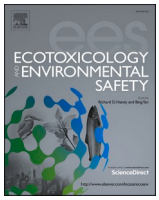
Ecotoxicology and Environmental Safety 252 (2023) 114577

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Microplastics affect soybean rhizosphere microbial composition and function during vegetative and reproductive stages

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ARTICLE INFO

Edited by Professor Bing Yan

Keywords:
Microplastics
Soybean rhizosphere
Biomarkers
Co-occurrence network
Function structure

ABSTRACT

Microplastics (MPs) are emerging contaminants in agricultural soil, whereas their effects on the rhizosphere microbial ecosystems and biogeochemical nitrogen cycles during plant growth remain unknown. Here, a 70-day greenhouse experiment was carried out with black and fluvo-aquic soil to evaluate the influence of polyamide (PA), polyethylene (PE), polyester (PES), and polyvinyl chloride (PVC) MPs on the bacterial communities and functions in the soybean rhizosphere. The PA treatment consistently affected the rhizobacterial alpha diversity in the fluvo-aquic soil at soybean vegetative and reproductive growth stages, whereas the PE, PES, and PVC treatments had a short-term effect on the bacterial alpha diversity. At two growth stages, 6 and 23 biomarkers were consistently abundant in the PA treatment in the black soil and fluvo-aquic soil, respectively, and order *Rhizobiales* was found to be a biomarker for PA MPs contamination in both soils. Additionally, PA treatment decreased bacterial network complexity and tightness, whereas the effects of the PE, PES, and PVC on bacterial co-occurrence patterns varied depending on the soil types. Furthermore, PES and PVC treatments inhibited ammonification processes in the soybean rhizosphere, and PE could temporarily inhibit ammonia oxidation and denitrification processes according to the variations of N-cycling gene abundances. These effects on soil N-cycling also varied with soil types and soybean growth stages. This study provides profound information for understanding of MPs residues on the assembly of the soybean rhizosphere communities and function during plant development.

1. Introduction

Microplastics (MPs, particles size < 5 mm) are deemed as an emerging contaminant due to their wide occurrence in a variety of environmental compartments and their negative effects on aquatic and terrestrial ecosystems (Law and Thompson, 2014; Wright et al., 2013). The study focus has turned to terrestrial ecosystems in the last five years (de Souza Machado et al., 2018, 2019; Li et al., 2021), after approximately a decade of research limited to ocean and freshwater ecosystems (Auta et al., 2017; Barker and Hill, 1981; do Sul and Costa, 2014; Rillig et al., 2019). Various environmental sources and human activities, such as soil amendments (e.g., organic fertilizer) (Weithmann et al., 2018; Zhang et al., 2020), plastic mulching (Qi et al., 2020, 2018), sewage sludge (Lehmann et al., 2020), atmospheric fallout (Mbachu et al., 2020), littering or street runoff (Zhao et al., 2021), may contribute to the contamination of MPs in terrestrial ecosystems. So far, MPs have been found in almost all soils worldwide, including cities and industrial areas (Fuller and Gautam, 2016), natural reserves (Scheurer and Bigalke, 2018), and agricultural fields (Piehl et al., 2018). Recent studies suggested that agricultural soils may store more MPs than oceans (Nizzetto et al., 2016) and more research should be carried out on its effects on the agricultural ecosystem.

The essential region that directly surrounds plant roots, known as the rhizosphere, has been identified as a hotspot of microbial interactions and activity in soils (Philippot et al., 2013). Importantly, the rhizosphere microbiome plays a vital role in directly and/or indirectly regulating plant growth and productivity, nutrient cycling, and ecosystem stability in agro-ecosystems (Hardoim et al., 2008; Powell and Rillig, 2018; Wang et al., 2017). Studies indicated that MPs particles may be transported to deeper soil layers by the action of soil biota, specifically animals,

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https://doi.org/10.1016/j.ecoenv.2023.114577
Received 9 December 2022; Received in revised form 8 January 2023; Accepted 23 January 2023
Available online 28 January 2023

thereby influencing roots and rhizosphere microbiome (Rillig et al., 2017). For instance, the relative abundances of the genus *Lysobacter* and *Sphingomonas* in the wheat rhizosphere soil were enhanced by the addition of polyethylene (PE) MPs (Qi et al., 2018). Also, the relative abundance of some species associated with pollution degradation in the wheat rhizosphere soil was miscellaneously increased by the PE, polyvinylchloride (PVC), and polystyrene (PS) MPs (Zhu et al., 2022a). Studies also reported that PE and polylactic acid (PLA) MPs influenced arbuscular mycorrhizal fungi community structure and diversity (Wang et al., 2020; Yang et al., 2021), and the PLA MPs altered soybean rhizosphere alpha diversity and the key bacteria of dinitrogen fixation (Lian et al., 2022). However, our understanding of how MPs affect the rhizosphere microbiome and function is still limited as their effects differed depending on the soil and polymer properties. Moreover, the amount and composition of root exudates vary significantly in different plant growth stages (Aulakh et al., 2001), which may result in changes in the bioavailability and toxicity of external contaminants including MPs. As far as we know, the changes in rhizobacteria driven by multiple types of MPs during plant growth and the potential impacts of MPs on ecological function in various agricultural ecosystems are still poorly known.

Soil types with distinct physicochemical properties (i.e., soil bulk density, water-stable aggregates, and water-holding capacity) can affect both the efficiency of the biogeochemical cycle (Zhang et al., 2022) and the communities of root-colonizing symbionts, including mycorrhizal fungi and N-fixers (Lowery and Ursell, 2019; Rillig et al., 2019). Therefore, considering different soil types in a study may improve the understanding of the changes of soil microbial communities and function caused by external contaminants. The fluvo-aquic soil and black soil present remarkable differences in their properties such as pH, soil organic matter content, microbial biomass carbon, etc (Table S1). In the present study, a 70-day greenhouse soil-pot experiment was designed to investigate the impacts of four common types of MPs (polyamide, PA; PE; polyester, PES; and PVC) on the rhizosphere microbiome and their related function in the fluvo-aquic and black soils. Soybean (*Glycine max*), one of the most important crops in the global agriculture, was selected as the tested plant (Karges et al., 2022). This study aimed to (1) reveal the effects of exposure to different MPs types on the soybean rhizosphere bacterial communities, N cycling function and its variation over plant growth stages, and (2) investigate if soil type can regulate MPs effects on soybean rhizosphere. This study significantly advances our systematic understanding of MPs residues on the assembly of rhizosphere bacterial communities and subsequently ecological function.

2. Materials and methods

2.1. Materials

PA MPs (nominal diameter < 50 μm) were purchased from Good Fellow (Good Fellow, AM306010; Cambridge, United Kingdom). PE (nominal diameter < 200 μm), PES (nominal diameter < 180 μm), and PVC MPs (nominal diameter < 200 μm) were acquired from GuanBu Electromechanical Technology Corporation (Shanghai, China). The tested soil was obtained from two major soybean cultivation area in China. Specifically, fluvo-aquic soil was collected from Langfang (39°82′ N, 116°70′ E), Hebei province, and black soil was collected from Suihua (46°36′ N, 126°54′ E), Heilongjiang province. The surface soil (0–15 cm) was collected, then filtered through a 2 mm mesh sieve and thoroughly mixed before use. The physicochemical features of the soil were analyzed at the Pony Testing International Group (Jilin, China) (Table S1). The soybean seeds (*Glycine max*) used in these experiments were Zhonghuang 37 strain and were cultivated by the Chinese Academy of Agricultural Sciences.

2.2. Experimental design

Four types of MPs, i.e., PE, PA, PES, and PVC, were added into the soil (fluvo-aquic and black soil), respectively, by a rate of 2% (w/w) for preparing the MPs contaminated soils. A control soil group was prepared without any MPs addition. Six replicates were used for each treatment and control, and the concentration of MPs was chosen based on previous studies (Chen et al., 2020; Rong et al., 2021). To yield a 2% (w/w) MPs residue mixture, 4 kg of soil (dry weight) received 80 g of the respective MPs, which were then evenly distributed throughout the soil.

