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Earthworms facilitated pepper (*Capsicum annuum* L.) growth via enhancing the population and function of arbuscular mycorrhizal fungi in a low-density polyethylene-contaminated soil

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Abstract

Microplastics (MPs) produced by the decomposition of plastics exist persistently, interfering with soil fertility and plant nutrition. Both arbuscular mycorrhizal (AM) fungi and earthworms are beneficial in terrestrial ecosystems, but their interactions under MPs contamination are unclear so far. Here, the influences of inoculating earthworms (*Eisenia fetida*) on indigenous AM fungi and pepper (*Capsicum annuum* L.) growth were investigated in a vegetable soil treated with 0.1% low-density polyethylene (LDPE), while the specific interactions of earthworm and AM fungus (*Funneliformis caledonium*) under LDPE contamination were further resolved in another experiment using sterilized soil. Inoculation of earthworms shifted soil AM fungal community structure, replacing the predominant genus *Glomus* by *Paraglomus*, and increased the abundance, diversity (i.e., Shannon) index, and root colonization rate of AM fungi by 108, 34.6 and 45.0%, respectively. Earthworms also significantly decreased soil pH, and significantly increased soil alkaline phosphatase (ALP) activity, shoot biomass and fruit yield of pepper by 394, 82.8 and 188%, respectively. In the sterilized soil, both *E. fetida* and *F. caledonium* improved pepper growth, while the latter noticeably increased phosphorus (P) translocation efficiency from root to shoot, and the combination induced the highest soil ALP activity and pepper fruit yield. Furthermore, the significantly interactive effects between earthworm and AM fungus were observed in soil pH and available P concentration, as well as in shoot P concentration and fruit yield of pepper. This study revealed the interaction between earthworms and AM fungi under MPs contamination conditions for the first time, indicating that earthworms could facilitate vegetable growth via enhancing the propagation and P-promoting function of AM fungi in LDPE-contaminated soils.

Keywords Alkaline phosphatase, *Eisenia fetida*, *Funneliformis caledonium*, Microplastics, P translocation efficiency

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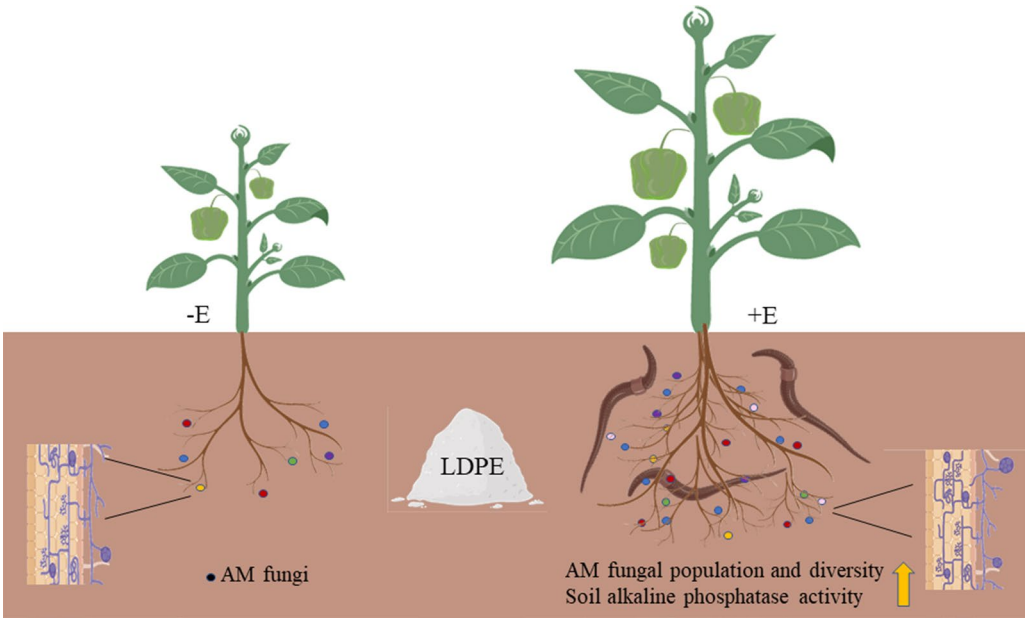
Graphical Abstract



Introduction

Owing to global economic, social and population development worldwide, there is an increasing demand for larger quantities and varieties of food and vegetables. This has led to the emergence and rapid development of intensive vegetable production systems in recent decades [1]. In order to achieve higher yields and better quality, approx. 1.4 million tonnes of agricultural plastic films were used to cover 17.4 million hectares in 2020 [2]. Plastic mulching serves primarily to reduce soil water loss and modify radiation budget, thereby improving water use efficiency, regulating soil temperature, plant growth, as well as insect and weed infestation [3]. However, overuse of mulches has resulted in excessive production of residues, causing detrimental white pollution in the field, which poses a threat to terrestrial ecosystems [4]. Residual mulches are known to decompose into fragments under environmental factors, resulting in particle smaller than 5 mm in size referred to as microplastics (MPs) [5]. MPs are emerging contaminants which have received significant global attention due to their potential environmental health risks.

As the main raw material of agricultural mulches, the stable chemical properties of low-density polyethylene (LDPE) could persist in the soil for 200–400 years before complete degradation [6]. The presence of MPs could hinder plant growth and inflict oxidative, genotoxic and antioxidant system damage [7], and potentially impede plant performance directly by altering soil physicochemical parameters, such as bulk density, water holding capacity [8, 9], and nutrient availability [10]. The presence of MPs could significantly decrease soil available phosphorus (P) concentration by approximately 50% [11]. Additionally, this alters soil microbial community and activity [9, 12], subsequently impacting plant nutrition [13]. As a result, the management and utilization of soil beneficial organisms for elevating nutrient (particularly P) utilization effectiveness by crops and/or vegetables under MPs contamination are critical areas of investigation.

