Effects of epigeic earthworm (*Eisenia fetida*) and arbuscular mycorrhizal fungus (*Glomus intraradices*) on enzyme activities of a sterilized soil–sand mixture and nutrient uptake by maize

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Abstract A pot experiment was conducted to investigate the effect of epigeic earthworm (*Eisenia fetida*) and arbuscular mycorrhizal (AM) fungi (*Glomus intraradices*) on soil enzyme activities and nutrient uptake by maize, which was grown on a mixture of sterilized soil and sand. Maize plants were grown in pots inoculated or not inoculated with AMF, treated or not treated with earthworms. Wheat straw was added as a feed source for earthworms. Mycorrhizal colonization of maize was markedly increased in AM fungi inoculated pots and further increased by addition of epigeic earthworms. AM fungi and epigeic earthworms increased maize shoot and root biomass, respectively. Soil acid phosphatase activity was increased by both earthworms and mycorrhiza, while urease and cellulase activities were only affected by earthworms. Inoculation with AM fungi significantly ($p < 0.001$) increased the activity of soil acid phosphatase but decreased soil available phosphorus (P) and potassium (K) concentrations at harvest. Addition of earthworms alone significantly ($p < 0.05$) increased soil ammonium-N content, but decreased soil available P and K contents. AM fungi increased maize shoot weight and root P content, while earthworms improved N, P, and K contents in shoots. AM fungi and earthworm interactively increased maize shoot and root biomass through their regulation

of soil enzyme activities and on the content of available soil N, P, and K.

Keywords Soil urease · Soil phosphatase · Soil cellulose · Mycorrhizal colonization · Shoot and root biomass

Introduction

Interactions between plants and soil organisms are widespread and essential for nutrient uptake by plants (Wurst et al. 2010). Earthworms, arbuscular mycorrhizal fungi (AM fungi), and plant roots are key components of soil biota (Six et al. 2002). AM fungi are symbiotically associated with the roots of two-thirds of terrestrial plant species (Smith and Read 2008). The extraradical mycelium of AM fungi increases nutrient uptake by the root system by extending the exploited soil volume (Tuffen et al. 2002). Earthworms influence plant growth by changing the spatiotemporal availability of P, N, and C in their casts and burrow walls (Milleret et al. 2009).

Earthworms and AM fungi have been extensively studied for their interactions in rhizosphere (Eisenhauer et al. 2009; Milleret et al. 2009; Wurst et al. 2004). These organisms belong to different guilds (Bardgett 2005) in soil and can create distinct niches for plants (Wurst et al. 2010). They impact plant growth by complementarily affecting soil nutrient availability. For example, AM fungi can increase soil P availability and earthworms can promote N availability (Li et al. 2012; Ortiz-Ceballos et al. 2007; Wurst et al. 2004), but their effect on plant growth depend on the presence of these nutrients in soil (Milleret et al. 2009). While soil fertility has been the focus of research, few studies have been conducted to examine interactive effects of earthworms

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and AM fungi on enzyme activities that play an essential role in soil biochemical transformation, organic residue decomposition, and nutrient cycling (Dilly and Imler 1998; McLatchey and Reddy 1998).

Soil enzymes originated from soil microorganisms, plant roots, plant debris, and animals. Enzyme activities can be used to monitor changes in soil microbial activity and soil fertility (Parthasarathi and Ranganathan 2000). AM fungi can increase the activity of soil phosphatase (Mar Vázquez et al. 2000), dehydrogenase, urease, acid phosphatase, protease, and B-glucosidase (Caravaca et al. 2004). The presence of earthworms in soil can also affect enzyme activities (Tao et al. 2009) such as protease, phosphatase, β -glucosidase, invertase, amylase, and cellulase activities (Aira et al. 2005; Parthasarathi and Ranganathan 2000; Zhang et al. 2000). However, information on interactions of AM fungi and earthworms on soil enzyme activities is lacking.