After being surface sterilized, soybean seeds germinated in the seedling trays for two days under moist and dark environments (Edwards et al., 2015). Eight pre-germinated seeds were aseptically transplanted into clay pots (20 cm height × 18 cm diameter) containing the above MPs contaminated soils and control soils. Each pot was randomly placed in a climate chamber to ensure relatively stable culture conditions (light intensity of 6000 lx, 16-h photoperiod, night/day temperature of 20 °C/25 °C, and relative humidity of 60%). Rhizosphere soil samples were collected during two periods (day 42 and day 70) after sowing. These two periods correspond to the vegetative stage (before flowering) and reproductive stage (before senescence) of soybean. The plants were manually removed from the pots after 60 pots were harvested each time. Rhizosphere soils were collected mainly based on the previously described protocols (Bulgarelli et al., 2012). Briefly, the roots were collected first, and the attached loose soils were removed by shaking and kneading with sterilized gloves. Then the roots were immersed in phosphate buffer solution (PBS) containing 6.33 g/L of $NaH_2PO_4{\cdot}H_2O$, 16.5 g/L of $Na_2HPO_4{\bullet}7\ H_2O$, and 200 μL/L of surfactant (Silwet L-77), and vortexed (15 s) for washing the roots. After that, the washing solution was collected and the washing step was repeated until the PBS was clear. The washing solutions were combined and filtered through a 100-μm nylon mesh cell strainer to remove broken roots and large sediment, and then centrifuged (3200 g, 15 min) to obtain the pellet of rhizosphere soil. The rhizosphere soil samples were then kept at − 80 °C before molecular analysis.

2.3. Microbial DNA extraction and real-time quantitative PCR (qPCR) analysis

Microbial DNA was isolated from 0.40 g soil using the DNeasy PowerSoil Kit (QIAGEN, Germany), following the manufacturer's instructions. The integrity of the obtained DNA was assessed by 1% gel electrophoresis, and the concentration of DNA was determined by NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, the USA).

QPCR targeting five functional marker genes (*AOAamoA*, *AOBamoA*, *nifH*, *nirK*, and *nirS*) and the 16 S rRNA gene was determined to understand the effects of MPs exposure on N cycling in rhizosphere soil. The information on primers and qPCR amplification conditions is listed in Table S2. In Table S3, the R^2 value and the target gene amplification efficiency are listed. More details of the instrument and reaction system were provided in our published study (Chen et al., 2020; Rong et al., 2021).

2.4. 16S rRNA amplicon sequencing and bioinformatic processing

We selected the primers pairs: 338 F and 806 R with a V3–V4 region of the 16 S rRNA gene to evaluate the taxonomic composition of bacteria (Xu et al., 2016). Detailed descriptions of PCR procedures are found in the previous study (Chen et al., 2020; Rong et al., 2021). The purified amplicons were paired-end sequenced (2 × 300) on an Illumina MiSeq PE300 platform (Meiji Biological Medicine Co., LTD, Shanghai, China) following a standard protocol. The raw reads were demultiplexed and quality-filtered by fastp software (version 0.19.6) (Chen et al., 2018) and merged by FLASH software (version 1.2.11) (Magoč and Salzberg, 2011) using the following criteria: (i) reads (300 bp) were truncated at any site

receiving an average quality score of < 20 over a 50 bp sliding window, and truncated reads shorter than 50 bp were discarded; (ii) according to their overlap sequence, pairs of reads were merged into a sequence with a minimum overlap of 10 bp, where the maximum permitted mismatch ratio in the overlapping region was 0.2 and the unqualified sequence was discarded; and (iii) the barcode and primers were used to identify the samples, and the sequence direction was adjusted with maximum mismatches (2 nucleotides) in the primer matching process. The high-quality sequences of the 16 S rRNA gene were clustered to operational taxonomic units (OTUs) according to 97% sequence similarity using UPARSE (version 7.0.1090) (Edgar, 2013). RDP classifier (version 2.11) was used to assign the taxonomy to the representative sequences of

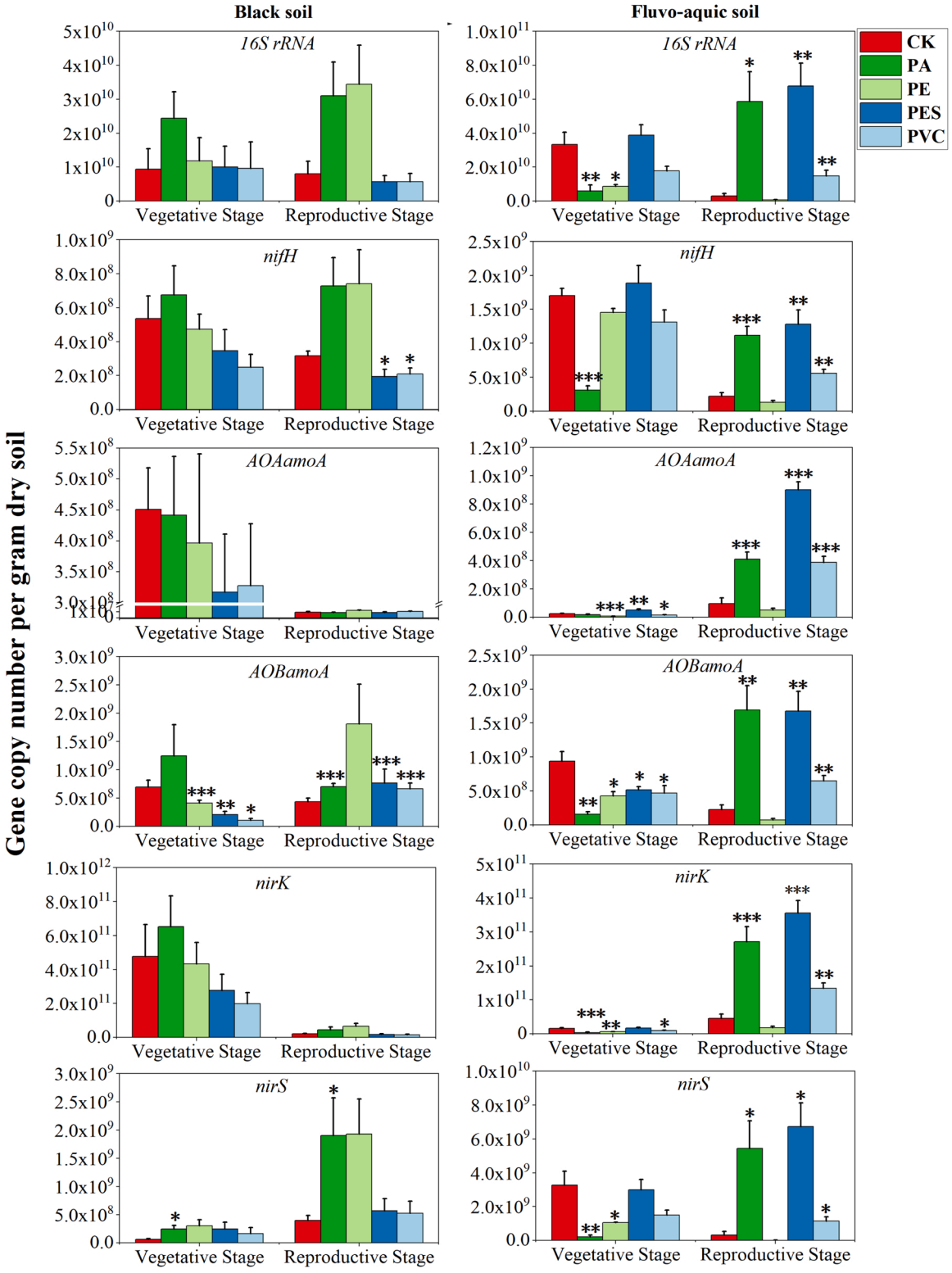

Fig. 1. The copy number of the 16 S rRNA and N-cycling functional genes in diverse agricultural rhizosphere soils. The levels of statistical significance between control and each MPs treatment (PA, PE, PES, and PVC) are represented by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Mean values (n = 5) ± S.E.

each OUT (Amato et al., 2013). The sequences obtained from all samples were rarefied at the same depth (30,531 reads per sample) for downstream analysis. All the raw sequences are available at the Sequence Read Archive of the NCBI (no. PRJNA807124).