Arbuscular mycorrhizal (AM) fungi are globally distributed in various soil ecosystems, including heathy, degraded, as well as contaminated [14], which can colonize more than 90% surveyed higher plants in terrestrial ecosystems [15]. Such colonization could facilitate host plants to uptake nutrient (notably P) [16], and enhance plant resistance [17]. On the one hand, AM fungi could improve soil physicochemical properties. For instance, the hyphae could promote the extension of root via improving soil physical structure, such as aggregation, bulk density and porosity [18], and the secretions could influence soil chemical parameters, resulting in a more favorable rhizosphere, including a decrease in phytoavailability of heavy metals [19]. On the other hand, the rhizosphere microbial community could be reshaped by AM

fungi [20]. It has been reported that AM fungi can stimulate the microbial groups correlated with polychlorinated biphenyls (PCBs) dissipation [21]. The performance, community structure, and diversity of AM fungi could be suppressed by MPs too [22], and there is a great prospect to decrease the negative effects of MPs using AM symbiosis. However, it is still not feasible to widely apply AM inocula since they are commonly propagated by mycorrhizal plants [23]. Thus, it is important to enhance the ecological function of indigenous AM fungal community.

As one of the most plentiful species, earthworms dramatically sustain the ecological function of soil [24]. Contributing to the behaviors such as moving, ingesting, excreting and secreting, earthworms improve the soil structure and fertility [25, 26], and interact with beneficial microbial groups to promote plant growth [27]. They also possess the ability to alleviate the toxicity of pollutants in soil, including arsenide and organic pollutants [28, 29]. A number of studies indicated that earthworms could decrease MPs by ingesting [30], and the bacteria extracted from earthworm's gut have been proven to reduce the size of LDPE [31]. These consequences demonstrate earthworms have great potential in soil MPs remediation. Furthermore, there are complex interactions between earthworms and AM fungi [32, 33]. Ma et al. [34] reported that both lead (Pb) and zinc (Zn) availabilities were significantly decreased upon the combination of earthworms and AM fungi. Several studies illustrated the translocation of AM fungal propagules attributed to the moving of earthworms increased the opportunity of mycorrhizal colonization [35, 36], and AM fungal community was stimulated by the activities and secretions of earthworms [37]. However, little is known about the interaction between earthworms and AM fungi in MPs-contaminated soils, and it would be great significance if earthworms could facilitate the growth and activity of indigenous AM fungi.

As highlighted above, it was hypothesized that earthworms could benefit AM fungi under MPs contamination, while improving P use effectiveness through interactions between them. Thus, a couple of greenhouse pot experiments were designed to identify the interactions between earthworms and AM fungi in a LDPE-treated soil. The first one aimed to answer how earthworms impact the performance and role of indigenous AM fungi, while the other one was implemented to reveal the interaction of earthworm and AM fungus on P use effectiveness by pepper (*Capsicum annuum* L.) plant. It was anticipated to provide a bio-technique for enhancing the facilitation of AM fungi for vegetable growth and the safe agricultural production in LDPE-contaminated fields.

Materials and methods

Preparation of the tested materials

The tested soil was collected from a vegetable field located in the Jiangning Area, Nanjing City, Jiangsu Province, China (31°43′12′′N, 118°46′24′′E). Plant residues, rocks and other debris were eliminated via sieving with 5-mm mesh. The following soil properties were then determined: pH 4.62 (soil/water = 1: 2.5), organic carbon (C) 16.3 g kg^{-1}, mineral nitrogen (N) 19.6 mg kg^{-1}, available P 34.5 mg kg^{-1}, available potassium (K) 98.6 mg kg^{-1}. The tested low-density polyethylene (LDPE) with the size of 500 nm was produced by DoPont, America. The soil was mixed uniformly with 0.1% of LDPE (*w*/*w*) for one week [8, 38], and then homogenized with mineral fertilizers (urea, superphosphate and potassium sulfate) with the application rate of N, P_2O_5 and K_2O at 135, 108, 162 kg ha^{-1}. Prior to mixing with LDPE, the soil used in the second experiment was sterilized twice using high-pressure steam (121 ℃, 1 h) with a 24 h interval.

The AM fungus *Funneliformis caledonium* (Nicolson & Gerd.) Walker & Schüßler (Isolate number: 90036) isolated from Fengqiu County, Henan Province, China was subject to testing [39]. It was propagated by cycles of white clover (*Trifolium repens* L.) and sudangrass (*Sorghum sudanense* Stapf.) with four months per cycle in the greenhouse of Institute of Soil Science, Chinese Academy of Sciences, China. The final inoculum, involving the rhizosphere soil containing spores, hyphae, and mycorrhizal root fragments, was air-dried and homogenized by sieving (2-mm mesh). Meanwhile, the non-mycorrhizal inoculum was also prepared using the same conditions. The tested earthworm *Eisenia fetida* L. (Sav.) was purchased from Wangjun Earthworms Farm, Jurong City, Jiangsu Province, China. To clean the intestine, all washed earthworms were put on wetting papers at the bottom of a box for 24 h. The seeds of pepper (Variety: Selway F1) were sterilized with 0.5% NaClO, and subsequently germinated in a hole tray filled with sterile seedling matrix for five weeks.

Pot experiment and harvest

Two pot experiments were carried out. The first experiment which used unsterilized soil was composed of two treatments: with earthworm inoculation (+E) and without (-E). The second experiment which used sterilized soil incorporated four treatments: the control, inoculation with earthworm (E), AM fungus (M), and both (E + M). On June 10, 2021, 2.4 kg of soil was put per pot (18 cm diameter × 19 cm depth) with three pepper seedlings and 0.6 kg of casing soil. Specially, a layer of 150 g of non-mycorrhizal or mycorrhizal inoculum was inoculated before pepper transplanting for the second experiment. For both experiments, every 15 earthworms were amended to each E-treated pot,

which was covered by a gauze to prevent earthworms from escaping. There were four replicates for each treatment. The experiments were carried out in a sunlit glasshouse with 30/22 ℃ day/night temperature and 40–60% relative humidity. Harvesting of mature peppers for yield, biomass and nutrition determination took place on September 8. Additionally, soil samples were taken from each pot for analysis of soil properties and DNA extraction.

Soil DNA extraction, quantitative PCR and Illumina sequencing

The extraction of genomic DNA from fresh soils was performed using the Fast DNA® Spin Kit for Soil (MP Biomedicals, OH, USA), and then assessment was carried out using the NanoDrop ND-2000 Spectrophotometer (Thermo Scientific). Enumeration of the copy number of AM fungal 18S rRNA gene fragment was achieved through quantitative PCR (qPCR), employing primer pairs AML2/NS31 on a CFX96 instrument (Bio-Rad, Shanghai, China) with an amplification efficiency of 96.7% ($R^2 = 0.997$), as instructed by Hu et al. [40].