According to their habitat and behavior, earthworms are epigeic (litter inhibitors, e.g. *Eisenia fetida*), endogeic (vertical burrowers, e.g. *Allolobophora chlorotica*), or anecic (mineral dwellers, e.g. *Lumbricus terrestris*) (Römbke et al. 2005). Studies of AM fungi and earthworm interaction have been focused on anecic species (such as *L. terrestris* L.) (Scheu 2003) or endogeic species (such as *Aporrectodea caliginosa* S.) (Parsch et al. 2006; Tuffen et al. 2002; Wurst et al. 2004), and little is known about the interaction of epigeic species and AM fungi and their effects on soil properties and plant growth. The objective of this study was to investigate the effects of epigeic species (*E. fetida*) and AM fungi (*Glomus intraradices*) on soil enzyme activities and nutrient uptake by maize, which was grown on a mixture of sterilized soil and sand. In order to avoid the effects of indigenous AM fungi and earthworms on enzyme activities and increase soil porosity and permeability, we used a sterilized soil–sand mixture (Hayman and Tavares 1985). Soil filtrate (0.45- μ m pore size) was also used to provide similar soil conditions for microbial communities (Calvet et al. 1993).

Materials and methods

Soil and organisms

Loamy sand soil was obtained from the top 0- to 20-cm layer of a crop field in Quzhou Experimental Station, China Agricultural University (36.5° N, 115.0° E). The soil was air-dried, sieved to pass 2.0 mm and autoclaved at 121 °C for 2 h. Each kilogram of soil contained 1.8 g total N, 13 g organic C, 7.72 mg available P (Olsen-P), and 33.6 mg available K ($\text{NH}_4\text{OAc-K}$). pH (H_2O , soil 2.5:1, v/v) was 8.44. Soil and river sand were sieved (<2 mm) and sterilized by autoclaving at 121 °C for 2 h. After drying at room temperature, the soil and river sand were mixed at a ratio of 1:1 (v/v).

Earthworms (*E. fetida*) obtained from the same field where the soil was collected were washed with distilled water and kept in sterilized glass vessels for 24 h to eliminate naturally occurring mycorrhizal propagules on their surfaces or in their gut contents. An AM (*G. intraradices* Schenck and Smith) inoculum (BEG141) was propagated on maize and white clover host plants in a growth chamber maintained at 30/18 °C, 16/8 h light/dark rotation and 50–75 % relative humidity.

Experimental design and inoculation

A completely randomized block design with a 2×2 factorial arrangement of treatments was utilized. The factors were AM fungi and earthworms, each having two levels (AM fungi inoculated or not inoculated; earthworms added or not added). Therefore, we had the following four treatments: soil–sand without earthworm and mycorrhizae (CK), soil–sand with earthworm addition (E), soil–sand with mycorrhizal addition (AM), and soil–sand with both earthworms and mycorrhizae (AM+E). The sterilized soil–sand mixture of the CK and E treatments were treated with sterilized mycorrhizae inoculum (see below). Each treatment was replicated four times.

Plastic pots (16 cm in diameter, 14 cm in height) were filled with 1.5 kg sieved (<2 mm) and sterilized soil–sand mixture that was mixed with 8 g oven-dried wheat straw. A 1-mm mesh with a 3-cm diameter hole in the center was placed at the top of the pot to prevent the escape of inoculated earthworms and to allow plant growth. Living or sterilized mycorrhiza inoculum was placed about 2 cm under the soil surface at 35 gkg⁻¹. The filtrate (0.45 μ m pore size) and sterilized mycorrhiza were used to provide similar nutrients of the live inoculum to soil (Calvet et al. 1993). Three adult earthworms of *E. fetida* with similar fresh weight were added to the appropriate pots when the third leaf emerged.

