background abundance of exotic earthworms in the studied soil (~ 53 individuals per square meter; see above).

Earthworm addition significantly increased the mass of earthworm casts (+ 120%; $P < 0.001$; Fig. S2a). Although the abundance of exotic earthworms was not significantly affected by earthworm addition (Fig. S2b), exotic earthworm addition decreased the abundance of native earthworms (Fig. S2c), indicating a shift in earthworm community composition and elevated exotic earthworm activity in the earthworm addition treatment and reduced effects of native earthworm species. One possible reason is that the results on abundance of earthworms came from only one sampling event in 2014. However, there should be an accumulated earthworm effect in plots that received exotic earthworms as the earthworm addition treatment was conducted in May 2009, 2012, 2013, and 2014. Overall, we refer to the treatments that received exotic earthworms as "earthworm addition (EA)" and "earthworm addition and planted shrubs (EAS)." Thus, we refer to experimentally induced differences in the dominance of exotic earthworms.

Soil sampling and analyses

Four soil cores (5 cm in diameter, 10 cm depth) were collected and combined into a single composite sample from each plot in June 2013 and June 2014, respectively. Litter was removed from the soil surface before soil samples were taken. Visible roots in the soil samples were picked out by hand as closely as possible. The soil cores from each plot were used for nematode community and soil microbial community analysis. We examined the dry mass of earthworm casts on the soil surface from each plot in December 2013 and June 2014 to assess earthworm activity (Zaller and Arnone 1997). Additionally, at the end of the experiment, we examined the density of exotic and native earthworms by sampling and hand-sorting two 30 × 30 × 40 cm soil samples from each plot in June 2014.

Phospholipid fatty acids (PLFAs) were extracted and quantified from the samples collected in June 2013 and June 2014 by gas chromatography (Bossio and Scow 1998). The biomass of bacteria was estimated by summing fatty acids, iso 15:0, anteiso 15:0, 15:0, iso 16:0, 16:1ω9c, iso 17:0, 17:0, anteiso 17:0, 17:0cy, 18:1ω5c and 19:0cy. The biomass of fungi was estimated as the sum of 18:2ω6c (Bossio et al. 1998).

Molecular techniques can be useful to link microbial identity to microbial diversity and ecological processes (Schöler et al. 2017; Vestergaard et al. 2017). Therefore, total genomic DNA was extracted directly from these samples collected in June 2014 using FastDNA® spin kit (MP bio, Santa Ana, USA) following the manufacturer's protocol. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. Prepared DNA samples were sent to Novegene (Beijing, China) for high-throughput sequencing analysis. The primers for 16S V4 and ITS1 were 515F-806R (Caporaso et al. 2011) and ITS1F- ITS2 (De Beeck et al. 2014), respectively. 16S /18S rRNA genes were amplified by using the specific primers with the barcode. All PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s, and an extension step at 72 °C for 5 min after cycling was complete. We mixed the same volume of 1X loading buffer (contained SYB green) with PCR products and conducted electrophoresis on 2% agarose gel for detection. Samples with bright main strip between 400 and 450 bp were chosen for further analyses. PCR products were mixed in equidensity ratios. Then, mixture PCR products was purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiSeq platform and 250 bp/300 bp paired-end reads were generated. The bacterial and fungal biodiversity was assessed by calculating Shannon's diversity index *H′* for the two groups.

Nematodes were extracted from 50 g of fresh soil using Baermann funnels for each composite soil sample (Barker 1985). After fixation in 4% formalin solution, nematodes were counted under an inverted microscope, and the first 100 individuals encountered were identified to genus or family level and classified into trophic groups (plant-feeding nematodes, bacterial-feeding nematodes, fungal-feeding nematodes, omnivorous-predatory nematodes) (Yeates et al. 1993). All nematodes were identified when the nematode number was lower than 100 individuals in a sample. The number of each nematode group was estimated according to their proportion in the 100 identified individuals and the total number of nematodes. The total biomass (wet mass) was estimated using the mean biomass of nematode genera or family (http://plpnemweb.ucdavis.edu/nemaplex/Ecology/nematode_weights.htm).

Nematode diversity indices (Shannon's diversity index *H′*, Evenness *J′*, Richness SR, Dominance λ) at the community level were calculated (Neher and Darby 2006; Yeates and Bongers 1999).

Nematode biomass C was calculated by multiplying the abundance of each taxon by their calculated fresh weight, and conversion assuming that 25% of wet weight is dry weight of nematodes (Yeates 1979) and that the C content is 50% of dry weight (Holtkamp et al. 2008). Calculation of nematode metabolic rates and energy fluxes was based on the methods described by Ehnes et al. (2011), Barnes et al. (2014), and Schwarz et al. (2017). More specifically, we calculated mean metabolic rates for each identified nematode taxon using metabolic rates of soil nematodes derived from scaling relationships of body mass and soil temperature. The following linear model was used: $\ln \mathrm{we} = 23.055335 + 0.695071 \times \ln \mathrm{M} - 0.68642 \times (1/\mathrm{kT})$, where we is the metabolic rate, M is the fresh body mass, k is the Boltzmann constant, and T the temperature in Kelvin (Schwarz et al. 2017). Then, we calculated the energy flux of each nematode trophic group based on the taxon-specific assimilation efficiencies and the summed metabolic rates of all individuals within the corresponding trophic group (Barnes et al. 2014; Ehnes et al. 2011; Schwarz et al. 2017). Energy fluxes were expressed as g C m^{-2} d^{-1}.