For the first experiment, the 18S rRNA gene fragment of AM fungi were again amplified by nested PCR with the primer pairs of GeoA2/AML2 and N31/AMDGR [41]. The PCR was conducted in a 20-μl reaction system, containing 4 μl of 5 × PCR buffer, 2 μl of 2.5 mmol L^{-1} of dNTPs, 0.8 μl of primers (5 μM each), 0.4 μl of FastPfu DNA polymerase, 0.2 μl of BSA, 10 ng of DNA template, and ddH_2O was added to make it up to a volume of 20 μl. The following program was conducted: predenaturing for 3 min at 95 ℃; 30 (first PCR) or 27 (second PCR) cycles of PCR at 95 ℃ for 30 s, 55 ℃ for 30 s, 72 ℃ for 45 s, and 72 ℃ for 10 min [42]. The products were purified with an AxyPrep NA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and subsequently subjected to 2 × 300 bp paired-end sequencing on the Illumina MiSeq platform at Majorbio Co., Ltd. (Shanghai, China).

The raw sequences were processed following the operational taxonomic unit (OTU) denoising pipeline, disposing by USEARCH *v*.11 (http://www.drive5.com/usearch/manual/uparse_pipeline.html) [43]. The OTUs underwent classification via a 97% similarity threshold and were BLASTed against the MaarjAM database [44]. The flattening OTU-table was utilized to compute the diversity index for avoiding bias. The AM fungi community's stacking diagram was generated by employing '*reshape*' in *R* 4.0.2. The '*vegan*' was applied to evaluate the observed richness, Chao1 index, Shannon index, and Pielou's evenness, and all figures were conducted by the '*ggplot2*'.

Soil and plant properties analyses

The air-dried soil samples were tested after homogenizing through a 0.25-mm mesh sieve. Soil pH was detected using a digital pH meter (soil/water = 1: 2.5), and available P was determined using the molybdenum blue method after extraction by hydrochloride-ammonium fluoride [45], while alkaline phosphatase (ALP) activity was quantified in line with Tabatabai [46]. After further sieving through a 0.15-mm mesh sieve, soil organic C was determined by $K_2Cr_2O_7$ colorimetric oxidization [47]. The fruits, shoots, and roots were harvested individually, and the fresh fruit yield was immediately recorded. All tissue samples, except sub-sample of fresh roots, were weighted after drying at 70 ℃ for 48 h. The fresh roots were examined microscopically for determining mycorrhizal colonization via line crossing method after be cleared with 10% (*m*/*m*) KOH, acidified with 1% (*m*/*m*) HCl and stained with trypan blue [48]. Dried plant samples collected from the second experiment were digested with H_2SO_4–H_2O_2 mixture, and the P concentration was determined using the molybdenum blue colorimetry [49].

Statistical analysis

Statistical analysis was conducted with SPSS 26.0, representing data as means with standard deviations. A one-way analysis of variance (ANOVA) was performed using Duncan's multiple range method ($p < 0.05$) to identify significant differences among the four treatments, and the Mann–Whitney U-test was applied to distinguish significant differences between −E and + E treatments or between M and E + M treatments. A two-way ANOVA was also performed to determine the interactions between factors E and M. The redundancy analysis (RDA), using Canoco 5.0, revealed the relationship among plant, soil, and mycorrhizal parameters. In *R* 4.3.1, the Pearson correlation was calculated by '*psych*' package, the randomforest model was constructed by '*randomForest*' package and tested for significance by '*A3*' package, and the '*vegan*' package was applied for variance partitioning analysis (VPA). For the construction of the structural equation model (SEM), Amos Graphics 21.0 was utilized.

Results

AM fungal diversity, colonization and plant growth in the unsterilized soil

A total of 120952 high-quality sequences and 85 OTUs were attained, encompassing *Paraglomus*, *Glomus*, *Claroideoglomus*, *Acaulospora*, *Archaeospora*, *Diversispora* and *Ambispora* (Fig. 1). In general, *Paraglomus* outcompeted *Glomus* as the dominant genus in the + E soil, whereas the other three genera (*Acaulospora*, *Diversispora* and *Ambispora*) were exclusively identified in the + E soil. The Shannon index (Fig. 2B) rather than the observed richness (Fig. 2A), Simpson's index (Fig. 2C) and Pielou's evenness (Fig. 2D), was obviously increased ($p < 0.05$) by 34.6% with + E.

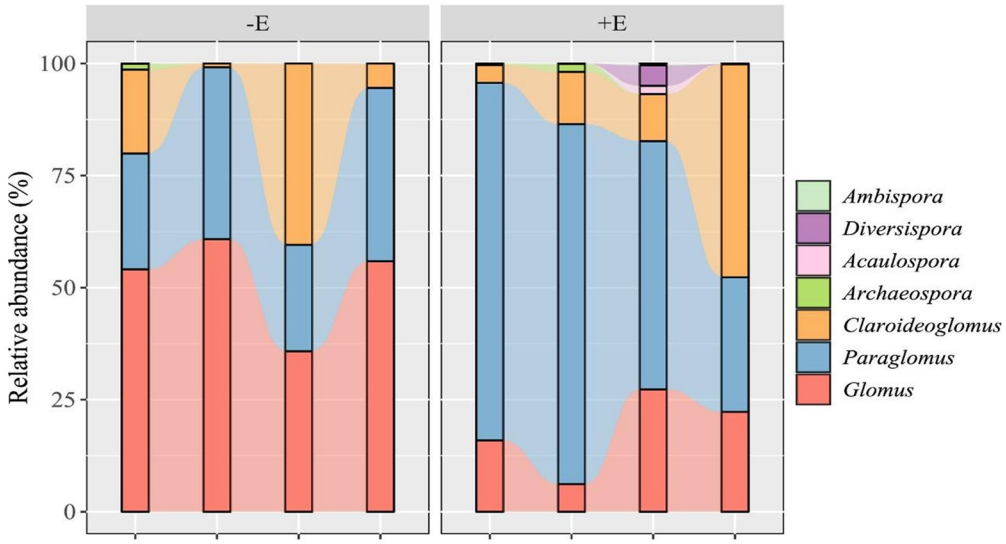


Fig. 1 Relative abundance of each genus of arbuscular mycorrhizal (AM) fungi in the soil. -E, non-inoculation of earthworm; + E, inoculation of earthworm