2.5. Statistics analysis

The alpha diversity indexes (Shannon and Faith'PD) were calculated by Mothur software. Principal coordinate analysis (PCoA) was used to investigate the changes in the distribution pattern of the rhizobacteria community in each treatment based on the Bray−Curtis distance. The Linear discriminant analysis coupled with effect size (LEfSe) was used to identify bacterial biomarkers (from phylum level to genus level) in the rhizosphere that respond significantly to different MPs treatments, with the threshold of the LDA score > 2 (Segata et al., 2011). Additionally, network analysis was used to estimate the co-occurrence patterns among bacterial taxa under the MPs exposure. The genera were selected using a relative abundance threshold higher than 0.01% to reduce network complexity. The associations of pairwise genera with Spearman's correlation coefficient (r) > 0.8 and the p-value < 0.01 were selected (Chen et al., 2019; Pérez-Jaramillo et al., 2019). Network properties calculation and visualization were conducted by the interactive platform Gephi (Bastian et al., 2009). The function profiles of the prokaryotic community were further inferred using the functional annotation of the prokaryotic taxa (FAPROTAX) database (Louca et al., 2016).

Comparisons across treatments for parameter properties (alpha diversity index, soil properties, and functional genes abundance) were analyzed in the SPSS 22.0 (IBM Corp., Armonk, NY, USA). Student's t-test was performed to compare the statistical difference in parameter properties. At each time point, statistically significant differences between the control (CK) and each MPs treatment (PA, PE, PES, and PVC) were shown with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results

3.1. Effect of MPs on soil properties

In black and fluvo-aquic soils, PA treatment had the highest soil nitrogen content at the soybean vegetative and reproductive stage ($p <$ 0.05, Fig. S2), and soil nitrogen content was significantly decreased by the PE, PES, and PVC treatments at the vegetative stage ($p < 0.05$, Fig. S2). Moreover, the shifts of C:N ratio were found to be different between black and fluvo-aquic soil. In the black soil, the C: N ratio was significantly decreased by the PA treatment at the soybean vegetative stage, whereas it was significantly increased by the PES treatment at the same stage ($p < 0.05$, Fig. S2). In the fluvo-aquic soil, C: N ratio was significantly decreased by the PA treatment, whereas it was significantly increased by the PE treatment at two growth stages ($p < 0.05$, Fig. S2).

3.2. Effect of MPs on rhizosphere bacterial abundance

The qPCR results showed that the effects of MPs on the copy number of the 16 S rRNA gene in the fluvo-aquic soil varied with the type of MPs (Fig. 1). The abundance of the 16 S rRNA gene was significantly inhibited by the PA and PE treatments at the soybean vegetative stage ($p < 0.05$, Fig. 1), whereas significant increases were observed in the PA, PES, and PVC treatments at the soybean reproductive stage ($p < 0.05$, Fig. 1).

3.3. Effect of MPs on rhizosphere bacterial diversity

Bacterial diversity of the rhizosphere was assessed based on 16 S rRNA sequencing data. After merging and quality filtering, totally, 6,289,789 sequences were obtained from 120 samples, with 52,414 sequences per sample. The rarefaction curves of 16 S rRNA attained the saturation plateau, indicating that all samples had sufficient sequencing depth (Fig. S1).

The MPs treatment did not alter the rhizosphere bacterial alpha diversity significantly in the black soil ($p > 0.05$, Fig. 2), except that the PA treatment significantly suppressed the alpha diversity (Shannon and Faith'PD indexes) of rhizosphere bacterial communities at the soybean vegetative stage ($p < 0.05$, Fig. 2a and c). In the fluvo-aquic soil, rhizosphere bacterial alpha diversity (Shannon and Faith'PD indexes) was significantly enhanced by the PA treatment at the soybean vegetative stage, while it was significantly decreased at the soybean reproductive stage ($p < 0.05$, Fig. 2b and d). Shannon and Faith'PD indexes in the PVC-treated rhizosphere soil were observed significantly higher than those in CK at the soybean vegetative stage ($p < 0.05$, Fig. 2b and d). Moreover, the Shannon index was significantly reduced in the PE treatment and Faith'PD index was significantly increased in the PES treatment at the soybean vegetative stage ($p < 0.05$, Fig. 2b and d).

3.4. Effect of MPs on rhizosphere microbial community and identification of sensitive bacterial species

PCoA plot revealed the distinct clustering of each MPs treatment. The first two principal components of the black soil and the fluvo-aquic soil explained 34.66% and 49.66% of the community variance, respectively. The OTUs from both soil samples (Fig. 3a and b) were separated as per the plant growth stage. We compared the dissimilarity distances among the five treatments at each soil type (Fig. 3c and d). The effect of MPs contamination on bacterial distribution patterns in the rhizosphere soil varied with two soil types. The microbial community structure was more susceptible to MPs treatment in the fluvo-aquic soil than that in the black soil.

The taxonomic composition of the bacterial phylum in rhizosphere soil was shown in Fig. 4. The dominant phyla were Proteobacteria (48.8%−79.6%), Acitinobacteriota (9.7%−21.6%), Bacteroidota (0.2%−31.5%), Firmicutes (0.4%−15.7%), Chloroflexi (1.3%−4.5%), Acidobacteriota (0.6%−3.6%), Gemmatimonadota (0.3%−1.6%), Verrucomicrobiota (0.2%−1.0%) and Cyanobacteria (0.01%−1.4%) in the black soil and Proteobacteria (51.3%−89.9%), Acitinobacteriota (4.0%−20.6%), Firmicutes (0.1%−26.2%), Bacteroidota (0.3%−11.6%), Chloroflexi (0.5%−2.9%), and Acidobacteriota (0.2%−3.0%) in the fluvo-aquic soil. In two soils, Proteobacteria was the dominant phylum at both growth stages. Moreover, the relative abundance of phylum Bacteroidota was observed to be higher at the vegetative stage than the reproductive stage, whereas the opposite phenomenon was observed for the relative abundances of phylum Firmicutes.

The LEfSe analysis showed that 114 biomarkers related to 12 phyla and 499 biomarkers related to 24 phyla were sensitive to MPs contamination in the black soil and the fluvo-aquic soil, respectively (Fig. 5 and Fig. S3, Supplementary excel 1 and Supplementary excel 2). Six biomarkers were significantly enriched in the PA treatment at both growth stages in the black soil, accounting for 7.9% of the total biomarkers at the vegetative stage and 15.8% at the reproductive stage (Fig. 5). These biomarkers were affiliated with the genera *Phycicoccus*, *Paenarthrobacter*, *Terrabacter*, *Kribbella*, *Methylobacterium*, and an unclassified genus within the family *Pseudonocardiaceae*. Similarly, in the fluvo-aquic soil, 23 biomarkers were affected by PA treatment at both growth stages, and these biomarkers accounted for 10.4% of the total at the vegetative stage and 8.3% at the reproductive stage (Fig. S3). These consistently increased biomarkers were affiliated with seven phyla, including Actinobacteriota, Patescibacteria, Firmicutes, Cyanobacteria, Deinococcota, Proteobacteria, and Bdellovibrionota. Besides, *Pontibacter* and *Flaviaesturariibacter*, the family *Hymenobacteraceae* (phylum Bacteroidota), *Paraliobacillus* (phylum Firmicutes), and the order *0319_7L14* (phylum Actinobacteriota) were significantly enriched at both growth stages in the fluvo-aquic soil after treated by PE ($p < 0.05$).

