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# Earthworms drive the effect of polyethylene and polylactic acid microplastics on soil bacterial communities

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## Abstract

Previous reports have highlighted the hazardous nature of microplastics (MPs) in the soil system. Various types of MPs (degradable/nondegradable) may exhibit distinct behaviors that can lead to diverse effects on soil ecosystems, in particular on soil microbiota. Soil fauna performs key ecological functions such as litter decomposition, nutrient cycling, and energy flow. MPs can pose a threat to soil fauna, compromising their ecological functions and presenting ecological risks. Meanwhile, soil fauna can act on MPs, inclouding MPs formation, degradation, migration and transfer. Thereby, the interactions between MPs and soil fauna needed long-term summarized. Here, we performed a 120-day soil microcosm with polyethylene (PE) and polylactic acid (PLA), and in absence/presence of earthworms (*Eisenia nordenskioldi*) to estimate their effects on the soil bacterial communities. We found that PE and PLA had different effects on the diversity and composition of soil bacteria. Compared to CK (no MPs or earthworms), PE decreased the alpha diversity, while PLA increased it. Patescibacteria were found to be significantly abundant in the PE group, and Actinobacteria and Gemmatimonadetes were enhanced in PE and PLA+EW (PLA and earthworms) groups. All treatments affected the community structure and network structure of soil bacteria. Earthworms may affect the impact of PE/PLA on soil bacteria by feeding on bacteria or by affecting soil properties. Meanwhile, the inoculation of earthworms increases the bioturbation among soil microbiota, affecting the aging of PLA. Altogether, our results provide novel insights into the soil-fauna-driven impact of degradable/nondegradable MPs exposure on the long-term environmental risks associated with soil microorganisms.

**Keywords:** degradable microplastics, earthworms, microbiota, ecological function, characterization.

## Highlights

- MPs showed different effects on bacterial diversity and structure.
- PE could be more damaging to the richness and diversity of soil bacteria than PLA.
- Inoculation of earthworms was beneficial for the stability of bacterial network.
- The presence of earthworms was more conducive to the aging of PLA.

## 1. Introduction

Microplastics (MPs) are emerging contaminants with a nearly permanent increase, posing a tremendous hazard to the sustainability of soil ecosystems (J.-J. Guo et al., 2020; Wang et al., 2019). Due to the slow degradation process, MPs persist in the soil for extended periods (Miri et al., 2022). The ubiquitous and persistent nature of MPs leads to inevitable changes in the composition of the soil environment, multiple ecosystem functions, and long-term soil quality (Chen et al., 2020; Li et al., 2021). Biodegradable MPs (BMPs) have been developed as an alternative to the environmental risks posed by nondegradable plastics (Fan et al., 2022). BMPs are broken down by naturally occurring microbial mineralisation into $CO_2$ and $H_2O$ as the end products (Folino et al., 2020; Harrison et al., 2018). These BMPs in the soil can alter the structure and function of the microbial communities and can change the physical and chemical properties of the soil (C. Li et al., 2022; Lian et al., 2022). Due to their biodegradability, the degradation process of BMPs can also affect soil microbiota (Qin et al., 2021). Therefore, the soil microbial community is an ideal model to assess the long-term ecotoxicity of MPs in soil. However, there is still limited research on the long-term effects of degradable/non-degradable MPs on soil microbial communities.

The role of soil microorganisms in the maintenance of terrestrial ecosystem stability (Van Der Heijden et al., 2008). Several studies have investigated the effects of degradable/nondegradable MPs on soil microbes. For example, polyethylene (PE) reduced the stability and complexity of microbial networks and decreased the activity of soil bacterial communities (Fei et al., 2020; J. Shi et al., 2022). Polybutylene succinate (PBS) and polylactic acid (PLA) affect the potential of soil microbiota to absorb exogenous carbohydrates and amino acids (Sun et al., 2022a). Soil microbiota play a key role in nutrient cycling and pollutant degradation, and are crucial to maintaining soil health and fertility (Saccá et al., 2017; Q. Zhou et al., 2020). Bacteria are the most abundant microbiota and are commonly used as indicators of soil quality and health (Hermans et al., 2020). Recent research has focused on the effects of soil bacterial communities to understand the biological and ecological behaviour of MPs in soil (Fei et al., 2020). To date, the relevant knowledge on this topic remains sparse. In terms of their physico-chemical and structural properties, aggregates of different sizes and stability in soils form complexes with different ecological niches. They are made up of many different types of aggregates, including mineral particles and organic material (Tiemann et al., 2015; Davinic et al., 2012). Although MPs had been reported to alter the stability of soil aggregates (Hou et al., 2021), the response of soil bacteria to soil aggregates and MPs needs further investigation.

Soil fauna are a significant component of terrestrial ecosystems, performing many prominent ecological functions, and are important promoters of soil health and productivity (Bender et al., 2016; Kibblewhite et al., 2008). As "ecosystem engineers", earthworms can repair acidified soils and contribute to the stability of reformed aggregates (Bronick and Lal, 2005; Mummey et al., 2006). In addition, earthworms can digest and degrade microbial cells as they consume soil, which can lead to a reduction in soil microbial biomass (Gomez-Brandon et al., 2011). Furthermore, earthworms can also limit the normal growth and activity of soil microbiota through competition for food resources (Jun-Zhu et al., 2012; Lubbers et al., 2017). Earthworm activity increases the surface area of organic matter, improves the physical and chemical properties of the soil, and has a beneficial effect on soil micribiota (X. Li et al., 2022). There is a strong interaction between MPs and soil fauna (Wang, Q., et al. 2022). The survival rate, body length and reproduction of *Caenorhabditis elegans* were significantly inhibited after 2 days of exposure to 5 mg/m$^2$ MPs (Liu et al., 2018). Low-density polyethylene (LDPE) MPs can cause oxidative stress response in *Eisenia fetida* (Chen et al., 2020), accumulation of polystyrene (PS) in the intestine can cause changes in intestinal cells and DNA damage in earthworms (Jiang et al., 2020). Prendergast Miller et al (2019) found that exposure and ingestion of polyester fibers were not lethal to *Lumbricus terestris*, but the number of burrows decreased. Kim et al (2019) observed that MPs can interfere the movement of *Folsomia Candida*. Meanwhile, MPs can also affect the gut microbes of soil fauna, which play a crucial role in the ecological services of terrestrial ecosystems (M. Sun et al., 2020). Several studies have shown that MPs can alter the gut microbes of earthworms (H.T. Wang et al., 2019; Xu et al., 2021), and springtails (Ju et al., 2019; Zhu, Chen et al., 2018). Studies have shown that earthworms may have MPs degrading bacteria that break down MPs into smaller particles, which may be related to gut microbes (Lwanga et al., 2018).

Numerous studies have shown that high concentrations of MPs have negative effects on soil fauna, causing osmotic imbalances, oxidative stress and tissue damage in earthworms (Cao et al., 2017; Huerta Lwanga et al., 2016; Sobhani et al., 2021). However, most of these studies focusing on the toxicity of MPs to individual species may neglect the important role that earthworms play in decomposing organic matter, cycling nutrients and maintaining soil structure and aggregates stability. Hitherto, there are still no universally accepted conclusions on the joint effects of degradable/nondegradable MPs in conjunction with soil fauna on soil bacteria.