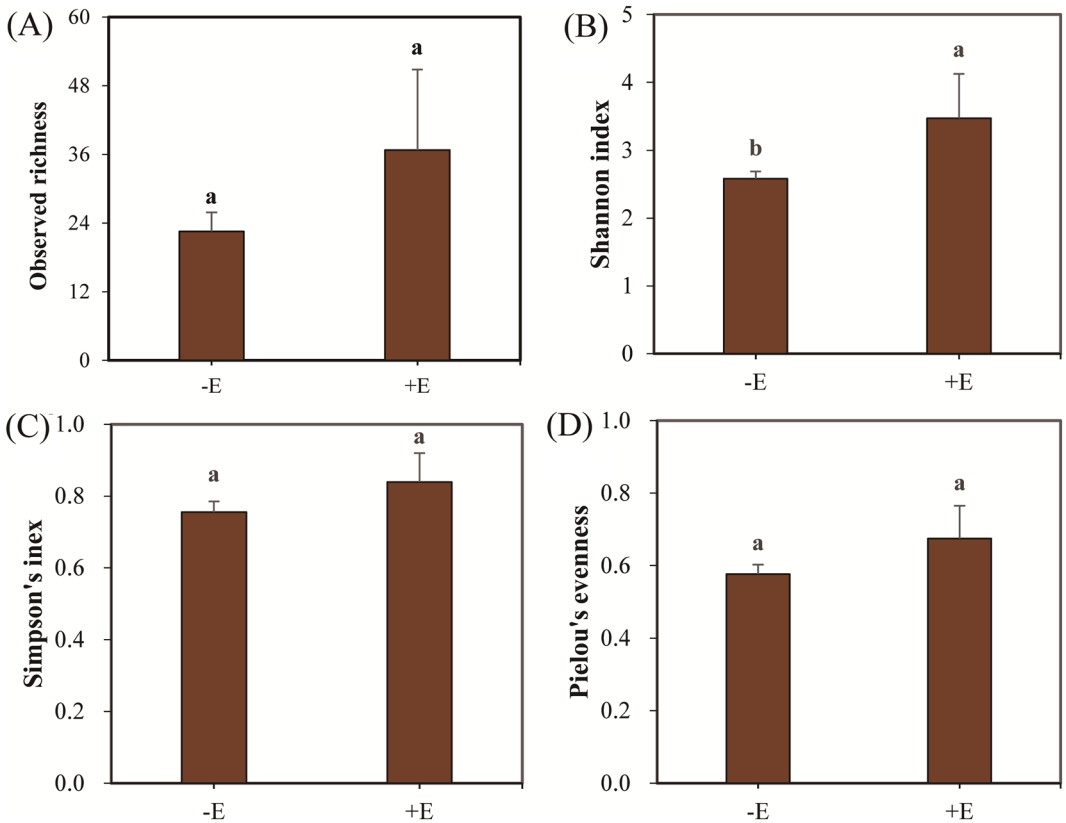


Fig. 2 The observed richness (**A**), Shannon index (**B**), Simpson's index (**C**), Pielou's evenness (**D**) of soil arbuscular mycorrhizal (AM) fungal community. -E, non-inoculation of earthworm; + E, inoculation of earthworm. Data are means with standard deviation ($n = 4$). Different letters indicate significant difference ($p < 0.05$)

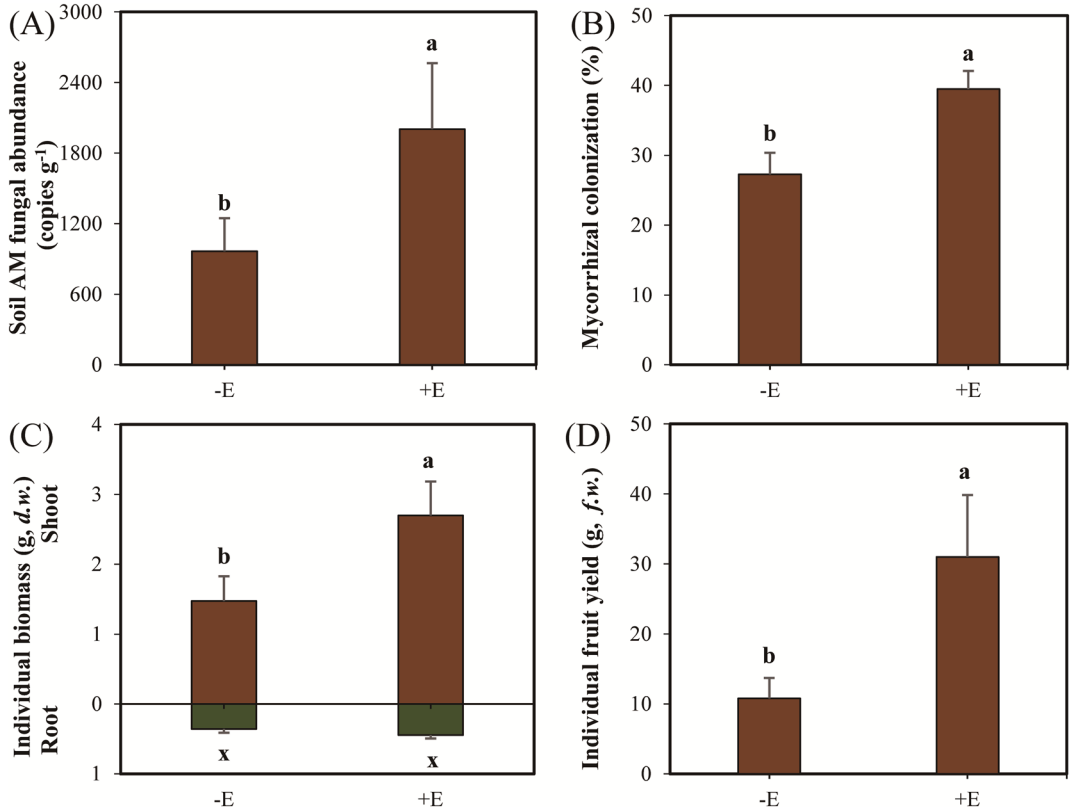


Fig. 3 Soil arbuscular mycorrhizal (AM) fungal abundance (**A**), root mycorrhizal colonization (**B**), and the individual plant biomass (**C**) and fruit yield (**D**) of pepper in the unsterilized soil. -E, non-inoculation of earthworm; + E, inoculation of earthworm. Data are means with standard deviation ($n = 4$). Different letters indicate significant difference ($p < 0.05$)