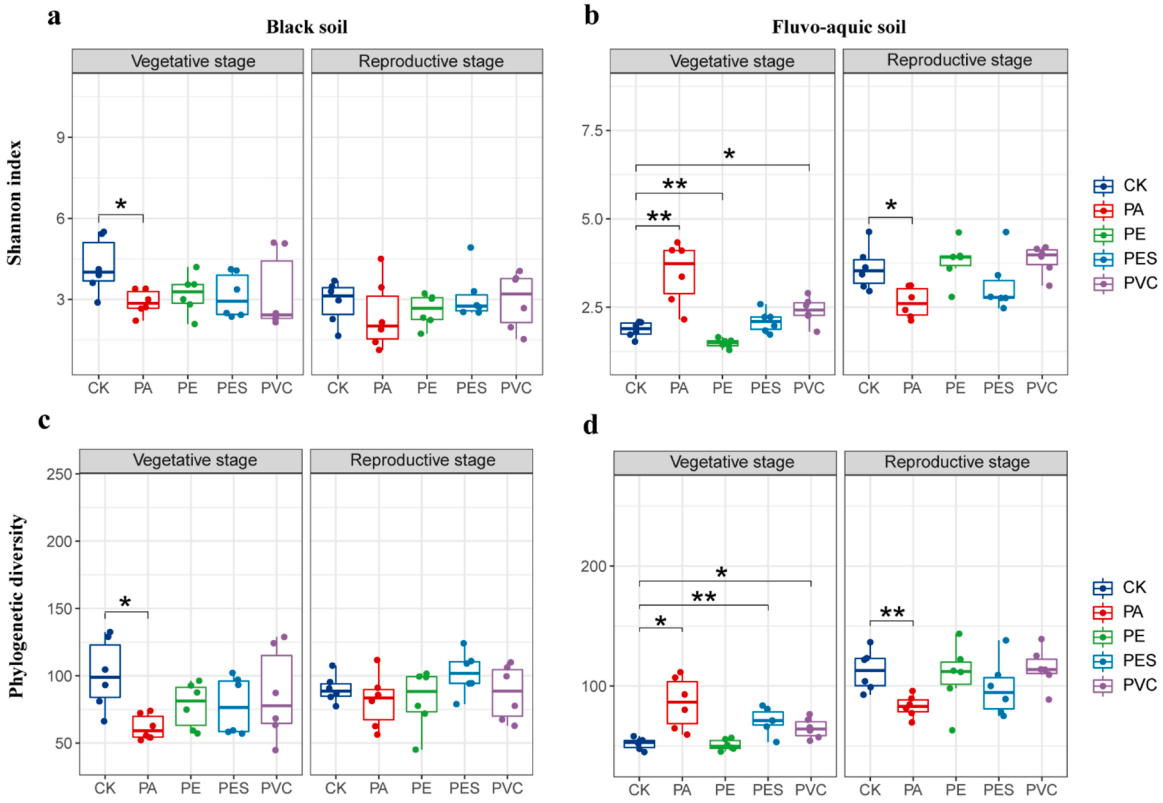

Fig. 2. Diversity indices (Shannon and Faith' PD) of rhizosphere bacterial community in diverse agricultural soils.

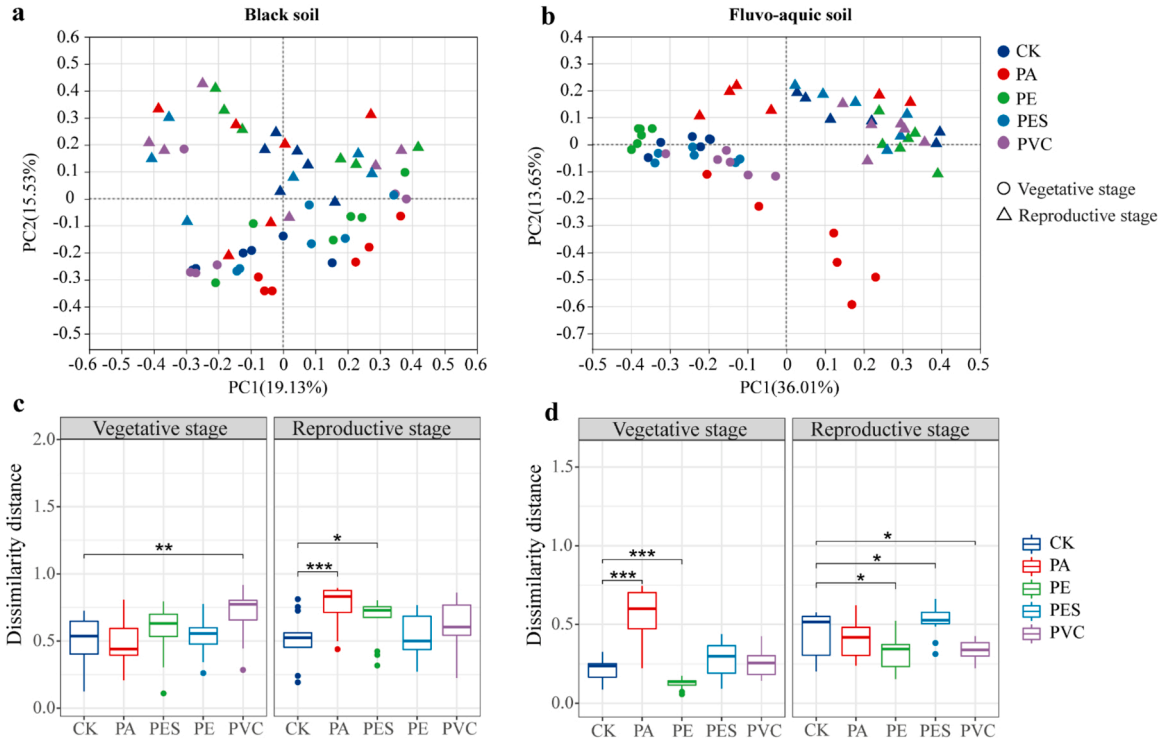

Fig. 3. Principal coordinate analysis (PCoA) of rhizobacteria community was performed on the Bray−Curtis distance in the black soil (a) and the fluvo-aquic soil (b). Dissimilarity distance in the black soil (c) and the fluvo-aquic soil (d).

3.5. Effect of MPs on the co-occurrence of rhizosphere microbiome

Co-occurrence network analysis further indicated that MPs treatment affected the complex network of bacterial interactions in the soybean rhizosphere. From the topological properties of the obtained networks, we found that the correlations in the networks of both black

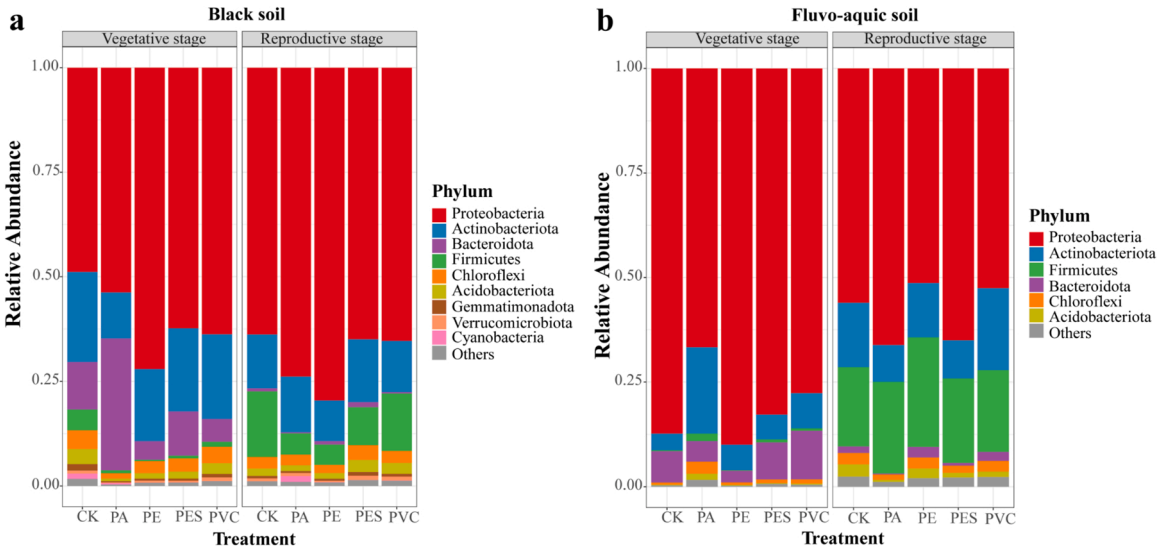

Fig. 4. Rhizosphere bacterial community composition in the black soil (a) and the fluvo-aquic soil (b) samples at the phylum level (>1% abundance).

and fluvo-aquic soils were primarily positive (> 96%), which represented both positive interactions and species occupying similar guilds or niches in the rhizosphere (Fig. 6 and Table 1).