Maize seeds (*Nong Da 108*) were surface sterilized with a 10 % (v/v) solution of H_2O_2 for 10 min, rinsed thoroughly with deionized water, placed on autoclaved filter paper soaked with sterile distilled water, and incubated at 25 °C for 24 h. Two seeds were planted in each plot. After the emergence of the third leaf, only one plant was kept. Each kilogram of the soil–sand mixture was fertilized with a solution containing: 200 mg N (NH_4NO_3), 50 mg P (KH_2PO_4), 200 mg K (K_2SO_4), 100 mg Mg ($\text{MgSO}_4 \cdot 0.7\text{H}_2\text{O}$), 5 mg Zn ($\text{ZnSO}_4 \cdot 0.7\text{H}_2\text{O}$), 5 mg Mn ($\text{MnSO}_4 \cdot 0.7\text{H}_2\text{O}$), and 5 mg Cu ($\text{CuSO}_4 \cdot 0.5\text{H}_2\text{O}$) (Zhang et al. 2008). Seedlings were grown from March to May in a greenhouse at China Agricultural University, Beijing, with 16 h/8 h (light/dark) photoperiods, 25 °C/16 °C (day/night), and 42 % relative humidity. All pots were arranged according to the experimental design and irrigated daily with deionized water.

Sampling and analysis

The experiment was carried out from April 4th to June 14th. Shoot, root, and rhizosphere samples were collected at the end of the experiment. Maize shoots were dried (65 °C, 48 h) and weighed. Roots were gently removed from soil by handpicking and sieving (2 mm); then they were washed, dried (65 °C, 48 h), and weighed. AMF colonization was determined on 30 random pieces of each root sample at 250× magnification using a modified line intersect method; 1 g roots was cut by 1-cm root pieces, which were cleared in 10 % KOH, acidified with 1 % HCl and stained with 0.05 % Trypan blue in lactoglycerol (McGonigle et al. 1990). Hyphal length density (HLD) was determined using the modified procedure by Jakobsen et al. (1992). Briefly, 4-g rhizosphere soil samples (three replicates in each pot) were dispersed in a sodium hexameta phosphate solution (35 g l⁻¹) and shaken for 30 s (end-over-end). After 30 min, the suspension was decanted quantitatively through a 40-μm sieve. Retained hyphae, roots, and organic matter were transferred with 200 ml of deionized water into a 250-ml flask. The contents were shaken vigorously by hand for 5 s. After 1 min, 4×1 ml aliquots (10-s interval) were pipetted onto Millipore RAWG02500 membranes (Millipore, Bedford, MA, USA). The filter was stained in 0.05 % Trypan blue. HLD was estimated with a gridline intersect method at 250× magnification (Newman 1966).

Dried roots and shoots were digested with mixed H₂SO₄ and H₂O₂ (5:2, v/v). The digest was analyzed for N with the Kjeldahl method (Axmann et al. 1990), for P using the molybdate blue colorimetric procedure (Murphy and Riley 1962), and for K by flame photometry (Jones and Case 1990). Ammonium was extracted from the soil–sand mixture with 2 M KCl and determined according to Keeney and Nelson (1982), available P was extracted with 0.5 mol l⁻¹ NaHCO₃ and determined according to Olsen et al. (1954), exchangeable K was extracted with N ammonium acetate and determined according to Metson (1956), and organic C (SOC) was determined by the K₂Cr₂O₇ colorimetric oxidation method by Walkley (1947).

Enzyme assays of the sterilized soil–sand mixture

Urease activity was determined as proposed by Kandeler and Gerber (1988). Soil–sand mixture (10 g) was treated with 10 ml urea (10 %, w/v), 20 ml citrate buffer (1 M, pH 6.7), and 1.5 ml methylbenzene and kept at room temperature for 15 min. The sample was then incubated at 37 °C for 24 h under shaking. The solution was filtered, and about 1.0 ml of the filtrate was mixed with 10 ml distilled water, 4 ml sodium phenolate hydroxide, and 3.0 ml sodium hypochlorite, and the optical density was determined 20 min later by a UV-2450 Spectrophotometer at 578 nm. Urease activity was expressed in milligram of NH₄⁺-N per gram of dry soil released in 24 h.

Acid (pH 6.5) phosphatase activity was determined according to the procedure of Tabatabai (1982) with minor modifications. Four milliliters of a modified universal buffer (MUB, pH 6.5) and 1 ml 25-mM sodium p-nitrophenyl phosphate in MUB were added to 1.0 g soil and then incubated at 37 °C. After 60 min, the reaction was stopped by adding 4 ml 0.5-M NaOH, and the sample was kept at room temperature for 20 min. The optical density of the solution was determined at 420 nm and by using 1 % p-nitrophenol as the standard.