Statistical analyses

Two-way repeated-measures ANOVA (planted shrubs and earthworm addition as main factors, replicate block as error term) was used to compare the effects of the experimental treatments and their interaction on the dry mass of earthworm casts, soil microbial PLFAs, the ratio of fungal to bacterial PLFAs, the abundance, biomass, diversity, and energy flux of nematodes. Two-way ANOVA was employed to compare the effect of planted shrubs, earthworm addition, and their interaction on the abundance of exotic and native earthworms, relative abundance of archaea, and Shannon's diversity index of bacteria and fungi (replicate block as error term). Levene's test was performed to test for homogeneity of variance. Tukey's honestly significant difference tests were used to compare differences among treatments for each sampling event when interactions between planted shrubs and earthworm addition were significant. Statistical significance was determined at $P < 0.05$. ANOVAs were performed using R version 2.15.1 (R Development Core Team 2009). Hierarchical clustering analysis was performed on the relative abundance of bacterial OTUs, fungal OTUs and nematode genera using the pheatmap package in R (R Development Core Team 2009). The 50 and 40 most dominant OTUs selected from the bacterial community and fungal community for the construction of heatmaps, respectively. Relationships between nematode diversity indices and total nematode energy flux were analyzed using linear or quadratic regression analyses. Models with the highest coefficient of determination (R^2) were chosen as the best-fit models (Aho et al. 2014).

Results

Soil microbial and nematode biomass/abundance

Planted shrubs resulted in a non-significant increase of bacterial biomass ($P = 0.09$; Fig. 1(a), Table 2) and significantly increased the abundance of bacterial-feeding ($P < 0.001$; Fig. 1(d), Table 2), fungal-feeding ($P < 0.001$; Fig. 1(e), Table 2), and plant-feeding nematodes ($P < 0.001$; Fig. 1(f), Table 2). Also, planted shrubs resulted in a non-significant increase of the abundance of omnivorous-predatory nematodes ($P = 0.08$; Fig. 1(g), Table 2) and significantly increased the abundance of total nematodes ($P < 0.001$; Fig. 1(h), Table 2). In addition, planted shrubs increased the total nematode biomass, while it decreased the mean individual biomass of soil nematodes (Fig. S3), indicating that planted shrubs had significant bottom-up effects on soil bacteria and nematodes, particularly on small-bodied nematodes. Neither planted shrubs nor the activity of exotic earthworms affected fungal biomass (Fig. 1(b), Table 2). The activity of exotic earthworms and planted shrubs alone resulted in a non-significant decrease of the relative abundance of soil archaea, while the presence of both exotic

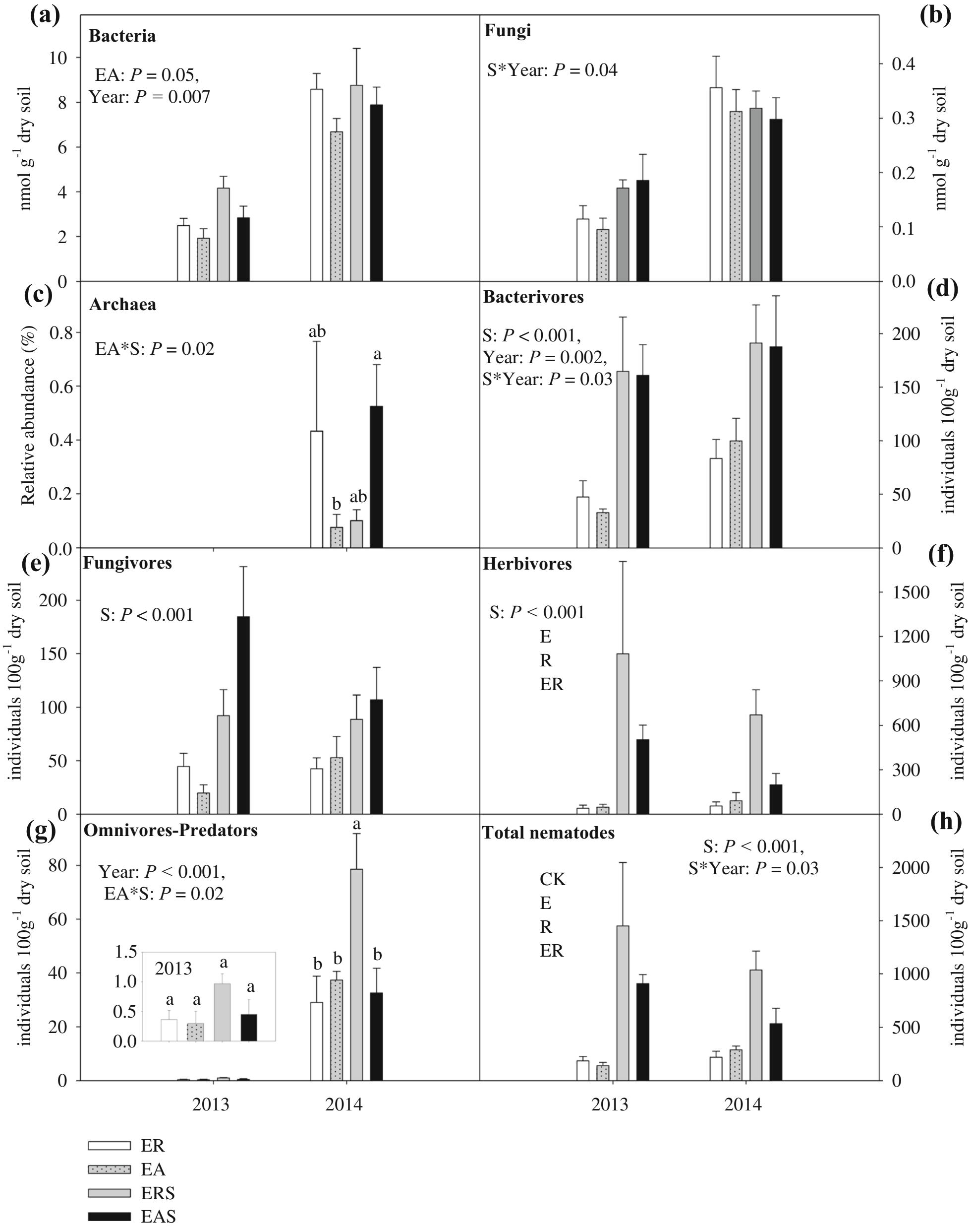


Fig. 1 Soil bacterial biomass (a) and fungal biomass (b), the relative abundance of soil archaea (c), and the density of bacterial-feeding (d), fungal-feeding (e), plant-feeding (f), omnivorous-predatory nematodes (g), and total nematodes (h) in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Data are means + SE ($n = 4$). Significant ($P < 0.05$) treatment and year effects from two-way repeated-measures ANOVA are provided in panels (a), (b), (d), (e), (f), (g), and (h), whereas significant ($P < 0.05$) treatment effects from the two-way ANOVA are provided in panel (c). Details of statistical analyses are provided in Table 2 and Table 3. Bars at a particular sampling time sharing the same superscript letter were not significantly different at $P = 0.05$ (Tukey HSD post hoc tests). Results of Tukey's HSD post hoc tests ($P = 0.05$) are provided when interaction effects in the ANOVA were significant