The networks of the PA treatment in the black soil and the fluvo-aquic soil incorporated 129 nodes and 461 edges, and 149 nodes and 871 edges, respectively, and were smaller than the networks of the control in the black soil (178 nodes and 1329 edges) and in the fluvo-aquic soil (191 nodes and 6948 edges). Besides, the clustering coefficient of the PA treatment was decreased by 0.024–0.186, and the network density of the PA treatment was reduced by 0.026–0.304 in both soils compared with the control (Table 1). Additionally, the clustering coefficient and network densities of the PE, PES, and PVC-treated black soils increased by 0.005–0.171 and 0.017–0.156, respectively, whereas those of the PE, PES, and PVC-treated fluvo-aquic soils decreased by 0.113–0.271 and 0.078–0.311, respectively, compared with the control. The clustering coefficient and network density of the PA-treated soils were lower than those of the other MPs-treated soils. The soil microbiome associations exhibited a greater lack of tightness with the PA contamination.

3.6. Effect of MPs on nitrogen-cycling functional genes in rhizosphere soil

Compared to the control, the copy number of the *nifH* gene was not changed significantly ($p > 0.05$, Fig. 1) by any MPs treatments in the black soil, except that the PES and PVC treatments inhibited the abundance of the *nifH* gene at the soybean reproductive stage ($p < 0.05$, Fig. 1). In contrast, the copy number of *nifH* gene was significantly decreased in the PA treatment at the soybean vegetative stage, whereas a significant increase was associated with the PA, PES, and PVC treatments at the soybean reproductive stage in the fluvo-aquic soil ($p < 0.05$, Fig. 1).

The abundance of the ammonia-oxidizing communities was estimated based on the archaeal *amoA* (*AOAamoA*) and bacterial *amoA* (*AOBamoA*) gene copy numbers. Compared with the control, the total number of the *AOAamoA* genes was not affected ($p > 0.05$, Fig. 1) by any MPs treatments in the black soil. However, in the fluvo-aquic soil the abundance of *AOAamoA* gene was significantly reduced in the PE and PVC treatments and upregulated in the PES treatment at the soybean vegetative stage, and enhanced in the PA, PES, and PVC treatments at the soybean reproductive stage ($p < 0.05$, Fig. 1). For the *AOBamoA* gene, the abundance was significantly decreased by the PE, PES, and PVC treatments at the soybean vegetative stage, whereas significant increases were found in the PA, PES, and PVC treatments at the soybean reproductive stage in both black and fluvo-aquic soils ($p < 0.05$, Fig. 1).

The abundance of bacteria capable of reducing nitrite was quantified by determining the *nirK* and *nirS* gene copy numbers in the rhizosphere soil. In the black soil, none of the MPs exposure affected the abundance of the *nirK* gene throughout the experiment ($p > 0.05$, Fig. 1). In the fluvo-aquic soil, the copy number of the *nirK* gene was significantly decreased in the PA, PE, and PVC treatments at the soybean vegetative stage, whereas it exhibited significant abundance in the PA, PES, and PVC treatments at soybean reproductive stage compared to the control ($p < 0.05$, Fig. 1). Regarding the *nirS* gene, the PE, PES, and PVC treatments did not affect its abundance throughout the experiment, whereas the PA treatment stimulated the abundance of the *nirS* gene at two growth stages ($p < 0.05$, Fig. 1) in the black soil. In the fluvo-aquic soil, the *nirS* gene copy number was significantly reduced in the PA and PE treatments at the soybean vegetative stage, whereas it was significantly increased for the PA, PES, and PVC treatments at the soybean reproductive stage ($p < 0.05$, Fig. 1).

3.7. Effect of MPs on potential functional profiling of rhizosphere microbiome

According to the OTU annotation results, a total of 52 and 49 ecological function groups were obtained using FAPROTAX in the fluvo-aquic soil and black soil, respectively (Fig. S4 and Fig. S5). The dominant functions were aerobic chemoheterotrophy and chemoheterotrophy across all soil samples. In the fluvo-aquic soil, six functional groups at both growth stages showed abundance advantage amended with PA. The addition of PA may induce the function associated with plastic degradation ($p < 0.05$, Fig. S6). Besides, after PA treatment, the abundance of bacteria associated with chitinolysis, ligninolysis, aliphatic non-methane hydrocarbon degradation, aromatic hydrocarbon degradation, and hydrocarbon degradation were significantly more abundant at the soybean vegetative stage and significantly decreased at the soybean reproductive stage ($p < 0.05$, Fig. S6). PE treatment significantly increased the level of dark hydrogen oxidation at two growth stages ($p < 0.05$, Fig. S6). Also, PVC treatment significantly activated the functional response of xylanolysis and cellulolysis ($p < 0.05$, Fig. S6). However, in the black soil, MPs treatments did not exhibit consistently significant effects on bacterial functional groups.