Therefore, a “MPs−earthworms” experiment system was set up in the present study to test the following questions: 1) What are the effects of PE and PLA on the soil bacterial community? 2) How do earthworms alter the effects of PE and PLA MPs on the soil bacterial community? The results not only provide a comprehensive understanding of the biological effects of earthworm-derived degradable/nondegradable MPs on the soil microbiome, but will also provide valuable insights for accurately assessing the long-term risk of degradable/nondegradable MPs in earthworm-affected soils. These findings would contribute to a better understanding of the impact of MPs and earthworms on soil ecosystems.

## 2. Materials and methods

### 2.1 Experimental MPs earthworms and soil

PE and PLA were purchased from Nari New Materials Supply Chain (Shanghai, China). These were all white irregular particles with densities of 0.93 and 1.24g/cm$^3$ for PE and PLA respectively. The two MPs were passed through a stainless steel sieve with a size of 500 microns. Surface soil sample was collected from Jingyuetan National Forest Park, Changchun, Jilin, northern China. Soil samples were air dried at room temperature and then passed through a 2 mm mesh for subsequent experiments. Earthworms had selected local dominant species *Eisenia nordenskioldi*.

### 2.2 Soil microcosm setup

The following treatments were used for 120 days of microcosm experiment: (1) control (CK), (2) control with earthworms (EW), (3) one dose of PE 1% (w/w) : (PE), (4) one dose of PE with earthworms : (PE+EW), (5) one dose of PLA 1% (w/w) : (PLA), (6) one dose of PLA with earthworms 1% (w/w) : (PLA+EW). Each treatment had five replicates. The dose of PE/PLA (1%) has been designed based on current knowledge of the occurrence of MPs in soils (Brown et al., 2023, 2022). The PE/PLA was thoroughly mixed into the soil to achieve the target doses. A 500mL glass bottle was used in the research to construct a controlled experiment. Each bottle weighed a total of 300 g of dry soil. Before the experiment, the experimental soil was adjusted to approximately 30% (w/w) water content and incubated at 20°C to restore the microbial population. After a 7-day pre-incubation period, two earthworms (*Eisenia nordenskioldi*) (~398 individuals $m^{-2}$) were added to the earthworm treatment (Wang et al., 2021). During the 120-day experimental period (dark, 20 ± 2 °C), no earthworms were found to be born or dead.

A column of three sieves was used to separate the aggregates: 2.00, 1.00 and 0.25 mm. Each treatment was replicated in triplicate. Aggregate stability for each sample was expressed as large aggregates content (R>0.25) and mean weight diameter (MWD, mm) for each treatment.

$R_{>0.25}$ is the cumulative mass of aggregates with a particle size greater than 0.25mm

$$MWD = \frac{\sum d * m}{100}$$

where d is the mean diameter between the two sieves (mm); and m the weight fraction of aggregates remaining on the sieve (%).

### 2.3 Characterisation of microplastics

Extraction of PE and PLA MPs from soil using the density method: the soil samples were added to the saturated sodium chloride solution, and after they were mixed evenly, the soil and contaminants sank to the bottom because their density is greater than that of the MPs, whereas the MPs would float in the solution because of their low density. The morphological change is an important index in the aging process of MPs, which provides theoretical support for identifying the aging degree of MPs. The surface morphology of MPs before and after 120 days is observed by scanning electron microscope (SEM) (Sigma 300). Fourier Transform Infrared spectroscopy (FTIR) (Nicolet iS 10) was used to study the molecular structure and chemical composition of substances. This method is often used to detect the changes in the functional group of the plastic. As one of the surface analysis techniques, X-ray photoelectron spectroscopy (XPS) (Thermo Kalpha) was used to study the elemental composition of the substance.

## 2.4 DNA extraction and sequencing analysis

According to the manufacturer's instructions, soil DNA was extracted from approximately 500 mg of soil using Fast DNA®SPIN (MP Biomedicals，Illkirch，France). The 338 F (5'-ACTCCTACGGGAGGCAGCAG-3) and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') primers were used to amplify the V3 - V4 regions of the genes. Each treatment was replicated fivefold.

Use of QIIME2 (Quantitative Insights into Microbial Ecology, v2020.2) to process sequencing data (Bolyen et al., 2019). Splicing and quality screening of offline data were performed using FLASH (v 1.2.11) and fastp (v 0.19.6), respectively. The processed sequence is denoised and clustered using DADA2 software (Callahan et al., 2016), to identify amplification sequence variants (ASVs). Compared to the traditional molecular Operational Classification Units (OTUs) clustering method, the core algorithm of DADA2 can preserve some real sequence mutations and has sufficient improvement in clustering performance and reproducibility.

## 2.5 Data analysis

R Studio (4.1.3) was used to analyse the data and plot the graphs. Prior to analysis, Shapiro-Wilk and Levene tests were performed on the data. One-way ANOVA was used to compare the effects of each treatment group on all indicators, $p < 0.05$ indicates significant difference. The diversity and composition of the soil bacterial community was characterised to assess the effects of the six treatment groups, and the number of replicates of the experiment is five. Significance was determined by one-way analysis of variance (ANOVA) followed by post-hoc Turkey's test. Nonmetric Multidimensional Scaling (NMDS) was used to investigate community dissimilarities between treatments. Non-parametric multivariate analysis of variance (Adonis) was used to determine the significance of distances between groups. To identify soil bacterial biomarkers in the community, Linear Discriminant Analysis (LDA) effect size (LEfSe) was used (the threshold of the logarithmic LDA score was set at 2.0). In addition, to compare the effect of all treatment groups on network stability, the co-occurrence network was visualised using Gephi (version 0.1.0).

# 3 Results

## 3.1 Characterization of microplastics

According to the SEM (Fig. S1), both PE and PLA were rough particles with an irregular surface and a diameter of less than 500 μm. The edges of PE were fibrous, while there were some pores on the surface of PLA. It can be seen that small soil particles adhere to the surface of PE after 120 days (Fig. 1). When inoculated with earthworms, there was no significant difference in morphology between PE+EW and PE (Fig. 1 a-d). PLA also showed similar results.

FTIR was used to verify the depolymerisation of PE and PLA. Compared to original PE, the chemical structure of PE and PE+EW groups remained unchanged. It can be observed that the characteristic peak at 1047 cm$^{-1}$, 2921 cm$^{-1}$ and 2852 cm$^{-1}$ represent C-H, while the peak at 705 cm$^{-1}$ represents C-$H_2$ (Fig. 2a). These functional groups still existed in the original PE, PE and PE+EW treatments, indicating that there were no obvious functional groups present in PE during the experimental process. The opposite was found for the PLA-treated groups. At these four wavelengths, 871 cm$^{-1}$, 1052 cm$^{-1}$, 1750 cm$^{-1}$, and 2998 cm$^{-1}$ represent C-C C-O, C=O and C-H, respectively (Fig. 2b). These are the absorption peaks of chemical bonds in PLA molecules. Compared to the original PLA, the peak of PLA at 871 cm$^{-1}$, 1052 cm$^{-1}$, 1750 cm$^{-1}$, and 2998 cm$^{-1}$ shifted, indicating that PLA had new chemical bonds after 120 days. Simultaneously, characteristic peaks in the infrared spectrum of PLA+EW undergo stretching compared to PLA, indicating that earthworm inoculation accelerates the aging process of PLA.

The main functional groups, C 1s and O 1s, of PE and PLA were determined by XPS. Both original PE, PE and PE+EW showed a significant carbon peak (C-C) at 384.8, 384.8 and 38.6 eV. PE+EW showed new chemical bonds C-N and N-C=N at 286.4 and 288.2 eV, respectively (Fig. 2c). In the original PLA, PLA and PLA+EW groups, the proportions of peaks 384.8eV (C-C), 286.9eV (C-N) and 288.9eV (N-C=N) decreased, indicating internal structural changes (Fig. 2d). The O 1s results showed that the oxygen peaks of original PE, PE and PE+EW had not changed significantly, indicating that there had been no change in lattice oxygen and material structure (Fig. 2e). In addition, the peak values of the O 1s in all types of PLA have been changed (Fig. 2f).