Table 2 Results (P values) of two-way repeated-measures ANOVA on the effects of earthworm addition (EA), planted shrubs (S), year (Y) and their interactions on dry mass of earthworm cast (Cast), bacterial biomass (B), fungal biomass (F), the abundance of bacterial-feeding (Ba), fungal-feeding (Fu), plant-feeding (Pl), omnivorous-predatory nematodes (OP), total nematodes (T), diversity indices of soil nematodes (Shannon's diversity index H', Evenness J', Richness SR, Dominance λ), energy flux of bacterial-feeding (EBa), fungal-feeding (EFu), plant-feeding (EPl), omnivorous (EOm), predatory nematodes (EPr), total nematodes (ET) and ratio of fungal to bacterial PLFAs (F:B)

	Cast	B	F	Ba	Fu	Pl	OP	T	H'	J'	SR	λ	EBa	EFu	EPl	EOm	EPr	ET	F:B
EA	*< 0.001*	0.05	0.46	0.90	0.95	0.53	0.20	0.28	0.50	0.86	0.99	0.71	0.11	0.06	*0.04*	0.08	*0.04*	0.05	*0.048*
S	*0.01*	0.09	0.31	*< 0.001*	*< 0.001*	*< 0.001*	0.08	*< 0.001*	*0.01*	*0.01*	*0.007*	*0.006*	*< 0.001*	*0.001*	*< 0.001*	*0.004*	*0.002*	*< 0.001*	0.99
EA*S	0.98	0.90	0.55	0.91	0.13	0.13	*0.02*	0.12	*0.03*	*0.03*	*0.11*	*0.04*	0.07	0.12	*0.02*	0.05	0.09	0.05	0.55
Y	*0.004*	*0.007*	0.11	*0.002*	0.71	0.58	*< 0.001*	0.91	0.16	0.25	0.36	0.20	0.47	0.29	0.25	0.38	0.20	0.28	0.23
Y*EA	0.08	0.71	0.53	0.64	0.63	0.51	0.85	0.80	0.16	0.26	0.05	0.15	0.95	0.63	0.91	0.43	0.98	0.87	0.20
Y*S	0.78	0.60	*0.04*	*0.03*	0.14	0.37	0.60	*0.03*	0.81	0.81	0.94	0.65	0.24	0.31	0.15	0.35	0.35	0.23	0.14
Y*EA*S	0.56	0.44	0.91	0.36	0.14	0.46	0.10	0.14	0.89	0.99	0.67	0.63	0.30	0.21	0.24	0.33	0.17	0.23	0.12

Italicized values indicate significant effects ($P < 0.05$)

earthworms and planted shrubs negated this trend (earthworm addition × planted shrubs interaction effect: $P = 0.02$; Fig. 1(c), Table 3).

Soil microbial and nematode community composition

Generally, bacterial community composition was affected by the activity of earthworms or planted shrubs by shifting the relative abundances of some OTUs. For instance, the top abundant OTU in the ER treatment was *Acidobacteria-2*. Exotic earthworms and planted shrubs did not decrease the abundance of *Acidobacteria-2,* their sole presence increased the abundance of *Koribacteraceae-1*. The joint presence of planted shrubs and earthworms decreased the abundance of *Acidobacteria-2,* but increased the abundance of *Sinobacteraceae*, *Actinomycetales*, *Rhodospirillaceae-2*, *Ellin-329*, and *Rhodospirillaceae-1* (Fig. 2). Also, fungal community composition changed in response to exotic earthworm addition or the presence of planted shrubs. For instance, exotic earthworms alone resulted in a non-significant increase of the abundance of *Oidiodendron*. Planted shrubs resulted in a non-significant increase of the abundance of *Thelephoraceae*. The planted shrubs and earthworm addition increased the abundance of *Penicillium,* but decreased the abundance of *Thelephoraceae* (Fig. 3).

Planted shrubs alone increased the abundance of *Pratylenchus* (plant-feeding nematodes), but decreased the abundance of *Iotonchus* (predatory nematodes) and *Boleodorus* (plant-feeding nematodes) in 2013 and 2014. Exotic earthworms alone increased the abundance of *Boleodorus* (plant-feeding nematodes) and *Enchodorus* (omnivorous nematodes), but decreased the abundance of *Longidorus* (plant-feeding nematodes) and *Trophurus* (plant-feeding nematodes) in 2013. Planted shrubs and earthworm addition increased the abundance of *Boleodorus* (plant-feeding nematodes), but decreased the abundance of *Iotonchus* (predatory nematodes) in 2013. Exotic earthworms alone increased the abundance of *Rotylenchus* (plant-feeding

Table 3 Results (P values) of two-way ANOVA on the effects of earthworm addition (EA), planted shrubs (S), and their interactions on abundance of exotic and native earthworms, relative abundance of soil archaea, bacteria Shannon's diversity index, and fungi Shannon's diversity index

	Abundance of exotic earthworms	Abundance of native earthworms	Relative abundance of soil archaea	Bacteria Shannon's diversity index	Fungi Shannon's diversity index
EA	0.32	*0.015*	0.83	0.77	0.91
S	*0.048*	*0.015*	0.70	0.60	0.34
EA*S	0.61	0.21	*0.02*	*0.025*	0.76