4. Discussion

Our findings demonstrated that MPs could impact the composition,

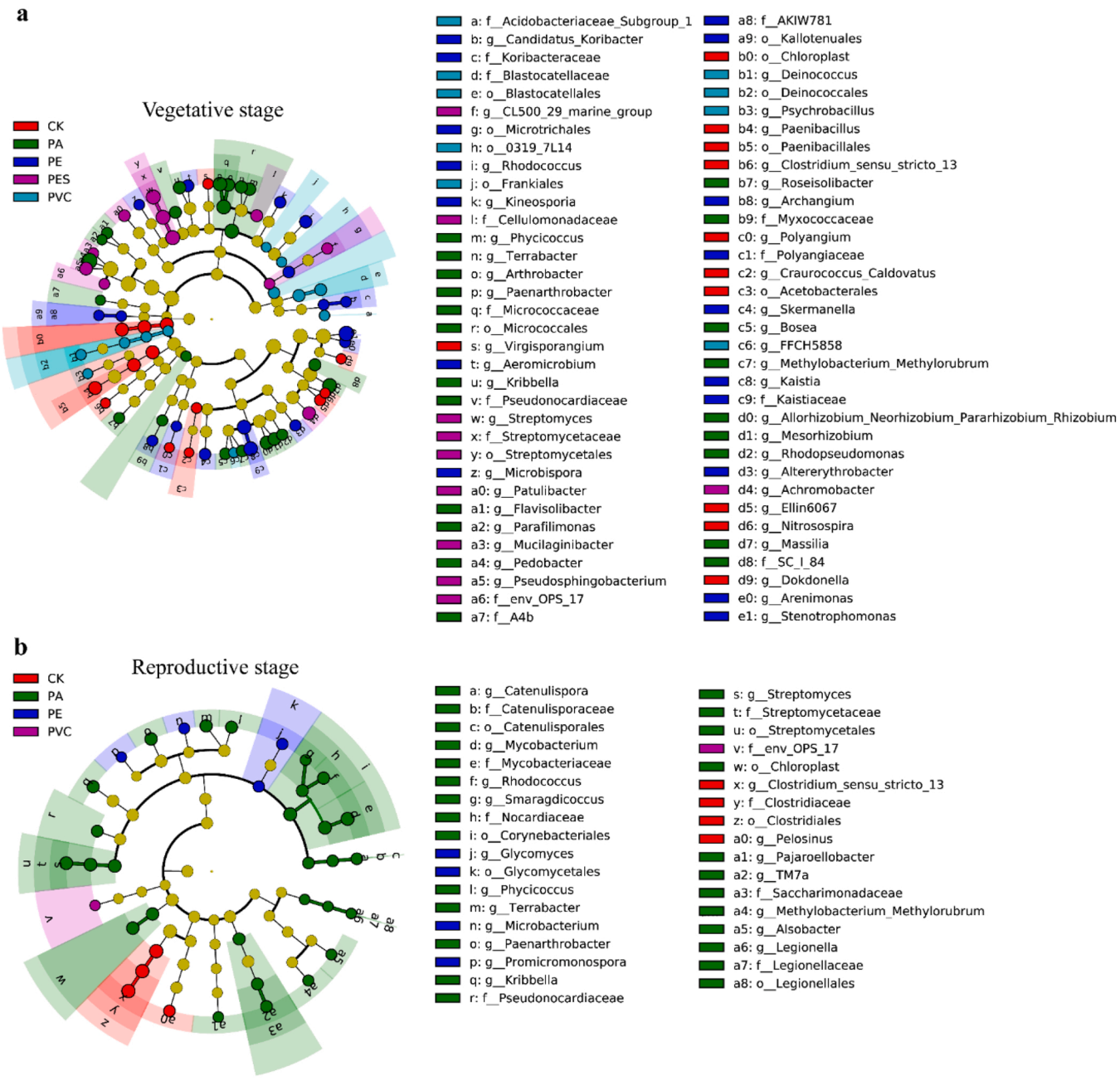

Fig. 5. LEfSe results reveal rhizosphere bacterial biomarkers (from phylum level to genus level) that were enriched in each MPs treatment in the black soil.

co-occurrence patterns, and N-cycling function of the soybean rhizobacteria. Further, these effects varied with MPs polymer types, soil types, and soybean growth stages. This study reveals the overall impact of MPs contamination on the rhizosphere ecosystem.

The response of the bacterial alpha and beta diversity in the rhizosphere soil to MPs varied according to the soil types. The fluvo-aquic soil, which has lower microbial biomass carbon and organic matter than black soil, was more affected by MPs contamination (Table S1) in terms of rhizobacterial diversity. The result was consistent with a prior study that revealed MPs had location-dependent impacts on the soil ecology (Bandopadhyay et al., 2020). The red soil was reported to be more sensitive to MPs contamination compared to paddy and fluvo-aquic soils (Li et al., 2021), and the impact of PE MPs on soil CO_2 and N_2O emissions varied with soil types (Yu et al., 2021). This might be due to the fact that different soil types exhibit distinct differences in microbial community compositions and soil properties, which in turn led to different responses to anthropogenic stressors.

The distinction of rhizobacterial diversities and community compositions among studied MPs-treated soils indicated that PA treatments more significantly impacted the rhizosphere bacterial community structure and distribution pattern than did PE, PES, and PVC treatments. In the current study, compared with the PE, PES, and PVC, PA significantly increased soil nitrogen content in the long term (Fig. S2), which was consistent with the report of (de Souza Machado et al., 2019), who revealed that the PA could affect the growth of spring onion by the fertilizer effect. The potential reason may explain that PA could act as the new substrates or provide nutrient resources, and thus promote microbial stochastic colonization. However, the previous study found no significant variation in the alpha diversity in the soil contaminated with PA MPs (Shi et al., 2022). The different effects of MPs on soil ecosystems are mostly triggered by the variation of the soil properties, plant species, as well as exposure duration (Ge et al., 2021).

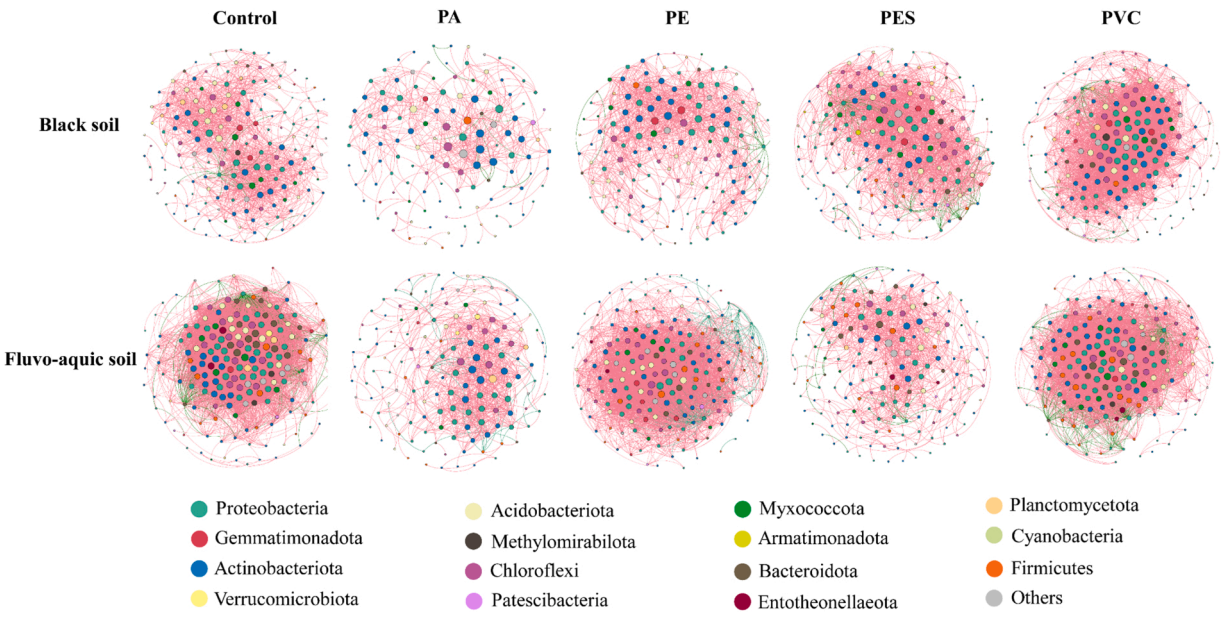

Fig. 6. Network analysis indicates the changes of the bacterial co-occurrence pattern in the rhizosphere under MPs exposure. For the node, the size of each node shows the number of connections (degree), and the bacterial phyla were used to color each node. Positive interaction between two nodes is labeled with red, whereas negative interaction is marked with blue.

Table 1
Topological properties of rhizosphere microbiome networks were obtained under the control and different MPs treatment in each soil.

	Black soil					Fluvo-aquic soil				
	Control	PA	PE	PES	PVC	Control	PA	PE	PES	PVC
Number of nodes	178	127	149	178	187	191	149	189	168	185
Number of edges	1329	461	1110	2122	4177	6948	871	4175	1005	5194
Positive edges	1307	450	1093	2041	4150	6582	841	4014	976	4998
Negative edges	22	11	17	81	27	366	30	161	29	196
Percentage of positive edges (%)	98.3%	97.6%	98.5%	96.2%	99.4%	94.7%	99.3%	96.1%	97.1%	96.2%
Percentage of negative edges (%)	1.7%	2.4%	1.5%	3.8%	0.6%	5.3%	0.7%	3.9%	2.9%	3.8%
Clustering coefficient	0.519	0.495	0.524	0.542	0.69	0.768	0.582	0.586	0.497	0.655
Network density	0.084	0.058	0.101	0.135	0.24	0.383	0.079	0.235	0.072	0.305
Modularity	0.433	0.514	0.441	0.341	0.197	0.08	0.415	0.204	0.413	0.148