### 3.2 Soil aggregates affected by microplastics

Weighing results of stable aggregates at all levels were collected as shown in Fig. S2. The stability analysis and calculation of $R_{>0.25}$ and MWD were shown in Fig. 3a, b. Compared to CK, PE, and PLA significantly reduced the content of soil macroaggregates (1-2mm) and $R_{>0.25}$ (Fig. S2, 3a, $p < 0.05$). After the addition of earthworms, the weight of large aggregates, R>0.25 and MWD in EW were significantly higher than those in CK (Fig. S2, 3a, b, $p < 0.05$), and the weight of large aggregates and MWD in PE+EW and PLA+EW were significantly higher than those in PE and PLA (Fig. S2, 3b, $p < 0.05$).

### 3.3 Soil bacterial diversity affected by microplastics

High quality sequence quantity can be support for our subsequent analysis (Table .S1). Richness estimates and diversity were compared for alpha diversity. Compared to PE, PLA improved Shannon and Simpson (Fig. 4a, b, $p < 0.05$), and PE+EW significantly improved Faith_pd (Fig. S3, $p < 0.05$). It was clear that the diversity of the bacterial communities in PE was much lower than in CK in the treated groups. PLA also increased the diversity of the bacterial community, although the Faith_pd and Shannon results were not significantly different. We found that different MPs have inconsistent effects on microbial diversity, as the effect of PE on alpha diversity differs from that of PLA, with overall PE decreasing while PLA increases. After inoculation with earthworms, the alpha diversity level was increased. In summary, PE may be more damaging to soil bacterial richness and diversity than PLA. In addition, earthworm inoculation had a positive effect on soil bacteria abundance and diversity.

Analysis of beta diversity in the soil showed that the NMDS plot showed clear clustering under all treatments, ASV was found to differ between treatments. In short, the ASV abundance of the CK, PE, PLA, EW, PE+EW and PLA+EW groups was separated from other groups. And in the treatment with or without earthworms, the greater the distance between points, the greater the difference in microbial communities under the earthworm condition (Fig. 4c, $R^2 = 0.461$, $p < 0.001$). In the PCoA plot of the treatment group with and without earthworms. There is a clear difference between CK, PE and PLA along the first principal coordinate. CK and PE were found to be significantly different from PLA (Fig. S4a, $R^2 = 0.382$, $p < 0.001$) and there are significant differences in the bacterial community structure between EW, PE+EW and PLA+EW (Fig. S4b, $R^2 = 0.378$, $p < 0.001$).

### 3.4 Effects of microplastics on the composition and major taxa of soil bacterial communities

The microbial composition of all treatment phyla was consistent and the major phyla included Proteobacteria (32.78% - 37.61%), Actinobacteria (22.53% - 32.57%), Acidobacteria (10.56% - 16.73%), Verrucomicrobia (5.54% - 11.39%) and Gemmatimonadetes (3.82% - 4.13%). Compared to CK, PE increased the abundance of Actinobacteria (34.92%), but decreased the abundance of Acidobacteria (26.09%) and Verrucomicrobia (38.58%). Compared to PLA, PLA+EW increased the abundance of Actinobacteria (38.37%), but decreased the abundance of Acidobacteria (36.31%) and Verrucomicrobia (45.65%) (Fig. 5).

In all categories, there were 12, 32, 49, 18, 41 and 49 taxa with significant abundance in the CK, PE, PLA, EW, PE+EW and PLA+EW groups, respectively (Fig. 6). At the phylum level, Verrucomicrobia, Cyanobacteria and Armatimonadetes showed an abundance advantage modified with CK, while Patescibacteria and WPS_2 were found to be significantly more abundant in the PE. Acidobacteria, Rokubacteria, Latescibacteria and Nitrospirae were significantly overrepresented in the PLA (Fig. 6). The Venn diagram showed different shared and unshared ASVs between the different treatment groups. For example, without earthworms, there were 7875, 5934 and 7340 unshared ASVs with CK, PE and PLA respectively, and only 7199, 6444 and 7499 unshared ASVs with EW, PE+EW and PLA+EW respectively (Fig. S5a). The random forest analysis shows the absolute abundance of the top 20 species in the different treatment groups. The most significant change was caused by earthworm inoculation.

(Fig. S5b), where ASV_163524 was found to be the most important biomarker with a higher relative abundance in EW. In addition, ASV_8048, ASV_200572 and ASV_54164 were important biomarkers for the difference between CK and EW+PLA, and ASV_8048 had a lower abundance in CK than the other treatment groups. UPGMA clustering of species composition data using Euclidean distance. CK, PLA, and EW had similar species composition. PE enriched Saccharimonadales, Pseudonocardia, Bacillus, and Jatrophihabitans. Meanwhile, PE+EW and PLA+EW also had a similar abundance (e.g., Gemmatimonas, 67-14, Ellin6067, Candidatus_Udaeobacter, Subgroup_6, and Pseudolabrys) (Fig. S5c).

### 3.5 Cooccurrence network in microplastics and earthworms treatment groups

Co-occurrence networks based on Spearman correlations between ASVs were constructed to investigate bacterial interactions after exposure to all treatment groups ($R >= 0.7$, $P < 0.05$). Both PE and PLA networks were less complex. The total number of nodes and links in CE, PE and PLA were fewer than in EW, PE+EW and PLA+EW respectively (Fig. 7, Table S2). In addition, nodes and links were found to be significantly higher in EW. In summary, these results indicate a clear difference in the microbial webs of PE and PLA, and an increase in the complexity of the microbial webs following earthworm inoculation. All these nodes represented the key species, contributing significantly to the structure of the network. Furthermore, the Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia and Gemmatimonadetes phyla were the key nodes in all treatment group networks. More key nodes were observed in the EW, PE+EW and PLA+EW networks, indicating greater complexity. Overall, the PLA and earthworm inoculated treatments showed greater microbial network complexity and stability.

## 4 Discussion

### 4.1 PLA aging accelerated by earthworms

Through SEM to characterize the morphology of MPs, we found that the surface of MPs was surrounded by soil particles after 120 days (Fig. 1), which may be due to the adsorption of MPs itself, and the electrostatic tension between MPs and soil particles. At the same time, the inoculation of earthworms may affect the distribution of aggregates, thus affecting the interaction between soil particles and MPs. The pits on the surface of MPs can also provide attachment sites for MPs microbiota. MPs are likely to provide substrates for some heterotrophic microbiota and act as "special microbial accumulators" (Shafea et al., 2023). Rougher MPs are more adsorbent and may attract more elements to their surface and recruit more degrading bacteria (Jin et al., 2022). MPs enter the soil environment and are influenced by soil aggregates to become adsorbed or encapsulated, which may reduce their interaction with environmental substances and prolong their aging process.