Cellulase activity was determined as reported by Mandels et al. (1976). Briefly, 1 ml 50-mM pH 4.8 citrate buffer, 1 ml filtered solution, 0.5 ml toluene, and a filter paper strip were incubated at 50 °C for 24 h. Reducing sugar concentration was estimated by the dinitrosalicylic acid method (Miller 1969). Glucose was used as the standard for the analysis.

Statistical analysis

Analysis of variance was carried out using the SPSS software, version 10.0 (SPSS Institute, Inc., Cary, NC, USA). Duncan's multiple range or Fisher's LSD was used to show significant differences between treatment means at $p < 5$ %, and correlation between the measured parameters were also carried out. Relationships between treatments were tested by Pearson's correlation analyses. The redundancy analysis (RDA) used the Canoco version 4.5 software package. Significance of the first and of all ordinations axes was calculated by the Monte Carlo permutation test.

Results

By the end of the experiment, no colonization of roots was found in the CK or E treatments. The AM treatment significantly enhanced colonization and all other mycorrhizal parameters ($p < 0.001$, Table 1). Adding earthworms to AM fungi significantly ($p < 0.05$) increased maize root colonization rate, hyphae frequency, and HLD.

Compared to the CK treatment, AM significantly increased acid phosphatase activity of the sterilized soil–sand mixture ($p < 0.05$) but had no effect on urease and cellulase activities (Table 2). Addition of earthworm alone or in combination with AM fungi increased urease, acid phosphatase, and cellulase activities when compared to the CK treatment ($p < 0.05$). Incubation with AM fungi alone increased acid phosphatase activity of the sterilized soil–sand mixture compared to adding earthworms alone but decreased cellulase activity ($p < 0.05$). Addition of both AM fungi and earthworms resulted in the greatest acid phosphatase activity. The RDA analysis showed that a relationship existed between AM fungi and earthworms and enzyme activities (Fig. 1a). Acid phosphatase activity was positively affected by the presence of both earthworms and

Table 1 Root colonization rate, hyphal length, and frequency of vesicle, arbuscular, and hyphae of mycorrhized and nonmycorrhized plants inoculated with or without earthworms

Inoculation treatment ¹	Root colonization rate (%)	Vesicle frequency (%)	Arbuscular frequency (%)	Hyphae frequency (%)	Hyphal length density (g m ⁻¹)
CK	—	—	—	—	—
AM	64±1a	15±2a	40±5a	57±1a	2.0±0.3a
E	—	—	—	—	—
AM+E	71±2b	15±2a	43±3a	66±2b	2.6±0.2b
Significance ^a due to					
AM inoculation	***	***	***	***	***
AM * E interaction	*	NS	NS	**	***

Data are the means±SD of four replicates, compared by Duncan's multiple range tests. Values sharing the same lower case letter in a row did not differ

CK sterilized soil–sand mixture without earthworm and mycorrhizae addition, E sterilized soil–sand mixture with earthworm addition, AM sterilized soil–sand mixture with mycorrhizal addition, AM+E sterilized soil–sand mixture with both earthworm and mycorrhizae addition, NS not significant

*** $p<0.001$; ** $p<0.01$; * $p<0.05$

¹ Root colonization rate (%): Intensity of the mycorrhizal colonization in the root fragments. Vesicle, Arbuscular, Hyphae frequency (%): Vesicle, Arbuscular, Hyphae abundance in mycorrhizal parts of root fragments. Hyphal length density (g m⁻¹): The extraradical hyphal abundance in soil

^a By analysis of variance

mycorrhiza, while urease and cellulose activity were only affected by earthworms.

Compared to the CK treatment, AM and AM+E significantly decreased available P and K (Table 3, $p<0.05$). Treatment E significantly enhanced ammonium-N content, but decreased soil available P and K contents ($p<0.05$). There was a significant interactive effect of earthworm and AM fungi on available P and K contents. Organic C content was lower ($p<0.05$) in the AM and E treatments, but higher ($p<0.01$) in the AM+E treatment than in the CK treatment (Table 3). The RDA analysis showed that the presence of

organisms had a significant influence on organic C and available N, P, and K contents (Fig. 1a).