Italicized values indicate significant effects ($P < 0.05$)

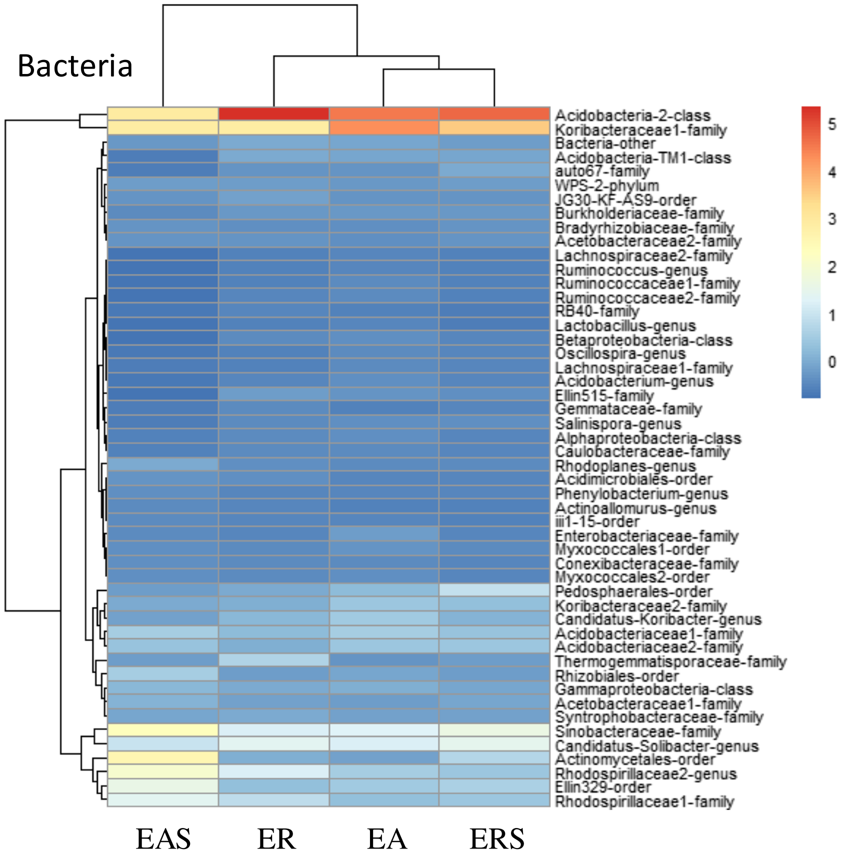


Fig. 2 A heatmap showing the bacterial composition in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Indicator scores are based on the OTU abundance in the bacterial assemblages. Only the 50 most abundant OTUs are shown in the figure

nematodes), *Tylenchus* (plant-feeding nematodes), and *Acrobeloides* (bacterial-feeding nematodes), but decreased the abundance of *Boleodorus* (plant-feeding nematodes) in 2014. Planted shrubs and earthworm addition increased the abundance of *Boleodorus* (plant-feeding nematodes), but decreased the abundance of *Trophurus* (plant-feeding nematodes) in 2014 (Fig. 4).

Soil microbial and nematode diversity

There was a statistically significant interaction effect of the activity of earthworms and planted shrubs on Shannon's diversity index of bacteria ($P = 0.025$; Fig. 5(a), Table 3), activity of exotic earthworms and planted shrubs alone resulted in a non-significant decrease of Shannon's diversity index of bacteria, while both the activity of earthworms and planted shrubs negated this trend. Neither planted shrubs nor activity of exotic earthworms affected Shannon's diversity index of fungi (Fig. 5(b), Table 3). However, interactions between the activity of exotic earthworms and planted shrubs affected nematode diversity indices. Planted shrubs decreased Shannon's diversity index of nematodes (Fig. 5(c), Table 2), evenness (Fig. 5(d), Table 2), and richness (Fig. 5(e), Table 2), and increased nematode dominance (Fig. 5(f), Table 2), but only in the plots where no exotic earthworms were added. This means that the activity of exotic earthworms counterbalanced the effects of planted shrubs on soil organisms (Fig. 5, Table 2). However, this opposing effect of earthworms was more pronounced in 2014 than in 2013, i.e., the activity of earthworms maintained soil biodiversity mostly in the second sampling year.

Nematode energy flux

Planted shrubs increased energy flux to the omnivorous ($P = 0.004$; Fig. S4a, Table 2), predatory ($P = 0.002$; Fig. S4b, Table 2), bacterial-feeding ($P = 0.0005$; Fig. S4c, Table 2), fungal-feeding ($P = 0.001$; Fig. S4d, Table 2), and plant-feeding nematode community ($P = 0.0006$; Fig. S4e, Table 2). The activity of exotic earthworms resulted in a non-significant decrease of the omnivorous ($P = 0.075$; Fig. S4a, Table 2) and fungal-feeding nematode energy flux ($P = 0.059$; Fig. S4d, Table 2); in addition, it decreased the predatory nematode ($P = 0.041$; Fig. S4b, Table 2) and plant-feeding nematode energy flux ($P = 0.045$; Fig. S4e, Table 2). Overall, planted shrubs increased the total nematode energy flux ($P = 0.0009$, Fig. 5(g), Table 2). The activity of exotic earthworms resulted in a non-significant decrease of the total nematode energy flux ($P = 0.052$, Fig. 5(g), Table 2). However, the activity of exotic earthworms and planted shrubs had an interactive effect on the total nematode energy flux ($P = 0.05$; Fig. 5(g), Table 2): while the presence of