Table 1 The pH, organic C, available P, and alkaline phosphatase (ALP) activity in the unsterilized soil

Treatments	pH	Organic C (g kg^{-1})	Available P (mg kg^{-1})	ALP activity (mg g^{-1} 24 h^{-1})
-E	5.15 ± 0.03a	18.2 ± 0.5a	36.9 ± 2.9a	0.051 ± 0.047b
+ E	5.03 ± 0.15b	16.5 ± 2.5a	41.1 ± 3.7a	0.252 ± 0.036a

-E, non-inoculation of earthworm; + E, inoculation of earthworm

Data are means with standard deviation ($n = 4$). Values within the same column followed by different letters differ significantly ($p < 0.05$)

The earthworms facilitated the propagation and symbiosis of AM fungi. Compared with -E, the soil AM fungal abundance (Fig. 3A) and root mycorrhizal colonization (Fig. 3B) in + E were increased by 108% and 45.0%, respectively. Similarly, + E significantly increased ($p < 0.05$) soil ALP activity (Table 1), and the shoot biomass (Fig. 3C) and fruit yield (Fig. 3D) of pepper by 394%, 82.8% and 188%, respectively. Contrarily, + E significantly decreased ($p < 0.05$) soil pH, however, it did not exhibit any remarkable impacts on the root biomass, and soil organic C and available P concentrations.

The roles of earthworms on AM fungi and plant growth in the unsterilized soil

The SEM plot (Fig. 4) showed the direct and indirect roles of earthworms on AM fungi and pepper growth. Earthworms had a direct positive effect on AM fungal population and colonization, leading to improve soil ALP activity and pepper growth indirectly. The randomforest model showed the top two factors influencing soil ALP activity were root mycorrhizal colonization and soil AM fungal abundance, and soil ALP activity demonstrated a positive correlation ($p < 0.01$) to both of them (Fig.S1).

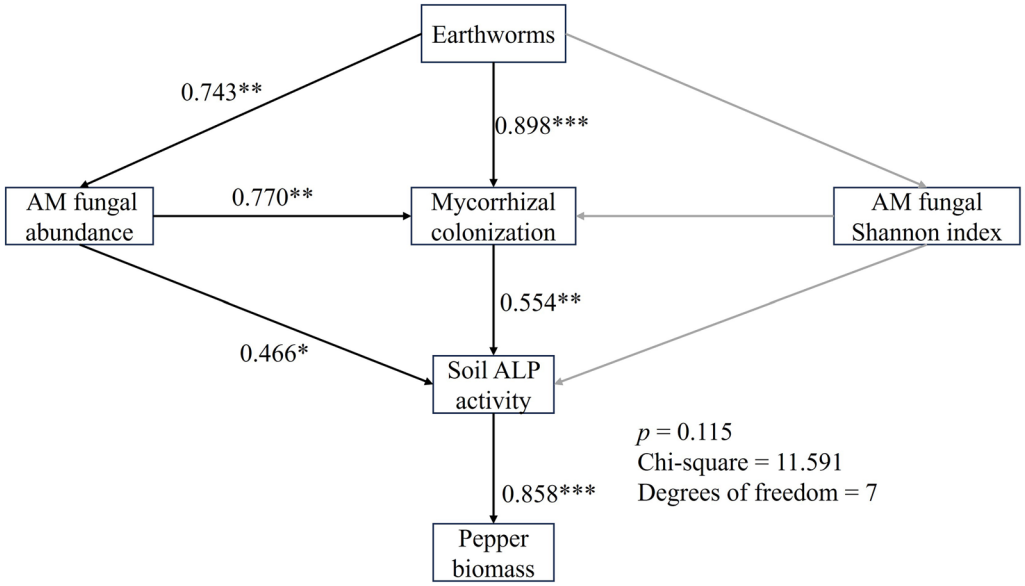


Fig. 4 The effects of earthworms on arbuscular mycorrhizal (AM) fungi (population size, Shannon index and colonization rate), soil alkaline phosphatase (ALP) activity and pepper growth. Blank and gray lines indicate significant and non-significant effects, respectively. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

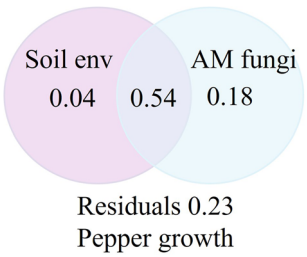


Fig. 5 Contributions made by soil environmental (env) factors, arbuscular mycorrhizal (AM) fungi and their interactions on pepper growth

The VPA plot (Fig. 5) elucidated the contributions made by soil environmental factors (soil pH, organic C, available P, and ALP activity), AM fungi (AM fungal population, Shannon index, and colonization rate) and their interactions to pepper growth (plant biomass and fruit yield), with 4%, 18% and 54% impact, respectively.

Mycorrhizal colonization and plant growth in the sterilized soil

Compared to the control, inoculation with either earthworm (E) or AM fungus (M) led to a significant increase ($p < 0.05$) in pepper fruit yield (Fig. 6D), as well as in the root and shoot biomasses (Fig. 6C). In addition, both E and M showed a tendency to increase plant total P acquisition (Fig. 7D), but only M significantly increased ($p < 0.05$) the P translocation efficiency from root to shoot (Fig. 7B), which resulted in a significantly lower ($p < 0.05$) root P concentration (Fig. 7A). Furthermore, E tended to decrease soil organic C and available P concentrations, as along with soil ALP activity, while M not only decreased soil pH ($p < 0.05$), but also showed a tendency to increase soil organic C concentration and ALP activity (Table 2). Based on the two-way ANOVA results (Table 3), inoculation of earthworm (E) had a significant systematic impact ($p < 0.01$) on plant biomass and fruit yield, while the inoculation of AM fungus (M) systematically affected ($p < 0.05$) all measured parameters except soil available P and fruit P concentrations. In addition, the significantly interactive effects of factors E and M were also observed ($p < 0.05$) in soil pH and available P concentration, as well as shoot P concentration and fruit yield of pepper.

When compared to either E or M alone, the combined inoculation (E + M) significantly increased ($p < 0.05$) soil ALP activity, root biomass, shoot P concentration, and fruit yield, and tended to increase plant total P acquisition. Compared with E, E + M also significantly increased ($p < 0.05$) soil available P concentration, shoot biomass and the root-to-shoot P translocation efficiency, and tended to decrease soil pH and raise organic C concentration. Compared to M, E + M also significantly increased ($p < 0.05$) soil AM fungal abundance (Fig. 6A), root mycorrhizal colonization rate (Fig. 6B), and tended to increase soil pH, available P concentration, and shoot biomass, but tended to decrease organic C concentration.