The E treatment significantly increased maize shoot and root biomass compared to the CK treatment ($p<0.05$), while AM only significantly improved maize shoot biomass (Table 4). The shoot and root biomass for the AM+E treatment was significantly higher than those of the CK treatment ($p<0.05$). However, the interaction between earthworm and AM fungi was only significant for the shoot biomass ($p<0.01$; Table 4). Compared to the CK treatment, E and AM+E treatments significantly ($p<0.05$) increased

Table 2 Urease activity, acid phosphatase activity, and cellulase activity of the soil–sand mixture at the harvest

Inoculation treatment	Urease (mg NH ₄ ⁺ -N g ⁻¹)	Acid phosphatase (μg PNP g ⁻¹ h ⁻¹)	Cellulase (μg glucose g ⁻¹ h ⁻¹)
CK	16.15±2.90c	24.76±0.78c	97.54±14.98b
AM	19.74±2.79bc	44.72±1.25a	89.63±21.26b
E	22.71±3.03b	28.80±1.27b	184.83±31.80a
AM+E	28.42±2.13a	42.72±1.91a	220.04±90.33a
Significance ^a due to			
AM inoculation	**	***	NS
E inoculation	***	NS	**
AM * E interaction	NS	**	NS

Data are the means±SD of four replicates, compared by Duncan's multiple range tests. Values sharing the same lower case letter in a row did not differ

CK sterilized soil–sand mixture without earthworm and mycorrhizae addition, E sterilized soil–sand mixture with earthworm addition, AM sterilized soil–sand mixture with mycorrhizal addition, AM+E sterilized soil–sand mixture with both earthworm and mycorrhizae addition, NS not significant

*** $p<0.001$; ** $p<0.01$; * $p<0.05$

Fig. 1 Independent and interactive action of earthworms and AM fungi on the properties of the sterilized soil–sand mixture (a) and maize nutrient uptake (b) in ordination diagrams from RDA. The coordinate from the first two ordination axes explained 62.6 % and 63.6 % of the variance in a and b, respectively. The significance (according to Monte Carlo permutation tests) of all canonical axes was $P=0.002$, indicating that the presence of soil organisms had a significant influence on the sterilized soil–sand mixture properties and maize nutrition uptake. CK is the sterilized soil–sand mixture without earthworm and mycorrhizae addition, E is the sterilized soil–sand mixture with earthworm addition, AM is the sterilized soil–sand mixture with mycorrhizal addition, AM+E is the sterilized soil–sand mixture with both earthworm and mycorrhizae addition

shoot N, P, and K contents but had no significant effects on root N, P, and K contents. The AM and AM+E treatments significantly improved shoot and root P content compared to the CK treatment ($p<0.01$). The RDA analysis showed that there was a relationship between organisms (AM fungi inoculated or not inoculated; earthworms added or not added) and maize nutrition uptake (Fig. 1b).

Discussion

Results of previous studies examining earthworm and mycorrhizal interactions have been inconsistent. Positive (Cheng et al. 2005, 2006; Zarea et al. 2009), negative (Gormsen et al. 2004; Pattinson et al. 1997; Welke and Parkinson 2003), and neutral (Eisenhaller et al. 2009; Ma

et al. 2006; Wurst et al. 2004) effects of earthworms on AM root colonization have been reported. Our results suggested that epigeic earthworms increased colonization rate and hyphae frequency on maize roots and HLD in the rhizosphere soil–sand mixture treated with wheat straw. It has been reported that earthworms can produce phytohormones stimulating mycorrhizal infections (Azcón et al. 1978). Unlike endogeic or anecic earthworms, epigeic earthworms live above the surface of soil mineral particles and form little burrows (Edwards and Fletcher 1988; Römcke et al. 2005; Scheu 1987), and this can reduce their negative effect on mycorrhizal colonization (Pattinson et al. 1997; Scheu 2003; Welke and Parkinson 2003). Alternatively, wheat straw may provide substrates to epigeic earthworms, minimizing potential damages to fungal hyphae (Li et al. 2012). All these factors might have contributed to the overall