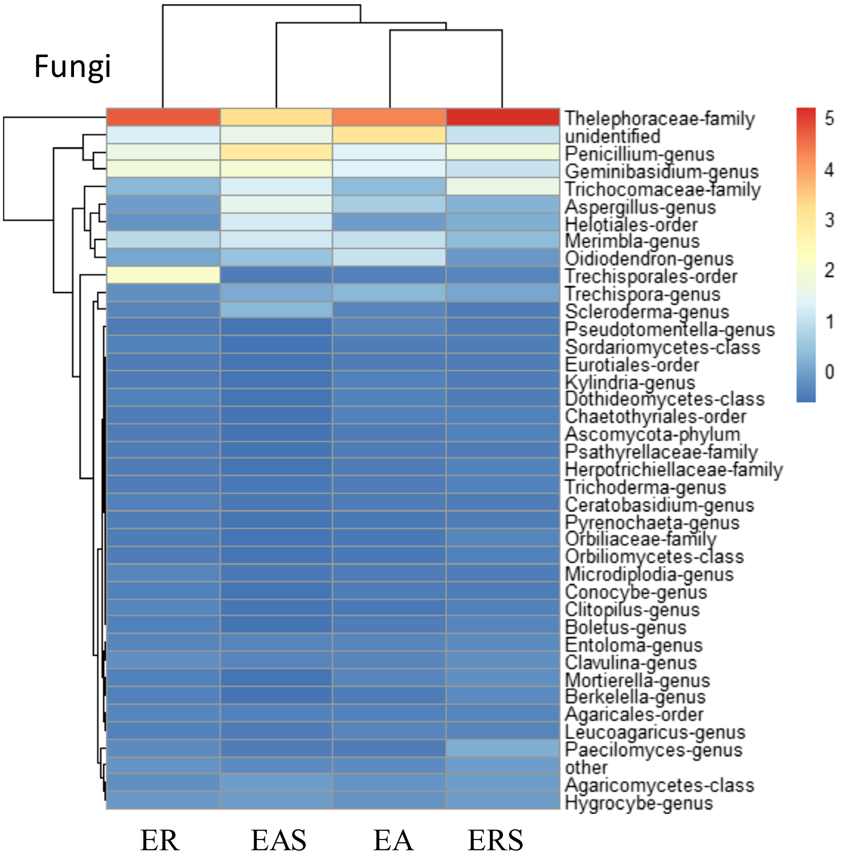


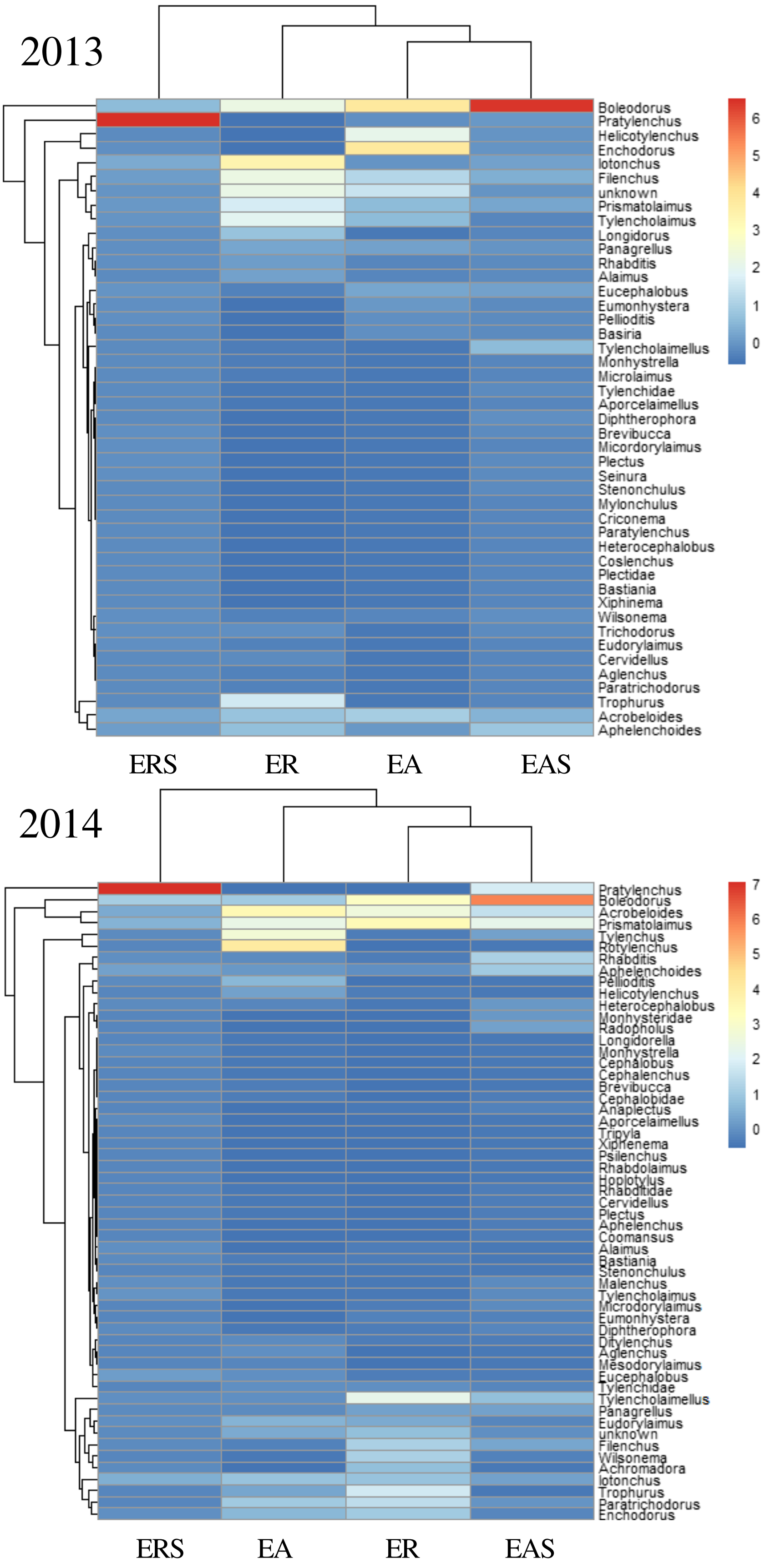
Fig. 3 A heatmap showing the fungal composition in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Indicator scores are based on the OTU abundance in the fungal assemblages. Only the 40 most abundant OTUs are shown in the figure

planted shrubs increased energy flux in general, exotic earthworms did not alter the total nematode energy flux in the absence of planted shrubs, but negated the planted shrubs effect by substantially decreasing energy flux.