The functional group and chemical structure of MPs were studied by FTIR technology. As shown in Fig 2 a, b, the peak value of C-H in the Fourier transform infrared spectrum did not change, which means that the functional group and chemical structure of PE did not change significantly (Bagheri et al., 2013; X. Guo et al., 2020). Compared to original PLA, the peak values observed in the range of 871 to 2998 $cm^{-1}$ for PLA exhibit typical tensile vibrations. These peaks may be due to skeletal stretching vibrations of C-C and bending vibrations of C-H in olefins, asymmetric stretching vibrations of aliphatic C-H and C=O vibrations of carboxylic acids (Dong et al., 2020; Y. Zhou et al., 2020). The tensile modulus peak of PLA+EW is extremely weak in the composite material spectrum, which may be due to the disturbance of soil elements by earthworm activity on PLA. The results showed that the chemical structure of PE was fully preserved, while PLA did not, and earthworms accelerated this effect. Aging increases the number of oxygen-containing functional groups on the surface of MPs, including carboxyl, aldehyde, and hydroxyl groups.

The chemical composition of the sample was examined by XPS, as shown in the Fig 2 c-f. The PE showed similar peak positions for C 1s and O 1s. For their XPS peaks, the spectra of C 1s can be deconvoluted into three peaks of the C-C, C-N, and N C-N double bond bands, located at 384.8, 286.4 and 288.2 eV, respectively (Li et al., 2019; Yang et al., 2023). The O 1s spectrum of PLA is divided into three peaks located at 532.8, 532 and 531.8 eV, which are attributed to the absorption of $H_2O$ on the surface by PLA lattice oxygen. However, after earthworm inoculation, the Binding energy of PLA+EW shifted slightly, indicating that there was charge transfer in the

PLA+EW process (Yang et al., 2022). Furthermore, there is a certain interaction between earthworms and PLA, which enhances the photoexcited carrier mobility between the contact interfaces. The change of lattice oxygen in PLA, PLA+EW indicates that redox may have occurred in the interior, while the electronic transition may have occurred in PLA+EW, accelerating the rightward shift of the O 1s peak. Polymeric materials in the environment use mineralization to convert elements such as C, H, O, etc. to the simplest inorganic form.

## 4.2 Interactions between earthworms, microplastics and bacteria in soil aggregates

Soil polysaccharides are important binders that facilitate aggregates formation and maintain structural stability (Costa et al., 2018), R>0.25 and MWD represent aggregates stability, with higher values indicating better soil aggregation and stability. Aggregates play an important role in regulating soil fertility and maintaining soil productivity as they are the basic structural unit of the soil and control its water holding capacity, permeability and erodibility (Manna et al., 2005). MPs deposited in the soil will gradually combine with soil particles (He et al., 2018). Once it enters the aggregates, it will alter the biological, physical and chemical processes within the aggregates, thus affecting the stability of the aggregates (Zhang and Liu, 2018). The stability of the soil after the addition of PE and PLA decreased to some extent compared to CK, which may be due to the increase in microaggregates. An increase in the number of microaggregates after soil aggregates fragmentation leads to a decrease in MWD, which is consistent with the results of this study (Spohn and Giani, 2010). The effects of MPs on soil aggregates are mainly twofold. On the one hand, MPs have a strong adsorption due to their high surface tension, which can accumulate a large amount of organic matter and microbiota and become the "aggregation zone" for the decomposition and transformation of soil organic matter, thereby affecting the stability of soil aggregates. On the other hand, due to their hydrophobicity and negative charge, MPs have a certain degree of repulsion with soil particles, which is not conducive to the formation of stable aggregates (G. S. Zhang et al., 2019). In the study, both PE and PLA reduced the stability of soil aggregates, but PLA had a stronger effect. The possible reason is that the adsorption of organic matter by MPs as an enrichment zone for organic matter decomposition and the provision of aggregates cement are limited, while the exclusion of soil particles by MPs becomes the dominant factor, leading to the destruction of soil aggregates. We have now found that the addition of earthworms improves the stability of soil aggregates. Earthworms can either directly affect the structure of soil aggregates through their burrowing activity, or increase the content of soil organic matter by feeding on soil microorganisms (mainly fungi) (Abiven et al., 2009) and excreting worm dung, thus indirectly affecting soil aggregates (Brown et al., 2000), which plays an important role in the formation and stability of soil structure. It can be seen that PE and PLA have different negative effects on the stability of soil aggregates at a 1% concentration load due to the different materials, and that earthworm inoculation could mitigate this negative effect.

### 4.3 Properties of microplastics influence bacterial selectivity

The results show that MPs and earthworms have a significant effect on soil bacterial diversity. Soil alpha diversity had a significant effect under low density PE (LDPE) (Gao et al., 2021). However, there are also reported mixed results. There was no significant effect of exposure to 2% (w/w) PLA on the alpha diversity community composition of bacteria in paddy soil (Chen et al., 2020). Therefore, these results indicate that the effects of MPs on soil microbiota do exist in different environments. However, under different conditions, the effect of different types of MPs on microbial community structure is not the same. PE is a nondegradable plastic that takes a long time to degrade. Once it breaks in the soil and the debris is buried underground, it can damage the permeability of the soil and affect plant and microbial communities. PLA is a polymer obtained by polymerisation using lactic acid as the main raw material. It is biodegradable and is widely used as plastic film in agricultural production (Zhong et al., 2020). Although intercellular enzymes can decompose PLA into carbon dioxide, water, or methane , but PLA took a long time to degrade in soil because it can resist microbial attack. Soil microbiota have different abilities to utilise different carbon sources. Some carbon sources are readily available, while others require a period of adaptation before use. The former are referred to as fast-acting carbon sources, while the latter are referred to as slow-acting carbon sources (Liu et al., 2012). A culture system using MPs as the sole carbon source means that the microbiota have only one slow-acting carbon source. Influenced by a single delayed carbon source, microbial communities tend to simplify (Goldford et al., 2018), leading to a significant reduction in diversity in the early stages. As the enrichment cycle progresses, some bacteria that can use MPs as a carbon source are recovered, showing a slight increase in diversity, due to targeted selection of microbial communities under pressure from a single carbon source (A. Shi et al., 2022). There is a significant difference in the composition of soil microbial communities with the addition of PLA compared to PE, which may be due to the influence of: 1) the physical and chemical characteristics of MPs on the bacterial communities that proliferate on the surface of MPs (Zhou et al., 2021b); and 2) aging of PLA as a rapid carbon source restores some bacterial diversity. On the basis of the above analysis, it is suggested that the type of MPs may be an important factor leading to the variability of the microbial communities. The significant aggregation of soil microbiota further suggests that MPs have selectivity for soil microbiota (Di Pippo et al., 2020; Wu et al., 2019). Interestingly, after inoculation with earthworms, they have different effects on the microbial community, suggesting that the effect of earthworms on soil microbes cannot be ignored compared to MPs.