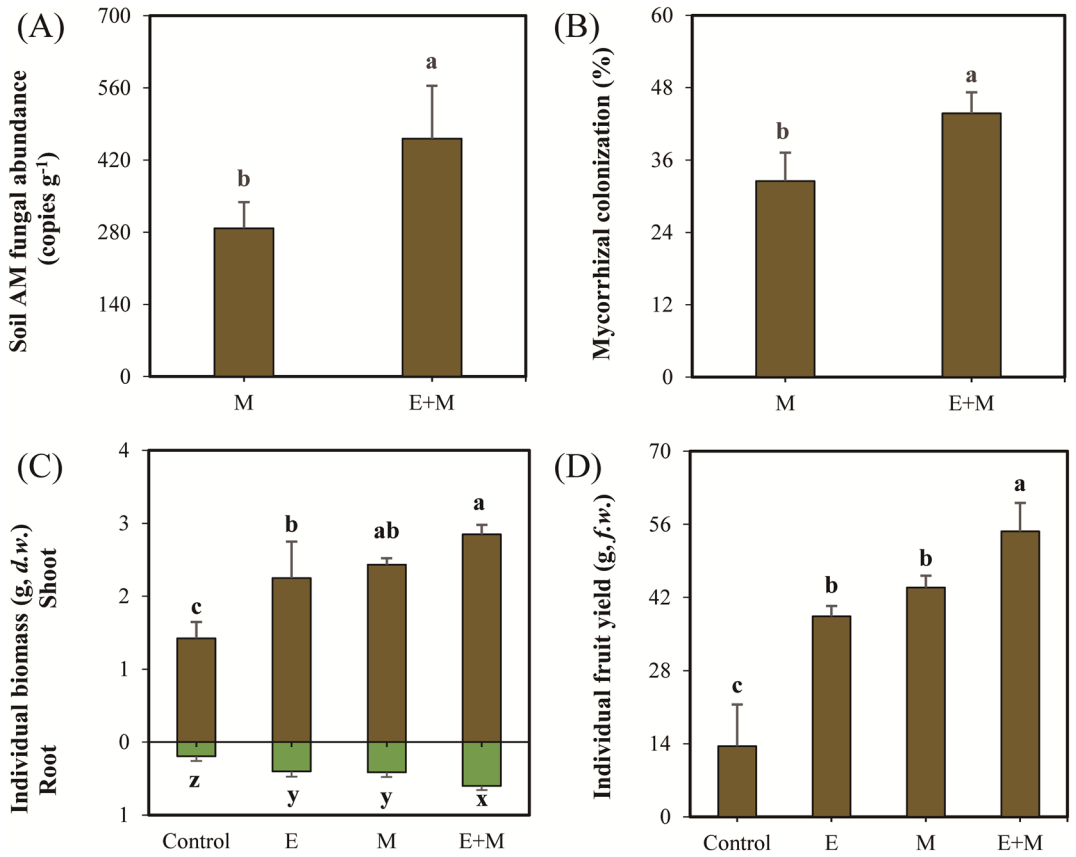


Fig. 6 Soil arbuscular mycorrhizal (AM) fungal abundance (**A**), and root mycorrhizal colonization (**B**), individual plant biomass (**C**), and fruit yield (**D**) of pepper in the sterilized soil. Control, non-inoculation; E, inoculation of earthworm; M, inoculation of AM fungus; E + M: inoculation of both. Data are means with standard deviation ($n = 4$). Different letters indicate significant difference ($p < 0.05$)

The effects of earthworms on AM fungi and plant growth in the sterilized soil

In the sterilized soil, both M and E + M had a more profound positive impact on soil ALP activity, plant total P acquisition, P translocation efficiency, plant biomass and fruit yield than E (Fig. 8). The plant biomass, fruit yield, and P translocation efficiency were all positively correlated ($p < 0.05$) to mycorrhizal colonization, AM fungal abundance, and soil ALP activity, which were also positively correlated ($p < 0.05$) to one another (Table 4). Besides, both soil organic C concentration and plant total P acquisition were positively correlated ($p < 0.01$) to mycorrhizal colonization, while soil organic C concentration was also positively correlated ($p < 0.01$) to AM fungal abundance. In contrast, the P translocation efficiency showed a negative correlation ($p < 0.05$) with soil pH. The VPA plot (Fig.S2) elucidated the contributions of AM fungi (population and colonization rate) and their interactions with soil environmental factors for pepper P nutrition (concentration, total acquisition and translocation efficiency), with 12% and 74% impact, respectively.

Discussion

The effects of AM fungi and earthworms on pepper growth

There is considerable evidence that AM fungi mitigate the negative effects of soil contaminants, such as petroleum [50], polycyclic aromatic hydrocarbons [51], and polychlorinated biphenyls [39]. In general, mycorrhizal plants have the ability to absorb more nutrients, exhibit increased antioxidant enzyme activity, upregulate expression of resistant genes [52], and regulate internal balances such as osmotic pressure [53] and hormone levels [54]. Meanwhile, AM fungi can also provide protection to hosts by secreting specific substances [55]. Sine plastic is a material made from hydrocarbons, AM fungi could theoretically mitigate the phytotoxicity of MPs. In addition, polyethylene (PE) has been shown to have severe toxic effects on seedling growth and can cause oxidative stress in plants [56]. Although the low input (0.1%) of LDPE might not have a notable effects on overall plant growth, it did decrease soil aggregation [57] and may be deemed valuable in the long term perspective [58]. In this study, as anticipated, the presence of AM fungus resulted