Generally, total energy flux decreased with increasing nematode Shannon's diversity index ($P = 0.009$; Fig. 6(a)), evenness ($P = 0.01$; Fig. 6(b)), and richness ($P = 0.0006$; Fig. 6(c)), while it increased with increasing nematode dominance ($P = 0.009$; Fig. 6(d)).

Discussion

In the present study, planted shrubs showed strong bottom-up effects on most groups of soil microorganisms and nematodes with a lower soil biodiversity in the plots where no exotic earthworms were added. In addition, planted shrubs increased the total nematode energy flux in the studied soil. Plants can influence soil biodiversity through altering resource supply (quantity and quality) (Wardle 2005, 2006) and creating a selective environment for microorganisms (Grayston et al. 1998). In the present study, the differences in the rhizodeposition (quantity and quality) of planted shrubs may have played an important role in fueling soil food webs. Different nematode groups feed on soil bacteria, soil fungi, roots, and root hairs, as well as on other nematodes (Coleman et al. 2004; Yeates et al. 1993). Plant-induced changes in resource supply can thus alter the energy flow from basal resources (roots, bacteria, and fungi) to nematodes. Confirming our hypothesis (1), bottom-up effects of planted shrubs induced changes in the bacterial and nematode community composition and dominance, and increased total nematode energy flux, whereas fungal communities were not affected. Especially bacteria may more strongly rely on the labile fraction of root-derived plant resources (Paterson et al. 2008), while most of the nematodes at lower trophic levels rely on plant roots and microorganisms, and these effects can cascade up to nematodes at higher trophic levels (Yeates 1999). In fact, planted shrubs and the activity of exotic earthworms counterbalanced their effects on soil microbial and nematode diversity. In the plots where no exotic earthworms were added, shifts in microbial community composition might have been due to the fact that only a few specific microbial taxa were favored by root-derived carbon inputs (Dennis et al. 2010; Huang et al. 2015), and if these taxa become dominant, it may result in a lower diversity. Indeed, the exotic endogeic earthworm species *P. corethrurus* has been shown to alter the composition of soil microbial communities in the rhizosphere (Huang et al. 2015), most likely through feeding, burrowing, and casting activities (Brown 1995).


2013
Boleodorus
Pratylenchus
Helicotylenchus
Enchodorus
Iotonchus
Filenchus
unknown
Prismatolaimus
Tylencholaimus
Longidorus
Panagrellus
Rhabditis
Alaimus
Eucephalobus
Eumonhystera
Pellioditis
Basiria
Tylencholaimellus
Monhystrella
Microlaimus
Tylenchidae
Aporcelaimellus
Diphtherophora
Brevibucca
Micordorylaimus
Plectus
Seinura
Stenonchulus
Mylonchulus
Criconema
Paratylenchus
Heterocephalobus
Coslenchus
Plectidae
Bastiania
Xiphinema
Wilsonema
Trichodorus
Eudorylaimus
Cervidellus
Aglenchus
Paratrichodorus
Trophurus
Acrobeloides
Aphelenchoides
6
5
4
3
2
1
0
ERS
ER
EA
EAS
2014
Pratylenchus
Boleodorus
Acrobeloides
Prismatolaimus
Tylenchus
Rotylenchus
Rhabditis
Aphelenchoides
Pellioditis
Helicotylenchus
Heterocephalobus
Monhysteridae
Radopholus
Longidorella
Monhystrella
Cephalobus
Cephalenchus
Brevibucca
Cephalobidae
Anaplectus
Aporcelaimellus
Tripyla
Xiphenema
Psilenchus
Rhabdolaimus
Hoplotylus
Rhabditidae
Cervidellus
Plectus
Aphelenchus
Coomansus
Alaimus
Bastiania
Stenonchulus
Malenchus
Tylencholaimus
Microdorylaimus
Eumonhystera
Diphtherophora
Ditylenchus
Aglenchus
Mesodorylaimus
Eucephalobus
Tylenchidae
Tylencholaimellus
Panagrellus
Eudorylaimus
unknown
Filenchus
Wilsonema
Achromadora
Iotonchus
Trophurus
Paratrichodorus
Enchodorus
7
6
5
4
3
2
1
0
ERS
EA
ER
EAS

◀ **Fig. 4** Heatmaps showing the nematode community composition in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Indicator scores are based on the abundances of nematode genera/families

In line with our hypothesis (2), earthworms counterbalanced the effects of plants on the main bacterial groups. In addition, the activity of exotic earthworms decreased elevated energy fluxes in soil micro-food webs in the presence of planted shrubs. This earthworm effect can be due to multiple, not mutually exclusive mechanisms. Firstly, exotic earthworms may feed on those microorganisms at the food web's basis (Schwarzmüller et al. 2015). It was shown that a respective increase in earthworm biomass channels away energy from micro-food webs (Schwarzmüller et al. 2015). Thus, in the present study, the activity of exotic earthworms might have reduced the energy flow in soil micro-food webs and mitigated the increase in total energy flux to nematodes in the presence of shrubs. We speculate that exotic earthworms grazed on the soil microbial community caused changes in soil microbial community composition, and thereby reduced bottom-up effects of planted shrubs on energy fluxes in the present study. Secondly, earthworms may affect soil