Furthermore, high-dimensional biomarker taxa with noticeably different abundances across MPs treatments were found by the LEfSe analysis. PA treatment exhibited sustained effects on the bacterial community assembly. In both black and fluvo-aquic soils, a biomarker associated with the order *Rhizobiales* was more abundant in PA treatment. It was reported that *Rhizobiales* might be plant pathogens such as liberobacter asiaticum (Song et al., 2022). On the other hand, *Rhizobiales* might be considered as bacteria capable of fixing nitrogen that could provide necessary support for their host plants in agricultural production (Banerjee et al., 2018; Carvalho et al., 2010). Therefore, the effects of PA contamination on the rhizosphere function are needed for further investigation. Furthermore, the natural equilibrium in the indigenous rhizosphere bacterial community was disrupted by the stress of the PE, PES, and PVC, and distinct dominating bacterial groups formed. In the black soil, 17 biomarkers in PE treatment and 12 biomarkers in PVC treatment emerged at the soybean vegetative stage but disappeared at the soybean reproductive stage. We also observed that 10 biomarkers in PES treatment and 56 biomarkers in PVC treatment appeared at the soybean vegetative stage, but disappeared at the soybean reproductive stage in the fluvo-aquic soil. Given the tremendous bacterial diversity in rhizosphere soil (Fierer, 2017), both resistance (insensitivity to disturbance) and resilience (rate of recovery after disturbance) drive the variance of sensitive microbiome taxa over time (Griffiths and Philippot, 2013; Shade et al., 2012). Additionally, growing plants could produce different amounts and compositions of root exudates, which alter the bioavailability and toxicity of external contaminants (Huang et al., 2017; Li et al., 2008). Thus, revealing the response of rhizobacterial communities to MPs contamination in multiple plant growth stages can provide a comprehensive understanding of its effects and risks on the soil-plant system.

Co-occurrence patterns help to elucidate potential microbiome interactions and evaluate subsequent changes in community structure (Faust and Raes, 2012). Interestingly, we discovered that the network connectivity (links) was predominantly positive (> 96%) in rhizosphere assemblages, with microorganisms occupying similar guilds or niches, and this highlighted the possibility of widespread cooperative or syntrophic interactions among rhizosphere microbiomes (Shi et al., 2016). Similarly, previous research has identified that the positive associations of the bacterial co-occurrence patterns occurred in the rhizosphere of plants (Hallam and McCutcheon, 2015; Ren et al., 2015; Shi et al., 2016). The decreased topological indexes (clustering coefficient and network density) in the PA treatment in both soils indicated that the bacterial community associations exhibited a remarkable lack of tightness under PA interference. Moreover, the variation in the network structure shows that MPs treatments impacted bacterial interactions (Fig. 6 and Table 1). Additionally, the network size (nodes) and connectivity (links) for PA, PE, PES, and PVC treatments decreased compared to those for the control in the fluvo-aquic soil, which implied that these four MPs contamination could decrease bacterial network complexity. Previous studies have reported that PA, PE, polystyrene

(PS), polyethylene terephthalate (PET), and PLA MPs could reduce the microbial interactive relationships (Chen et al., 2020; Rong et al., 2021; Sun et al., 2022) and the community stability (Shi et al., 2022). The potential reason may be that the MPs are more recalcitrant and have lower perviousness compared to the natural soil particles, which may induce the physical block effect and thus cut off the interactions between soil microorganisms (Shi et al., 2022). Additionally, the clustering coefficient and network densities of the PE, PES, and PVC-treated black soils were increased, indicating that rhizosphere soil microorganism associations were more tightened and consequently increased system stability to resist adverse environmental interference of these MPs in the black soil. The structure and dynamics of ecological communities are primarily driven by the co-occurrence interactions between taxa (Schmidt et al., 2017). Therefore, mechanisms of the observed variations in the rhizosphere soil microbial co-occurrence network structures of different soil types yet need to be identified.

Additionally, this research examined how MPs affected the rhizosphere function structure, including the N-cycling. Nitrogen-fixing microorganisms are essential for soil NH_4^+-N equilibrium (Zhang et al., 2006). The different effects of MPs on the abundance of *nifH* indicated different impacts on microbial N fixation. Our results suggested that PES and PVC could inhibit ammonification processes in the soybean rhizosphere in the black soil, and PA could stimulate the growth of nitrogen-fixing bacteria in the fluvo-aquic soil. Studies have shown that LDPE MPs stimulate the nitrogen-fixing bacteria *Burkholderia*, which may affect the nitrogen-fixing process in the soil (Huang et al., 2019). A critical enzyme in nitrification, an ammonia monooxygenase (encoded by the *amoA* gene), oxidizes ammonia (NH_4^+) to hydroxylamine (NH_2OH) (Seeley et al., 2020). The changes of the copy number of *AOAamoA* and *AOBamoA* genes in the PE treatment in the fluvo-aquic soil, suggest that PE could temporarily inhibit ammonia oxidation. It was reported that the abundance of *amoA* gene was stimulated by the PUF and PLA MPs in sediments (Seeley et al., 2020). However, different MPs types, soil properties, and related communities may contribute to the varying results among studies (Ge et al., 2018). Denitrification is widely regarded as being primarily regulated by two nitrite reductases responsible for nitrite reduction, either a cytochrome cd1-encoding enzyme encoded by the *nirS* gene or a Cu-containing enzyme encoded by the *nirK* gene (Kandeler et al., 2006). In the fluvo-aquic soil, the abundance variation of the *nirS* and *nirK* genes suggested that PE treatment could inhibit the growth of *nirS*- and *nirK*-type bacteria in short term, whereas PA, PES, and PVC treatment could stimulate the growth of *nirS*- and *nirK*-type bacteria. Our observations showed that different soil ecosystems have different responses to MPs regarding nitrogen cycling in rhizosphere soil. Nitrogen-cycling microorganisms were more sensitive in the fluvo-aquic soil than in the black soil.

Interestingly, PA, PE, and PVC contamination induced significant shifts of soil C cycling functional groups and plastic degradation groups. As we know, previous research has demonstrated that the abundance of C, N, and P cycling genes in the compound fertilizers-treated soil was obviously increased by PE MPs exposure (Li et al., 2021), however, the sensitive taxa varied between studies and soil types. The levels of some functional groups of rhizosphere associated with pollutants degradation, such as xenobiotics biodegradation and metabolism, were found to be improved under PVC MPs treatment (Zhu et al., 2022b), which indicated that some species could use the PVC MPs as the carbon source in the rhizosphere soils. All these findings point to the potential ecological effects of MPs: once the functional groups of rhizospheres have been altered due to MPs contamination, biogeochemical cycling of C and N involving these functional groups could be impacted, which may have potential effects on plant growth and nutritional value.

5. Conclusions

In this study, the response of the rhizosphere bacterial community to MPs contamination at soybean vegetative and reproductive stages in black and fluvo-aquic soils was investigated systematically. The impact of MPs treatment was found to be more significant on the soybean rhizobacteria in the fluvo-aquic soil than that in the black soil, which might be caused by the difference in the inherent composition of the original soil. Additionally, the results revealed a greater impact of PA than those of PE, PES, and PVC, as reflected by more remarkable community dissimilarities, less bacterial network complexity and tightness, and more sensitive species and function changes under the PA contamination. Importantly, the shifts of the abundance of N cycling function genes observed in MPs treated soils suggested that MPs contamination inhibited ammonification, ammonia oxidation, and denitrification processes in the soybean rhizosphere. The present study shed new light on the comprehensive understanding of the effects of MPs contamination on the rhizosphere ecosystems and emphasized the critical role of agricultural soil types and plant growth stages on these effects.

CRediT authorship contribution statement

Lili Rong: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Yu Wang**: Conceptualization, Writing – review & editing, Supervision. **Peter Meidl**: Writing – review & editing. **Lei Wang**: Methodology, Investigation. **Hongwen Sun**: Funding acquisition, Writing – review & editing, Supervision.

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.

Data availability

All sequence data was then downloaded from the Sequence Read Archive of the National Center for Biotechnology Information with accession number PRJNA807124.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. U21A20291, No. 41773109, No. 42007328, No. 42077336) and the Ministry of Education of China (No. T2017002).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.114577.