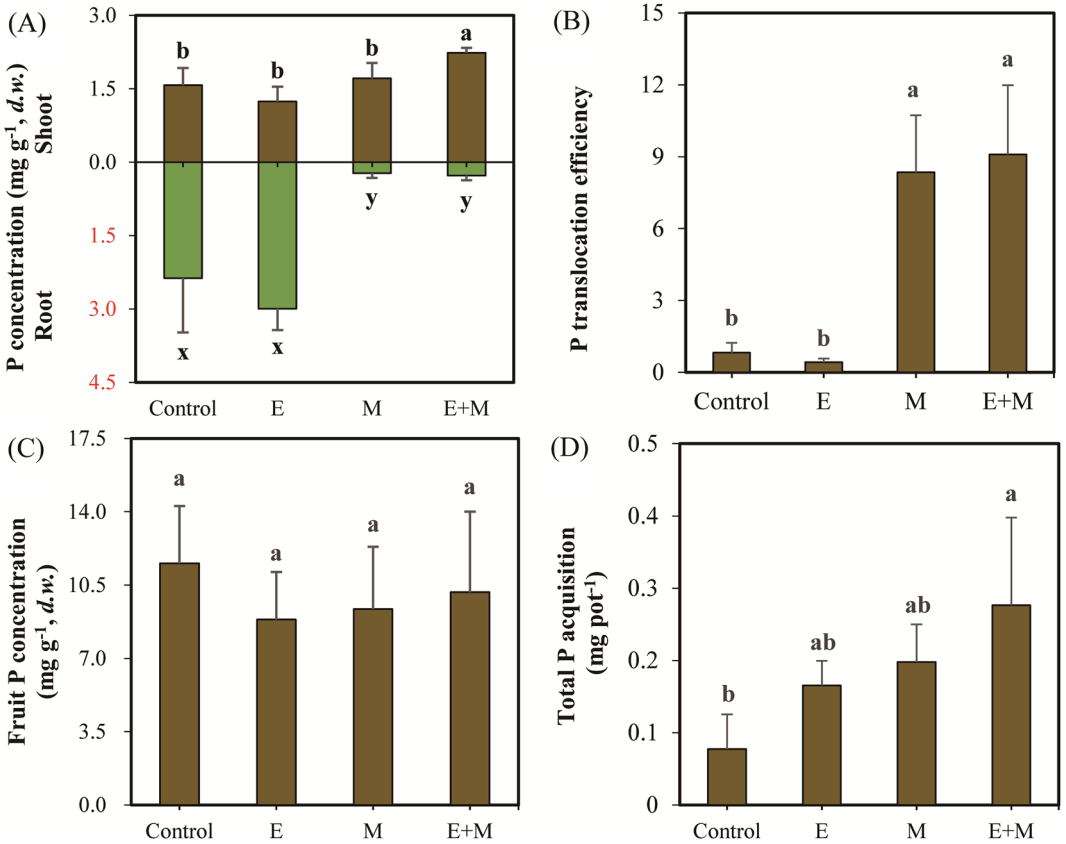


Fig. 7 Shoot and root P concentration (**A**), root-to-shoot P translocation efficiency (**B**), fruit P concentration (**C**), and total P acquisition (**D**) of pepper in the sterilized soil. Control, non-inoculation; E, inoculation of earthworm; M, inoculation of arbuscular mycorrhizal (AM) fungus; E + M: inoculation of both. Data are means with standard deviation ($n = 4$). Different letters indicate significant difference ($p < 0.05$)

Table 2 The pH, organic C, available P and alkaline phosphatase (ALP) activity in the sterilized soil

Treatments	pH	Organic C (g kg^{-1})	Available P (mg kg^{-1})	ALP activity (mg g^{-1} 24 h^{-1})
Control	5.46 ± 0.13a	17.1 ± 0.9ab	194 ± 13ab	0.135 ± 0.086bc
E	5.38 ± 0.04a	16.4 ± 0.7b	175 ± 14b	0.095 ± 0.033c
M	5.23 ± 0.03b	18.7 ± 2.0a	187 ± 19ab	0.268 ± 0.084b
E + M	5.36 ± 0.08ab	18.4 ± 0.8ab	210 ± 20a	0.412 ± 0.101a

Control, non-inoculation; E, inoculation of earthworm; M, inoculation of arbuscular mycorrhizal (AM) fungus; E + M: inoculation of both

Values are means ± standard deviations ($n = 4$). Different letters in the same column indicate significant difference ($p < 0.05$)

in a better growth of pepper plants (Fig. 6C). On the one hand, AM fungus improved plant P utilization (Fig. 7D). On the other hand, AM fungi altered partial soil properties (Table 2), in particular, the secretion of organic acids with potentially adverse effects on MPs decreased soil pH [59]. It has been suggested that AM fungi can gain support from other soil microbes by altering soil physicochemical properties [50]. Nevertheless, further experiments are still imperative to determine the effects of AM fungi in alleviating MPs pressure on pepper growth.

The widespread adaptability of earthworms in terrestrial ecosystems has garnered attentions in recent times. It is generally supposed that earthworms can promote plant growth via increasing soil nutrient availability [33]. There are two routines of earthworm here, one is

Table 3 Two-way analysis results of variance (*P* values) for the experiment conducted in the sterilized soil

Factors	Root biomass	Shoot biomass	Fruit yield	Root P concentration	Shoot P concentration	P translocation efficiency	Fruit P concentration	Total P acquisition	Soil pH	Soil organic C	Soil available P	Soil ALP activity
E	0.002**	0.003**	< 0.001***	0.351	0.584	0.873	0.599	0.069	0.518	0.518	0.818	0.286
M	0.001**	< 0.001***	< 0.001***	< 0.001***	0.005**	< 0.001***	0.805	0.017*	0.017*	0.020*	0.167	< 0.001***
E × M	0.131	0.233	0.033*	0.418	0.022*	0.608	0.333	0.915	0.038*	0.806	0.047*	0.072

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ALP, alkaline phosphatase

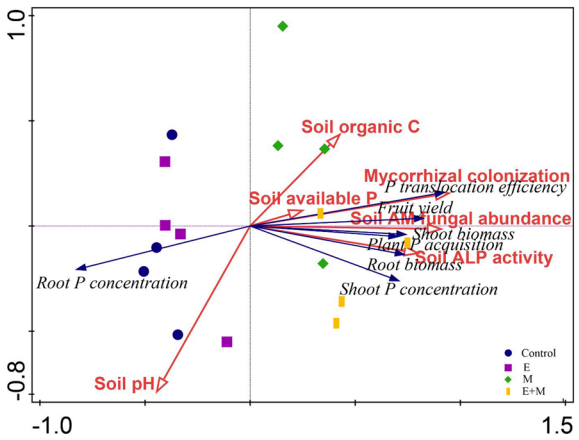


Fig. 8 Redundant analysis (RDA) plot of plant parameters with soil chemical and arbuscular mycorrhizal (AM) properties as affected by different inoculation treatments. Control, non-inoculation; E, inoculation of earthworm; M, inoculation of AM fungus; E + M: inoculation of both; ALP, alkaline phosphatase