Table 3 Organic C, $\text{NH}_4^+\text{-N}$, and available P and K concentrations of the soil–sand mixture at harvest

Inoculation treatment	Ammonium-N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	SOC (%)
CK	4.82±0.45b	1.55±0.10a	16.44±0.28a	1.18±0.02b
AM	5.16±0.09ab	0.97±0.05c	13.09±0.25b	1.12±0.02c
E	5.39±0.31a	1.46±0.11b	14.47±0.68b	1.12±0.03c
AM+E	5.23±0.23ab	1.12±0.06c	13.69±0.53b	1.25±0.02a
Significance ^a due to				
AM inoculation	NS	***	**	*
E inoculation	*	NS	NS	*
AM * E interaction	NS	*	*	***

Data are the means±SD of four replicates, compared by Duncan's multiple range tests. Values sharing the same lower case letter in a row did not differ

CK sterilized soil–sand mixture without earthworm and mycorrhizae addition, E sterilized soil–sand mixture with earthworm addition, AM sterilized soil–sand mixture with mycorrhizal addition AM+E sterilized soil–sand mixture with both earthworm and mycorrhizae addition, NS not significant

*** $p<0.001$; ** $p<0.01$; * $p<0.05$

positive impact of epigeic earthworms on the colonization rate and HLD of AM fungi observed in the present study.

Earthworms contribute to enzyme activities of soil through their casts (including surface and underground casts) (Tao et al. 2009). Although, the present enzyme assays do not give valid information on the distribution and comparative importance of reactions mediated by microbes (Nannipieri et al. 2002), soil microbial diversity can also affect enzyme activity

(Nannipieri et al. 2003). It has been reported that AM fungi can increase soil neutral phosphatase and alkaline phosphatase activities by altering the root exudation pattern or fungal exudates (Wang et al. 2006, 2007; Zhang et al. 2011). In the present study, the activity of cellulase, phosphatase, and urease of soil–sand mixture were all significantly increased by earthworms ($p<0.05$), confirming what has already been reported. (Don et al. 2008; Tiwari et al. 1989; Zhang et al.

Table 4 Mean values of shoot and root biomass (g dry matter wt) and nutrient uptake at harvest

	Shoot				Root			
	Biomass (g/plant)	N content (mg/plant)	P content (mg/plant)	K content (mg/plant)	Biomass (g/plant)	N content (mg/plant)	P content (mg/plant)	K content (mg/plant)
CK	10.37±0.14c	12.05±0.32b	0.92±0.04c	44.61±2.41b	4.93±0.30b	4.22±0.42a	0.34±0.02b	5.32±1.39a
AM	13.29±0.17b	11.87±0.41b	1.74±0.05a	45.84±2.12b	5.39±0.22ab	4.06±0.13a	0.55±0.04a	5.84±0.12a
E	13.03±0.26b	14.24±0.582a	1.17±0.05b	52.42±2.24a	5.57±0.05a	4.25±0.19a	0.40±0.03b	4.80±0.52a
AM+E	14.33±0.21a	14.50±0.18a	1.86±0.07a	50.19±1.95a	5.62±0.08a	4.27±0.25a	0.56±0.03a	5.30±0.63a
Significance ^a due to								
AM inoculation		***	NS	***	NS	NS	NS	***
NS								
E inoculation		***	***	**	***	*	NS	NS
NS								
AM * E interaction	**	NS	NS	NS	NS	NS	NS	NS

Data are the means±SD of four replicates, compared by Duncan's multiple range tests. Values sharing the same lower case letter in a row did not differ

CK sterilized soil–sand mixture without earthworm and mycorrhizae addition, E sterilized soil–sand mixture with earthworm addition, AM sterilized soil–sand mixture with mycorrhizal addition, AM+E sterilized soil–sand mixture with both earthworm and mycorrhizae addition, NS not significant

*** $p<0.001$; ** $p<0.01$; * $p<0.05$

2000). In addition to releasing enzymes with casts, earthworms can affect enzyme activity of the soil by stimulating soil microbial activity (Zhang et al. 2000). Stimulation of ureolytic microorganisms, and thus increase in urease activity, can also occur in soil (Caravaca et al. 2003). However, more detailed investigations are needed to understand the interactive influence between microbial diversity and earthworm on soil enzyme activities.