High abundance in all groups of Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia and Gemmatimonadetes (Fig. 5, 6, 7), showing that these five groups of bacteria have a strong adaptability and play an important role in the aging of MPs and earthworm activities, which is similar to the previous results (Sun et al., 2022c). Previous studies suggest that PE may have an effect on the relative abundance of Proteobacteria (Hou et al., 2021; Ren et al., 2020; Zhu et al., 2022). This is inconsistent with our conclusion, as we found that PE+EW significantly increased the abundance of Proteobacteria compared to CK. One possibility is that our test soil is a black soil with high organic matter content. Bacterial taxa such as Actinobacteria and Proteobacteria that degrade polyethylene have been shown to enrich on MPs surfaces

(F. Wang et al., 2022). Acidobacteria are more likely to predominate in oligotrophic environments (Zhou et al., 2021a). Patescibacteria were found to be significantly abundant in the PE group and were found to be enriched on the PE-MPs, causing the polymer's molecular chain to break (Sun et al., 2022b; Zhelezova et al., 2021). PE could increase soil porosity and aeration through reduction of soil bulk density (Zhu et al., 2022), which may inhibit the growth of anaerobic bacteria such as phylum Patescibacteria. Patescibacteria contribute to degrading the PE (Hou et al., 2021). Actinomyces and Gemmatimonadetes, for example, are capable of synthesising hydrolytic enzymes that degrade plastics and are also involved in breaking down complex organic materials (M. Zhang et al., 2019), and their abundance was increased in the PE and PLA+EW groups. In summary, we found that PLA and PE have different effects on the soil bacterial communities. There are two possible explanations for this phenomenon: 1) the chemical structures of the two are different, and the multiple oxygen atoms possessed by PLA may stimulate changes in the microbial community; 2) PLA may participate in the assimilation process of microorganisms during aging, thereby directly affecting the microbial community. In addition, MPs may affect soil properties, thereby affecting soil bacteria. After the cultivation experiment, PE and PLA decreased by 0.14 and 0.1 respectively compared to CK (Table S3, $p < 0.05$). Lauber et al., (2009) showed that soil pH is one of the important factors affecting microbial richness and diversity. In this study, the inoculation of earthworms significantly increased the soil pH (Table S3), which may lead to significant changes in soil bacterial richness, diversity, and uniformity. PLA treatment significantly increased soil Dissolved organic carbon (DOC) content, while there was no significant difference between CK and PE (Table S3). Our result indicated that PLA was subject to ageing and even biodegradation. The effect of MPs on soil DOC depends on soil type, MPs type, exposure time and MPs concentration. For example, Liu et al. (2017) found that PP significantly increased soil DOC concentration, while Hou et al. (2021) found that long-term exposure to PE reduced DOC content in various soil aggregate components. Sun et al. (2022) have shown that biodegradable MPs such as PBS and PLA can significantly increase soil DOC concentration. PLA can be hydrolysed to form water-soluble low molecular weight oligomers (Elsawy et al., 2017; Gigli et al., 2012), which can directly increase soil active carbon. In addition, due to their slow degradation, degradable MPs may also indirectly affect soil carbon by altering soil physical conditions. Since the increased DOC may come from MPs, they may have different organic molecules compared to the original soil DOC, leading to changes in the soil microbial community (Li et al., 2019; Ward et al., 2017).

### 4.4 Earthworms influence the behaviour of microplastics on soil bacteria

In addition, earthworm inoculation has a significant effect on the effect of MPs on soil bacteria. The effect of earthworms on the abundance of soil bacteria has many aspects: on the one hand, the feeding effect of earthworms on bacteria can directly change their community composition. On the other hand, the activity of earthworms in the soil can accelerate the decomposition of organic matter and the release of mineral nutrients, which can effectively activate dormant or semi dormant bacteria in the soil (Furlong et al., 2002). The physical structural changes caused by earthworm inoculation may also affect the abundance and diversity of soil bacteria (Al-Maliki et al., 2021). In this study, earthworms significantly increased Actinobacteria while decreasing the relative abundance of Verrucomicrobia, suggesting that earthworms may have a feeding selectivity towards Verrucomicrobia. We also found that earthworm inoculation significantly increased the proportion of soil macroaggregates. The complex pore structure in soil aggregates provides a favourable living microenvironment for soil microbiota, and the microbial community structure is limited by the content of air, water and matrix in this microenvironment. Compared to large aggregates, microaggregates with high spatial heterogeneity can provide a larger ecological niche for bacteria and maintain higher bacterial community diversity (Tecon and Or, 2017)' (Bach et al., 2018). The presence of earthworms not only promotes the formation of microaggregates within large aggregates, but also leads to the release of microaggregates once fragmented, which may have a certain regulatory effect on the soil bacterial community. Earthworm inoculation can have a significant impact on the structure of soil bacterial communities by significantly increasing the number of soil macroaggregates (Bu et al., 2020). The endophytic activity of earthworms can strongly influence soil structure. Soil microbiota are considered to be at the center of earthworm aggregates formation, and the bacterial communities in different soil aggregates size components are affected differently by earthworm activity. Earthworms reconstruct the soil environment through burrowing, casting, and consumption. The biological disturbance of earthworms can affect soil aggregation, soil physicochemical properties, and microbial communities.

Earthworm activity, soil aggregates, and soil microbial communities interact in complex ways to influence the decomposition and storage of soil organic matter (SOM) and soil organic carbon (SOC). DOC is a small fraction of soil SOC, derived primarily from soil microbiota. Sensitive to changes in the soil environment, this study found that earthworm inoculation reduced the content of DOC. In this study, earthworms reduced soil DOC content, which may be due to their activity in improving soil physical structure, increasing soil porosity and permeability, and promoting the growth and reproduction of aerobic microorganisms (Subler et al., 2018). The formation process of microaggregates can occur gradually through microbial treatment of organic matter and physical processes in large aggregates (Six et al., 1999; Dexter et al., 1988).The formation rate of microaggregates after earthworm treatment is relatively fast. Compared with microaggregates, the turnover time of large aggregates is shorter, and the SOM contained in large aggregates is more easily degraded by soil microbiota (Bossuyt et al., 2006). In addition, the highly stable micro aggregates cast by earthworms provide a higher level of physical protection, thereby enhancing the composition of organic minerals (Shipitalo and Protz, 1989). The formation of microaggregates and the activity of microorganisms affect soil microbes (Mummey et al., 2006). Meanwhile, the gut microbiota of soil animals may also indirectly affect soil microbiota. The ingestion of MPs by soil fauna had caused many physiological changes. The change in gut microbiome composition was a direct result of MPs exposure and had implications for soil microbial diversity, function and ecosystem services (Filippo et al., 2022). A 42 day microscopic experiment have showed that earthworms gut microbes may affect the effect of nanoplastics on soil microbes (Zhou et al., 2023). There is still a lack of research on the effects of earthworm gut microbes on MPs and soil microbes.

Past research has shown the effect of MPs on soil microbiota, but little has been done to assess its impact on the complex interactions among soil microbiota. We found that the PLA community formed a significantly larger and more complex network than the PE community (Fig. 7). In addition, the molecular ecological network of the earthworm inoculated treatment group showed greater robustness than other groups. The increased network complexity may be due to several mechanisms. On the one hand, as PLA decomposes, soil becomes an enriched compartment where microbial competition is reduced and more species can maintain a mutualistic structure (Hibbing et al., 2010; Sun et al., 2022c). On the other hand, earthworms feed on soil bacteria and their impact on soil aggregates may affect the interaction between bacteria. Therefore, the PLA and earthworm treatment groups may reflect greater ecological stability, and their microbially linked ecosystem functions may be less fragile. This is important for resisting changes in the soil environment. In short, our research showed that using BMPs and earthworm inoculation can increase soil bacterial community complexity and robustness compared to nonbiodegradable MPs. However, we should be cautious about extending interaction-based theories to explain ecological networks, as network parameters alone are not sufficient to predict the stability of microbial ecosystems.

## 5. Conclusion

In summary, our study provides a better understanding of the effects of degradable/nondegradable MPs and earthworms on the diversity, composition, and network structure of soil bacterial communities. The results showed that the type of MPs and the presence of earthworm inoculation affected the diversity, composition and structure of the bacterial community. Nevertheless, in comparison with PE, PLA may be a fast carbon source that can quickly affect soil microbiota and might provide more nutrients and habitats for microbes, which mitigated both cooperation and competition among microbial communities. At the same time, earthworm inoculation had a positive effect on the levels of macroaggregates and may have contributed to the diversity and stability of the soil bacterial community, as well as increasing the complexity and robustness of the bacterial network. Interestingly, the presence of earthworms also accelerated the aging of PLA. Future research should consider the interactive effects of MPs, earthworms and soil properties on the stability of soil organic matter and fungi in different soil types, and the functions performed by soil microbiota. Our research provides new insights into the interactions between soil animals, MPs and soil microbes.