swallowing plastic fragments, while the other relates to enhancing soil microbial activities that contribute to degradation [60]. The survival inhibition of earthworms was observed even at relatively high concentrations of MPs [61]. Previous finding has proposed that earthworms remained unharmed in the 0.1% LDPE-contaminated soil [62], indicating their resilience under such conditions. In LDPE-contaminated soils, earthworms had a positive effect on plant growth, but soil organic C and available P concentrations showed a downward trend (Table 2). The consumption of soil organic C might be linked to the feeding of earthworms, which indirectly decreased the soil ALP activity, since there was a positive correlation between soil organic C and ALP activity [63]. Meanwhile, the enhancement of pepper P absorption led to a decrease in soil available P concentration in the presence of earthworms [64]. Furthermore, the presence of earthworms may improve the tolerant capacity of plants directly or indirectly, potentially decreasing soil contamination's toxicity and stimulating plant's immunity [65].

The facilitation of earthworms on the performance of AM fungi

Both earthworms and AM fungi are ubiquitous in soil systems. Whereas, there may be multiple and uncertain impacts of earthworms on AM fungi under LDPE contamination. On the one hand, the earthworm casts could provide physical protection to a large number of AM fungal spores, resulting in an increase in population and colonization. Furthermore, earthworm activities such as burrowing, feeding, and movement could lead to the deposition of viable propagules of AM fungi [40, 66]. On the other hand, MPs had a toxic impact on AM fungi [67], whereas earthworms could directly consume MPs [68], thereby protecting AM fungi indirectly. Meanwhile, the preference of earthworms affects the community composition of AM fungi [69], leading to the displacement of the prevailing genus *Glomus* by *Paragl*omus in this study (Fig. 1). Similar to Yu et al. [70] and Dempsey et al. [71], there were higher abundance and colonization of AM fungi when inoculated with earthworms in the present tests (Fig. 3A, B; and Fig. 6A, B). The favorable resources provided by earthworms may have contributed to this benefit [72]. The significantly positive correlation between mycorrhizal colonization and AM fungal abundance (Table 4) implies that more propagules may provide more opportunities to colonize plant root, while various species (Fig. 2B) may also provide plant chances to get more harvest via establishing association with more effective fungal partners [73].

With regard to the effects of earthworms on AM fungal diversity, although there is a shortage of published evidence, the alterations in community species diversity in response to environmental changes posse significant ecological functions. One of the most significant is the improvement of plant productivity [74]. According to Tiwari and Mishra [75], earthworm casts contained all soil fungal species, and the diversity was boosted in no-tillage coupled farming combined with straw return was increased due to earthworms [76]. In this study, the increased diversity (Fig. 2B) of AM fungi was again

Table 4 Pearson's correlation coefficients (*R* values) between plant, arbuscular mycorrhizal (AM) fungal and soil parameters in the sterilized soil

	Root biomass	Shoot biomass	Fruit yield	Plant P acquisition	P translocation efficiency	Mycorrhizal colonization	AM fungal abundance
Mycorrhizal colonization	0.649**	0.706**	0.766**	0.513*	0.879**		
AM fungal abundance	0.610*	0.671**	0.731**	0.490	0.805**	0.965**	
Soil pH	-0.237	-0.301	-0.429	-0.198	-0.530*	-0.462	-0.430
Soil organic C	0.242	0.312	0.339	0.065	0.385	-0.652**	0.630**
Soil available P	0.071	0.185	0.122	-0.059	0.307	0.399	0.392
Soil ALP activity	0.577*	0.564*	0.635**	0.409	0.701**	0.812**	0.879**

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

observed in soils treated with earthworms. The activities of earthworms were used to explain the increased species of AM fungi, such as *Ambispora*, *Acaulospora*, and *Diversispora* (Fig. 1), which were located in the corner when earthworms absent. As a result of earthworms' existence, this minority was relocated to a better location for growth, regardless of whether the soils were contaminated by MPs.

It was evident that mycorrhizal plants could improve P nutrition by secreting more phosphatase enzymes to hydrolyze soil P [77]. When coexisted with earthworms in LDPE-contaminated soil, soil ALP activity was significantly improved (Tables 1 and 2), since the colonization of AM fungi got easier due to earthworm for young root feeding [78]. Although the two-way ANOVA showed the significantly interactive effect of factors (earthworm and AM fungus) was not appeared in soil ALP activity (Table 3), the enhancement of soil ALP activity was real occurrence. Meanwhile, the soil available P and shoot P concentrations, as well as pepper fruit yield (Table 3) suggested notable interactive effects between earthworms and AM fungi in LDPE-contaminated soil. Simply put, earthworms may boost pepper growth by increasing the mobility of soil P and promoting P uptake by AM fungi in certain conditions. However, proofs on the effects of earthworms on AM fungal function are inadequate since there were only limited replicates in both greenhouse pot experiments in this study, and the positive effects are inconclusive and other processes require further exploration.

Conclusions

Both *Eisenia fetida* and *Funneliformis caledonium* were effective in improving pepper growth in LDPE-contaminated soil, while the combined application could induce higher soil ALP activity and fruit yield. *E. fetida* could alter the community structure and increase the Shannon index of soil AM fungi, and could increase AM fungal abundance and the root colonization rate, and tended to increase plant P acquisition. In summary, earthworms have the potential to improve plant growth via enhancing the propagation, colonization, and mobilization and utilization of P by AM fungi. This indicates that earthworms could be used to exploit the potential of soil indigenous AM fungi to enhance the P use effectiveness in LDPE-contaminated fields.

Supplementary Information

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Additional file 1: Figure S1. The factors influencing soil alkaline phosphatase (ALP) activity (**A**), and relationship between ALP activity and mycorrhizal colonization (**B**) or arbuscular mycorrhizal (AM) fungal abundance (**C**) in the unsterilized soil. **Figure S2.** Contributions made by soil environmental (soil env) factors, arbuscular mycorrhizal (AM) fungi and their interactions on pepper P nutrition.

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Author contributions
YL, BH, and JH conducted the experiment, prepared figures and tables, and wrote the original manuscript. All authors reviewed and edited the manuscript.

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Availability of data and materials
The data that support the findings of this study are available within the article and/or its Additional file 1.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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