Enzyme activity can be related to the availability of nutrients in soil (He et al. 2010). For example, urease activity hydrolyzes urea N to $\text{NH}_4^+\text{-N}$ (Don et al. 2008). Indeed, we have observed a positive and a significant ($p < 0.05$) correlation between urease activity and ammonium-N content ($p < 0.05$) in the present study. However, the activity of acid phosphatase was negatively correlated with available P and K ($p < 0.001$). Probably earthworms decreased available P, thus stimulating the activity of acid phosphatase. AM fungi also significantly increased the activity of acid phosphatase with the consequent decrease in available P and K concentrations. Earthworms and AM fungi interacted in their effects on acid phosphatase activity.

The observed reduction in organic C content with AM possibly resulted from hyphal growth of *G. intraradices* in response to specific compounds such as 3,4,5-substituted benzyl structures. These compounds have been reported to enhance soil organic matter decomposition and decrease organic C content (Gryndler et al. 2009). The content of organic C was reduced in the E treatment. Probably the digestion activity of earthworms increased mineralization and humification of organic matter (Lavelle 1988). However, the effects of earthworms on organic matter transformations are not limited by the short and transit gut activities because the earthworm casts can prolong microbial activities, thus affecting soil organic matter transformation (Lavelle 1988).

Earthworms and arbuscular mycorrhiza fungi (AMF) are important organisms of the rhizosphere soil. AM fungi form prominent symbioses with roots, increasing their potential to mobilize nutrients, particularly P (Bago et al. 2000; Artursson et al. 2006), which can affect growth and physiology of most plants (Jeffries et al. 2003; Smith and Read 2008). Although the propagules in the native soil were lower than those in mycorrhizal inocula, the indigenous AM fungi of soil could colonize the host plants in the nonmycorrhized treatments (Wang et al. 2006; Zarea et al. 2009). In order to avoid the effects of the indigenous AM fungi and increase soil porosity and permeability, we used the sterilized soil–sand mixture (Hayman and Tavares 1985). Compared to the CK treatment, the AM treatment significantly increased maize shoot and root P content as well as shoot biomass. Similarly, the E treatment also increased biomass of maize and shoot N and P contents. These results are consistent with reports that earthworms influence plant growth by altering the spatiotemporal

availability of P and N contents (Edwards 2004; Eisenhauer and Scheu 2008; Laossi et al. 2010). In addition, shoot K content was increased in the E treatment, and the activities of soil cellulase showed a significant ($p < 0.05$) positive correlation with the shoot K content.

Earthworms and AM fungi function as different nutrient providers and create distinct niches for plants (Wurst et al. 2010). They can increase plant nutrient availability in soil and synergically can increase plant nutrient uptake (Eisenhauer et al. 2009). In the present study, shoot and root biomass and their N and P contents were increased in the AM+E treatment compared to the CK treatment. In addition, earthworms and AM fungi increased maize shoot biomass in an interactive way. Results suggest that earthworms and AM fungi can enhance the availability of N, P, and K contents in the soil–sand mixture and their uptake by the plant, resulting in an improved plant growth (Bardgett 2005; Li et al. 2012; Wurst et al. 2004).

Conclusion

Epigeic earthworms (*E. fetida*) increased maize shoot and root biomass by affecting the activities of urease, acid phosphatase, and cellulase and increased availability of N, P, and K contents in the sterilized soil–sand mixture and their uptake by maize. AM fungi (*G. intraradices*) increased the maize shoot biomass by enhancing acid phosphatase activity and shoot and root P content. AM fungi and earthworm interactively increased maize shoot and root biomass probably through their effects on enzyme activities and on availability of N, P, and K in the sterilized soil–sand mixture, which complementarily improved maize nutrient uptake and growth. This research shed light on the possibility to improve plant nutrient uptake by regulating biology, which could explore the potential for soil biological fertility in the sustainable agricultural system.

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