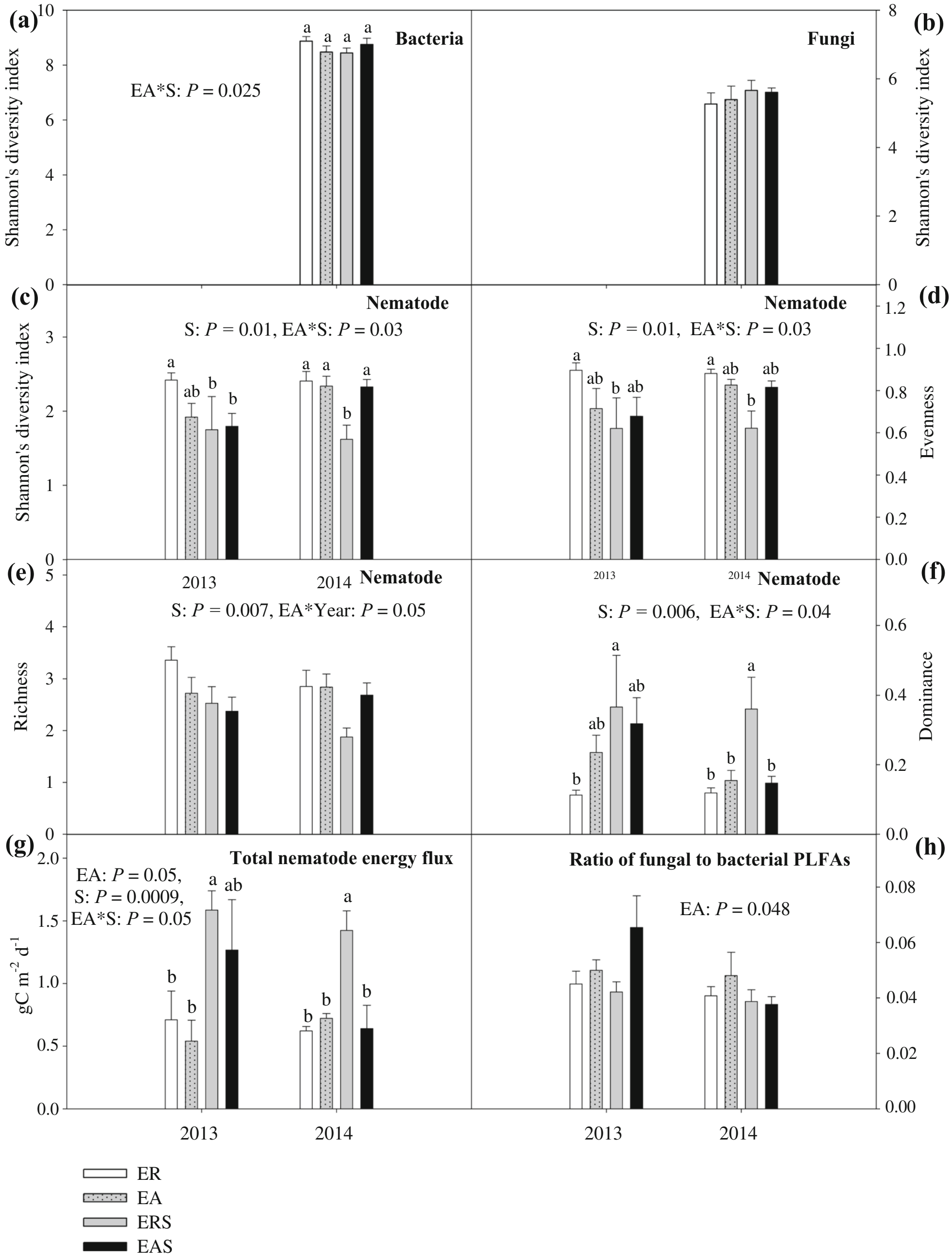


Fig. 5 Diversity indices of soil bacteria (a), fungi (b), nematodes (c, d, e, f), total nematode energy flux (g), and ratios of fungal and bacterial PLFAs (h) in earthworm reduction (ER), earthworm addition (EA), earthworm reduction and planted shrubs (ERS), and earthworm addition plus planted shrubs (EAS) treatments in a field experiment. Data are means + SE ($n = 4$). Significant ($P < 0.05$) treatment effects from two-way ANOVA are provided in panels (a) and (b), whereas significant ($P < 0.05$) treatment and year effects from two-way repeated-measures ANOVA are provided in panels (c), (d), (e), and (f). Details of statistical analyses are provided in Table 2 and Table 3. Bars at a particular sampling time sharing the same superscript letter were not significantly different at $P = 0.05$ (Tukey HSD post hoc tests). Results of Tukey's HSD post hoc tests ($P = 0.05$) are provided when interaction effects in the ANOVA were significant

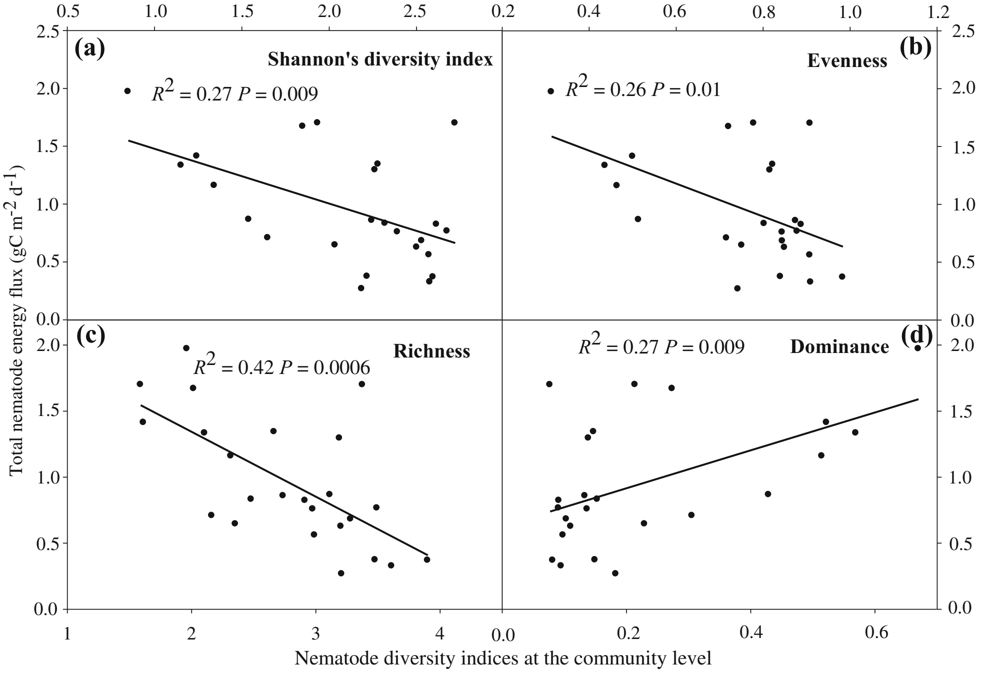


Fig. 6 Relationships between diversity indices of nematodes and total nematode energy flux. (a) Shannon's diversity index of nematodes and total nematode energy flux. (b) Nematode evenness and total nematode energy flux. (c) Nematode richness and total nematode energy flux. (d). Nematode dominance and total nematode energy flux