References

Amato, K.R., et al., 2013. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. ISME J. 7, 1344–1353.
Aulakh, M., et al., 2001. Characterization of root exudates at different growth stages of ten rice (Oryza sativa L.) cultivars. Plant Biol. 3, 139–148.
Auta, H.S., et al., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176.
Bandopadhyay, S., et al., 2020. Effects of biodegradable plastic film mulching on soil microbial communities in two agroecosystems. PeerJ 8, e9015.
Banerjee, S., et al., 2018. Keystone taxa as drivers of microbiome structure and functioning. Nat. Rev. Microbiol. 16, 567–576.
Barker, P., Hill, I., 1981. Philosophical transactions of the royal society of London.
Bastian, M., et al., Gephi: an open source software for exploring and manipulating networks. In: Proceedings of the International AAAI Conference on Web and Social Media, Vol. 3, 2009, pp. 361–362.
Bulgarelli, D., et al., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–95.
Carvalho, F.M., et al., 2010. Genomic and evolutionary comparisons of diazotrophic and pathogenic bacteria of the order Rhizobiales. BMC Microbiol. 10, 1–15.
Chen, H., et al., 2020. Mixing effect of polylactic acid microplastic and straw residue on soil property and ecological function. Chemosphere 243, 125271.

Chen, L., et al., 2019. Competitive interaction with keystone taxa induced negative priming under biochar amendments. Microbiome 7, 1–18.
Chen, S., et al., 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.
Edwards, J., et al., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. USA 112, E911–E920.
Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.
Fuller, S., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780.
Ge, J., et al., 2021. Review of the toxic effect of microplastics on terrestrial and aquatic plants. Sci. Total Environ. 791, 148333.
Ge, Y., et al., 2018. Carbonaceous nanomaterials have higher effects on soybean rhizosphere prokaryotic communities during the reproductive growth phase than during vegetative growth. Environ. Sci. Technol. 52, 6636–6646.
Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37, 112–129.
Hallam, S.J., McCutcheon, J.P., 2015. Microbes don't play solitaire: how cooperation trumps isolation in the microbial world. Environ. Microbiol. Rep. 7.
Hardoim, P.R., et al., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 16, 463–471.
Huang, Y., et al., 2017. Interactions, transformations, and bioavailability of nano-copper exposed to root exudates. Environ. Sci. Technol. 51, 9774–9783.
Huang, Y., et al., 2019. LDPE microplastic films alter microbial community composition and enzymatic activities in soil. Environ. Pollut. 254, 112983.
Kandeler, E., et al., 2006. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. Appl. Environ. Microbiol. 72, 5957–5962.
Karges, K., et al., 2022. Agro-economic prospects for expanding soybean production beyond its current northerly limit in Europe. Eur. J. Agron. 133, 126415.
Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. Science 345, 144–145.
Lehmann, A., et al., 2020. Microplastic fiber and drought effects on plants and soil are only slightly modified by arbuscular mycorrhizal fungi. Soil Ecol. Lett. 1–13.
Li, D., et al., 2008. Effect of soil sorption and aquatic natural organic matter on the antibacterial activity of a fullerene water suspension. Environ. Toxicol. Chem. Int. J. 27, 1888–1894.
Li, H.-Z., et al., 2021. Long-term fertilization history alters effects of microplastics on soil properties, microbial communities, and functions in diverse farmland ecosystem. Environ. Sci. Technol. 55, 4658–4668.
Lian, Y., et al., 2022. Effects of polyethylene and polylactic acid microplastics on plant growth and bacterial community in the soil. J. Hazard. Mater. 435, 129057.
Louca, S., et al., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353, 1272–1277.
Lowery, N.V., Ursell, T., 2019. Structured environments fundamentally alter dynamics and stability of ecological communities. Proc. Natl. Acad. Sci. USA 116, 379–388.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Mbachu, O., et al., 2020. A new contaminant superhighway? A review of sources, measurement techniques and fate of atmospheric microplastics. Water Air Soil Pollut. 231, 1–27.
Nizzetto, L., et al., 2016. Are Agricultural Soils Dumps for Microplastics of Urban Origin? ACS Publications.
Pérez-Jaramillo, J.E., et al., 2019. Deciphering rhizosphere microbiome assembly of wild and modern common bean (Phaseolus vulgaris) in native and agricultural soils from Colombia. Microbiome 7, 1–16.
Philippot, L., et al., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799.
Piehl, S., et al., 2018. Identification and quantification of macro-and microplastics on an agricultural farmland. Sci. Rep. 8, 1–9.
Powell, J.R., Rillig, M.C., 2018. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytol. 220, 1059–1075.
Qi, R., et al., 2020. Behavior of microplastics and plastic film residues in the soil environment: a critical review. Sci. Total Environ. 703, 134722.
Qi, Y., et al., 2018. Macro-and micro-plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci. Total Environ. 645, 1048–1056.
Ren, D., et al., 2015. High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. ISME J. 9, 81–89.
Rillig, M.C., et al., 2017. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8.
Rillig, M.C., et al., 2019. Microplastic effects on plants. New Phytol. 223, 1066–1070.
Rong, L., et al., 2021. LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci. Total Environ. 773, 145640.
Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss floodplain soils. Environ. Sci. Technol. 52, 3591–3598.
Schmidt, T.S.B., et al., 2017. A family of interaction-adjusted indices of community similarity. ISME J. 11, 791–807.
Seeley, M.E., et al., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. Nat. Commun. 11, 1–10.
Segata, N., et al., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, 1–18.
Shade, A., et al., 2012. Fundamentals of microbial community resistance and resilience. Front. Microbiol. 3, 417.
Shi, J., et al., 2022. Microplastics reduce soil microbial network complexity and ecological deterministic selection. Environ. Microbiol. 24, 2157–2169.
Shi, S., et al., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. 19, 926–936.
Song, Y., et al., 2022. Effects of common microplastics on aerobic composting of cow manure: physiochemical characteristics, humification and microbial community. J. Environ. Chem. Eng. 10, 108681.
de Souza Machado, A.A., et al., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Change Biol. 24, 1405–1416.
de Souza Machado, A.A., et al., 2019. Microplastics can change soil properties and affect plant performance. Environ. Sci. Technol. 53, 6044–6052.
do Sul, J.A.I., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364.
Sun, Y., et al., 2022. Biodegradable microplastics enhance soil microbial network complexity and ecological stochasticity. J. Hazard. Mater. 439, 129610.
Wang, F., et al., 2020. Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere 254, 126791.
Wang, Y., et al., 2017. Agglomeration determines effects of carbonaceous nanomaterials on soybean nodulation, dinitrogen fixation potential, and growth in soil. ACS nano 11, 5753–5765.
Weithmann, N., et al., 2018. Organic fertilizer as a vehicle for the entry of microplastic into the environment. Sci. Adv. 4, eaap8060.
Wright, S.L., et al., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Xu, N., et al., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74, 1–8.
Yang, W., et al., 2021. Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with ZnO nanoparticles. Soil Biol. Biochem. 155, 108179.
Yu, H., et al., 2021. Effects of microplastics on soil organic carbon and greenhouse gas emissions in the context of straw incorporation: a comparison with different types of soil. Environ. Pollut. 288, 117733.
Zhang, L., et al., 2020. An overlooked entry pathway of microplastics into agricultural soils from application of sludge-based fertilizers. Environ. Sci. Technol. 54, 4248–4255.
Zhang, Y., et al., 2006. Molecular diversity of nitrogen-fixing bacteria from the Tibetan Plateau, China. FEMS Microbiol. Lett. 260, 134–142.
Zhang, Y., et al., 2022. Application of thifluzamide alters microbial network structure and affects methane cycle genes in rice-paddy soil. Sci. Total Environ. 838, 155769.
Zhao, T., et al., 2021. Microplastics increase soil pH and decrease microbial activities as a function of microplastic shape, polymer type, and exposure time. Front. Environ. Sci. 9.
Zhu, J., et al., 2022a. Microplastic particles alter wheat rhizosphere soil microbial community composition and function. J. Hazard. Mater., 129176
Zhu, J., et al., 2022b. Microplastic particles alter wheat rhizosphere soil microbial community composition and function. J. Hazard. Mater. 436, 129176.