## CRediT authorship contribution statement

**Siyuan Lu**: Conceptualization, Data curation, Writing - review & editing. **Jiahua Hao**: Investigation. **Hao Yang**: Investigation. **Mengya Chen**: Writing - review & editing. **Jiapan Lian**: Investigation. **Yalan Chen**: Formal analysis. **Zhuoma Wan**: Formal analysis. **Wei Wang**: Investigation. **Wenjin Chang**: Investigation. **Donghui Wu**: Methodology, Investigation, Formal analysis.

## Declaration of Competing Interest

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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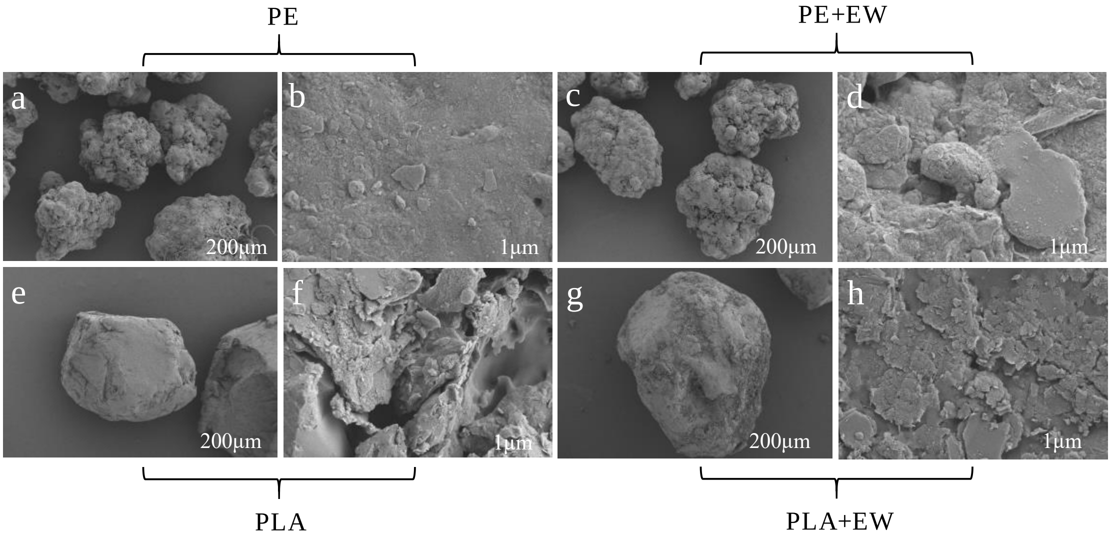

Fig. 1 SEM images of PE (a-b), PE+EW (c-d), PLA (e-f) and PLA+EW (g-h).

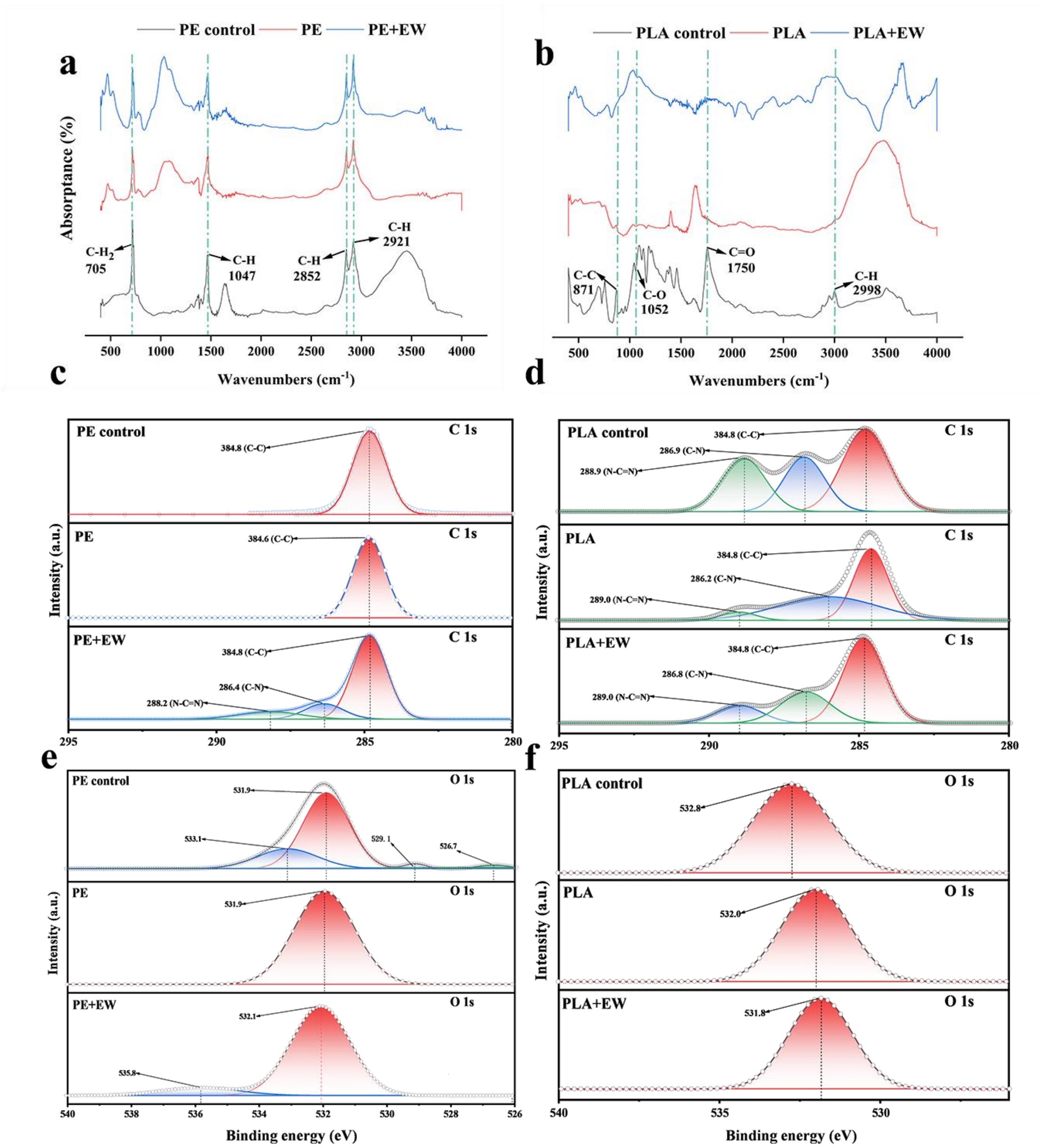

Fig.2 FITR of original PE, PE and PE+EW (a), original PLA, PLA and PLA+EW (b). XPS spectra of (c) C 1 s for original PE, PE and PE+EW, (d) C 1 s for original PLA, PLA and PLA+EW, (e) O 1 s for original PE, PE and PE+EW and (f) O 1 s for original PLA, PLA and PLA+EW. Control represents the original state of the material.