biodiversity by creating higher habitat complexity (Lavelle et al. 2006; Wardle 2006), which is important for maintaining soil biodiversity by enhancing resource partitioning and reducing competitive exclusion through enabling the physical separation of organisms in the soil matrix (Ferris and Tuomisto 2015; Wardle 2002). Thirdly, earthworms can affect the diversity of soil bacteria through gut processes (Nechitaylo et al. 2010) or through physical attachment of microbes to the body surface of exotic earthworms (Brown 1995; Wardle 2006). As a consequence, the rhizodeposition inputs of planted shrubs and exotic earthworm activity may have favored the coexistence of different bacterial taxa leading to bacterial diversity levels (based on dominant OTUs) that are similar to the earthworm reduction treatment.

We did not calculate the diversity of archaea because of the low number of taxa in the studied soil. However, we found a similar trend for the relative abundance of soil archaea as for Shannon's diversity index of soil bacteria in response to planted shrubs and/or exotic earthworms addition. However, the underlying mechanisms and functional consequences need to be explored in future studies. Although fungal community composition changed in response to exotic earthworm addition and planted shrubs, some dominant OTUs were not significantly affected by the treatments, because no dominant fungal taxa were suppressed or stimulated. The missing effects may have been due to the fact that new root-derived C inputs by the planted shrubs in our studied soils might have had a stronger influence on the composition of bacterial communities than on the composition of fungal communities as these groups utilize different C resources (Paterson et al. 2008).

Notably, earthworm addition reduced the dominance of certain nematode taxa and maintained nematode diversity in the treatment with planted shrubs. Specifically, planted shrubs favored the dominance of the plant-feeding nematode *Pratylenchus* in the treatment with reduced abundance of exotic earthworms, which coincided with a reduction in soil biodiversity. A possible explanation could be that plant roots or root hairs are main food resources for the plant-feeding nematode *Pratylenchus* spp., which is why they might have been directly affected by planted shrubs (Ferris and Bongers 2006; Neher 2010; Yeates 1999). In line with our hypothesis (2), exotic earthworm addition increased the abundance of the plant-feeding nematode *Boleodorus*, but it decreased the dominance of the plant-feeding nematode *Pratylenchus* in the presence of planted shrubs, which coincided with the maintenance of higher soil biodiversity. However, *Pratylenchus* spp. have an intimate association with plants, because they can mainly feed on deeper cell layers in the roots (Bongers and Bongers 1998). By contrast, *Boleodorus* spp. are often treated as plant-associated nematodes, because they are less plant host-specific, or perhaps more dependent on rhizodeposits and soil microorganisms (Yeates 1999, 2007). Therefore, *Boleodorus* spp. may have benefitted from the positive effects of earthworms on plant growth and/or soil shifts in soil microbial community composition in the present study. As a result, changes in nematode community composition could enhance the growth of plants in the long term, because exotic earthworms resulted in a non-significant increase of the abundance of plant-feeding nematode taxa that are less detrimental to plant growth (*Boleodorus*), while they significantly reduced the abundance of more harmful plant-feeding nematodes

(*Pratylenchus*) (Shao et al. 2017). Additionally, just like soil microbes, small-bodied nematodes have limited ability to move within the soil, but may have benefitted from dispersal by earthworms to suitable food resources (Brown 1995; Wardle 2006).

In the present study, the activity of exotic earthworms increased the ratio of fungal to bacterial PLFAs and negated the increase in total nematode energy flux in response to the presence of planted shrubs. Many studies on soil food web analyses suggest that relative dominance of the fungal channel, i.e., relatively slower energy channel in comparison to bacterial dominated soil communities, can imply higher stability (de Ruiter et al. 1995; Moore et al. 1996; Rooney et al. 2006; Rooney and McCann 2012; Schwarz et al. 2017). Therefore, our results indicate that the activity of exotic earthworms can increase the stability of soil micro-food webs in conditions of elevated basal resource availability. Although we cannot disentangle the relative contribution of trophic and non-trophic interactions to soil biodiversity and energy flux in soil micro-food webs, the present study shows how plants and exotic earthworms can interactively influence ecological communities and biodiversity. Notably, the invasion of exotic earthworms may alter plant effects at the base of the soil food web that cascade up to higher trophic levels with unknown consequences for the functioning of the respective soils. However, some potential research gaps need to be addressed in future studies. First, only one plant species (*E. lepta*) was planted in the studied soils, and it remains to be tested if the results are generalizable across plant species. Second, other soil faunal groups, such as enchytraeids and microarthropods often play decisive functional roles in soils and should also be considered in future studies. Third, long-term monitoring plots should be established to check dynamics of earthworm populations, because there may be cumulative effects in plots that received exotic earthworms. Otherwise, a higher soil sampling frequency is needed to validate the temporal variation of earthworm populations and other soil properties. Finally, future studies should explore casts deposited at the soil surface and casts deposited beneath the surface to evaluate the activity of earthworms, because *P. corethrurus* can produce casts on the soil surface or beneath the surface (Lavelle et al. 1992). In addition to identifying these critical future research directions, our study presents novel information on the influence of exotic earthworms on soil biodiversity, which could reconcile different effects of plants on the resource availability for and biodiversity of soil organisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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