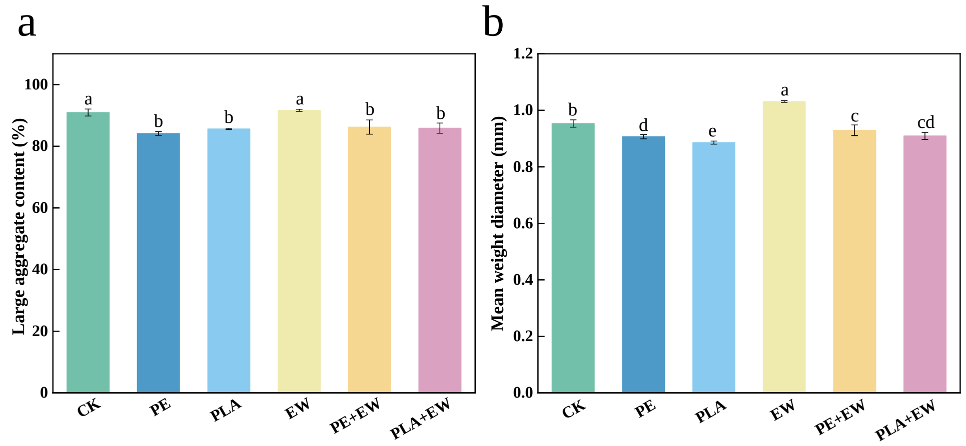

Fig.3 Effects of Microplastics and earthworms on soil large aggregates content (a), and mean weight diameter (b).

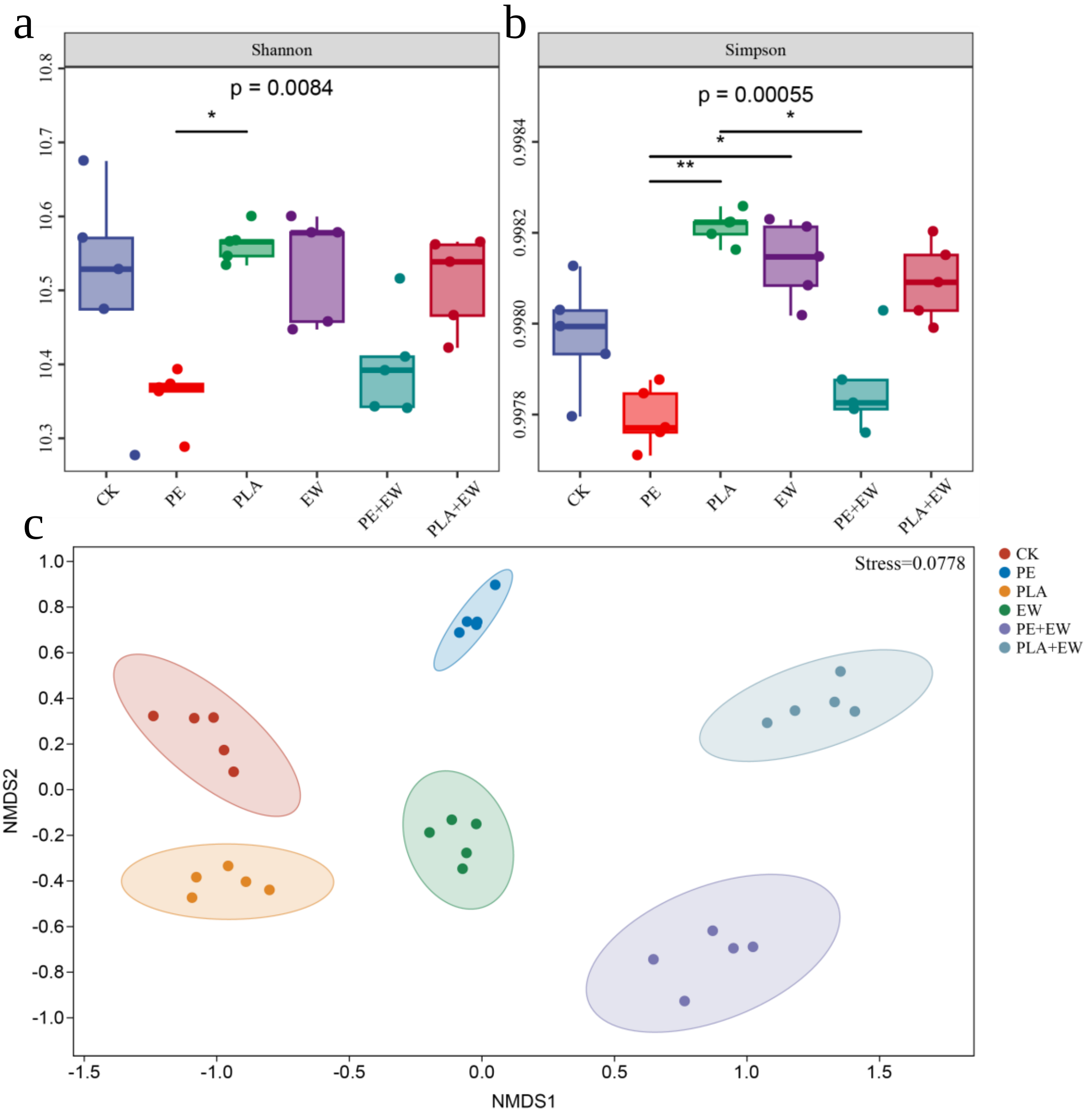

Fig.4 Effects of MPs and earthworms on Shannon indices (a) and Simpon indices (b).

NMDS rarefied abundances of ASVs and depiction of beta diversity patterns for bacteria (c).

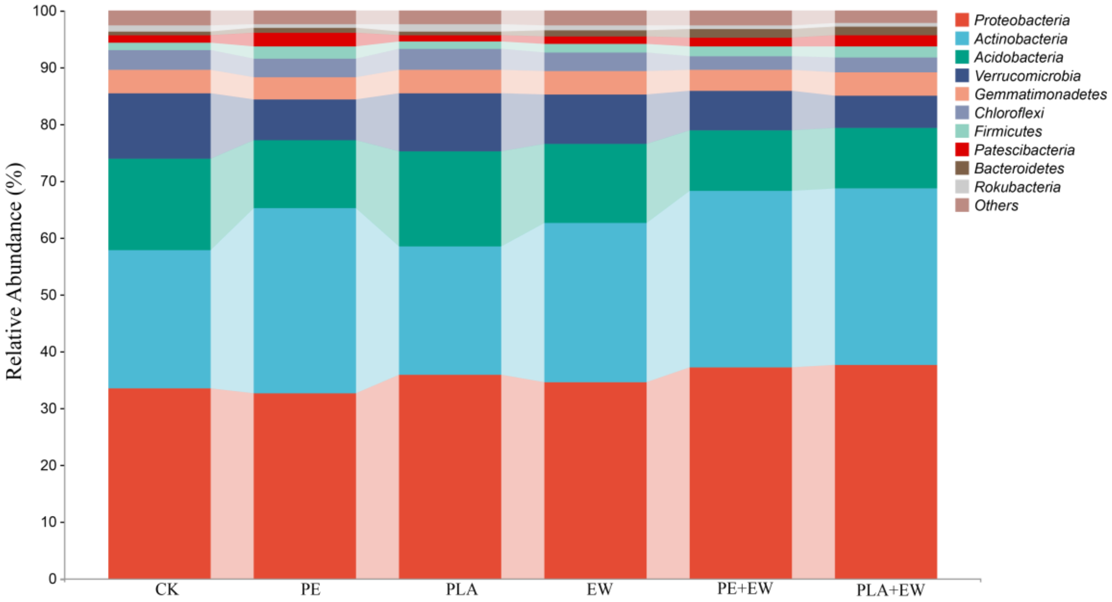

**Figure 5.** Bar chart showing the top 10 most abundant bacterial phyla in the community structure of each of the individual treatments (n = 5).

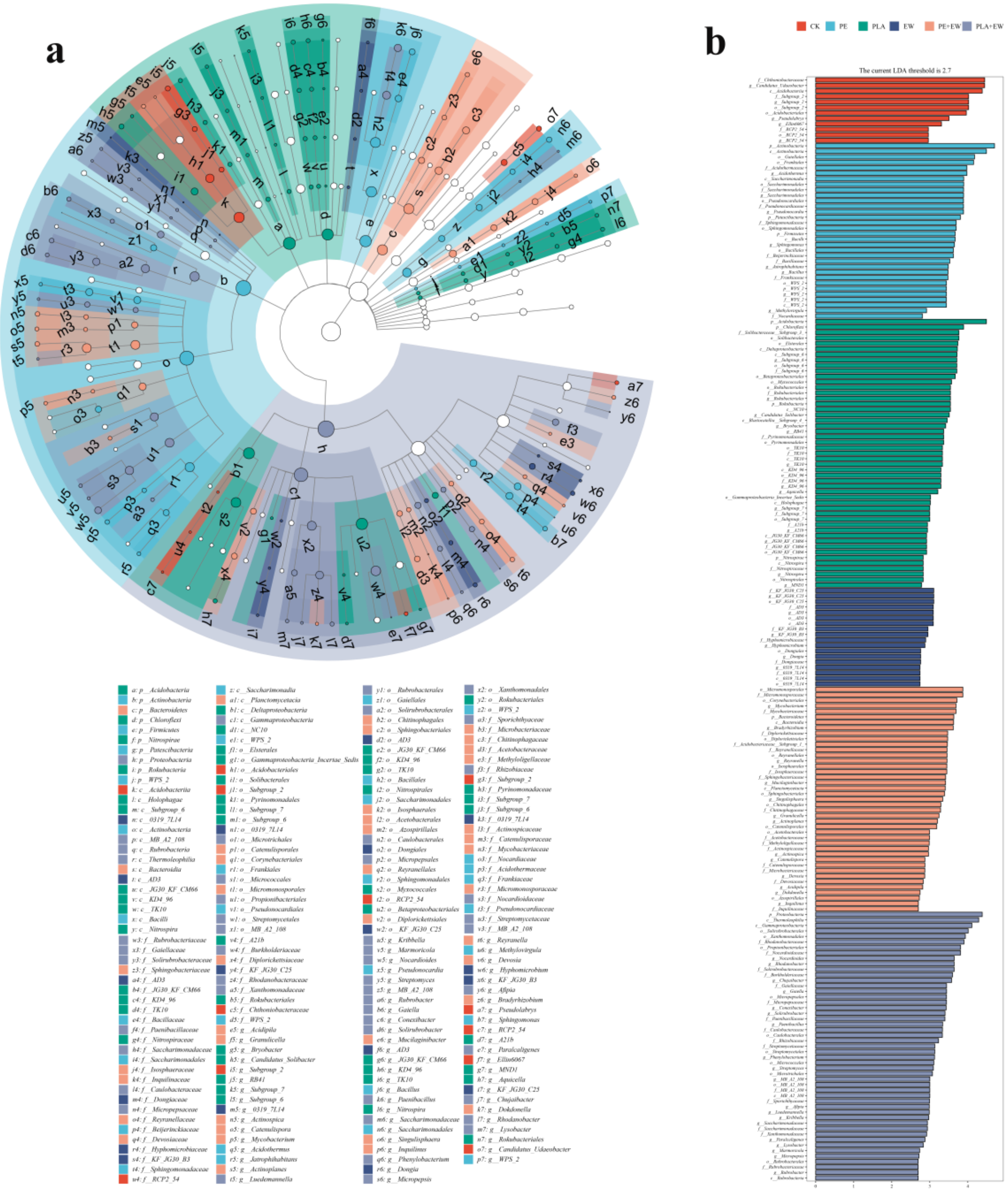

Fig. 6 Cladogram showing the phylogenetic distribution of bacterial lineages associated with different treatments (a). Indicator bacteria with LDA scores of 2 or higher in bacterial communities associated with different treatments (b). Different coloured regions represent different treatments. Circles indicate phylogenetic levels from phylum to genus. The diameter of each circle is proportional to the abundance of the group. p: phylum, o: order, c: class, f: family, g: genus.

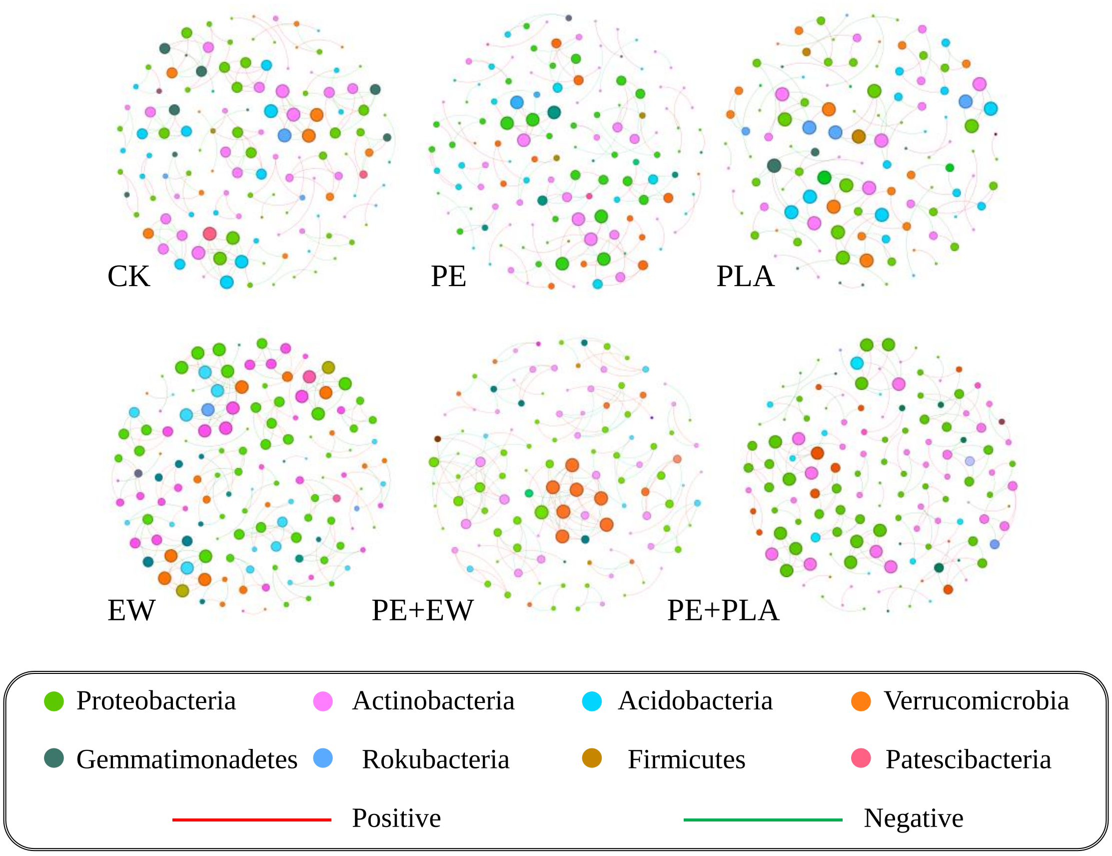

Fig.7 Co-occurrence networks from ASV profiles of the soil bacterial community under different treatments. The different colours represent different phyla levels and the sizes represent abundance (Top 8).