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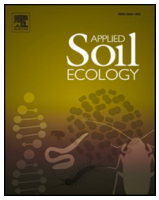

Effects of microplastics on common bean rhizosphere bacterial communities

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ABSTRACT

Microplastic pollution in terrestrial ecosystems is a growing concern due to its potential influences on soil properties and crop growth. Little is known about the effects of microplastics on the microbiome in the rhizosphere. Here, we studied the effects of two types of microplastics (MPs), low density polyethylene (LDPE-MPs) and biodegradable microplastic (Bio-MPs) of poly-butylene-adipate-*co*-terephthalate (PBAT) mixed with polylactic acid (PLA), on rhizosphere bacterial communities of *Phaseolus vulgaris* at doses of 0.5 %, 1.0 % and 2.5 % (w/w, dry weight ratio between MPs and soil). Bio-MPs and LDPE-MPs showed significant higher α-diversity (Chao 1, ACE, Shannon and Simpson) than control. For each type of microplastic material, 2.5 % of LDPE-MPs and Bio-MPs showed lowest α-diversity as compared to doses of 0.5 % and 1.0 %, indicating 2.5 % dose of MPs might pose selective effect on rhizosphere bacterial communities. β-Diversity of 1.0 % and 2.5 % Bio-MPs were distinctive from the control and other treatments. Microplastics also affected the relative abundance at family level, i.e. as compared to control, *Comamonadaceae* was higher in all the MPs treatments, *Rhizobiaceae* was highest in 2.5 % LDPE-MPs and lowest in 2.5 % Bio-MPs. LefSe results showed, as compared to control, Bio-MPs induced more indictive taxa than LDPE-MPs. Our findings evidenced that LDPE-MPs and Bio-MPs exerted profound effects on rhizosphere bacterial communities, and these effects might have far-reaching effects on soil nutrient cycling and plant health in agroecosystems.

1. Introduction

Microplastics (MPs) are generally defined as plastic particles smaller than 5 mm and are considered to be environmental pollutants (Nizzetto et al., 2016; Thompson et al., 2009). Previous research examining microplastic pollution was mainly focused on marine and sediment systems (Andrady, 2011; Ivar do Sul and Costa, 2014; Koelmans et al., 2013). After realizing that about 80 % of the microplastics in marine systems originate from land-based sources (Li et al., 2016), researchers began to examine microplastic pollution in terrestrial ecosystems and soils more closely. Microplastics can reach to agroecosystems via different input pathways, such as sewage water irrigation, compost and organic fertilization and plastic residues degradation (Corradini et al., 2019; de Souza Machado et al., 2018a; Ng et al., 2018; Nizzetto et al., 2016; R. Qi et al., 2020; Van den Berg et al., 2020; Zhu et al., 2019). Plastic mulching has been identified as one of the main input fluxes in agricultural ecosystems (Huang et al., 2020; Steinmetz et al., 2016). Study by Zhang et al. (2020) found 107 particles·kg^{-1} of low density polyethylene microplastics (LDPE-MPs) in plastic mulching fields in northeast China. Liu et al. (2018) found 62–78 particles·kg^{-1} of microplastics in vegetable fields with plastic mulching films in Shanghai.

To prevent agricultural plastic pollution arising from mulching residues, biodegradable plastics were introduced into agricultural production systems. Biodegradable plastics were designed to maintain the advantages of conventional polyethylene film while also having the benefit of being biodegradable so they can be tilled into soils and decomposed into carbon dioxide, water, and microbial biomass (Bandopadhyay et al., 2018; Siwek et al., 2019). Unfortunately, although biodegradable plastic mulches were designed to be degraded in agricultural fields, this doesn't mean that they can (Sintim and Flury, 2017). Recent research have found that biodegradable materials, such as polylactide (PLA)-based films and starch-based films, can actually break down into smaller plastic particles rather than completely biodegrade under natural field conditions, resulting in an accumulation of bio-microplastics in agricultural soils (Briassoulis, 2004; de Souza Machado et al., 2018a; Whitacre, 2014), however, their potentially

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ecological impacts and environmental risks remained unclear.

After reaching at agricultural soils, microplastics can affect soil processes in many ways. Several studies found microplastics can reduce soil bulk density and soil aggregate stability, increase soil water evaporation, and alter soil water repellency (de Souza Machado et al., 2018b; Lehmann et al., 2019; Y. Qi et al., 2020a). Soil fauna activity and fitness can also be altered by microplastics, for instance, Kim and An (2019) found that polystyrene microplastics (PS, 0.47–0.53 μm) can inhibit the movement of springtails. Huerta Lwanga et al. (2016) observed higher mortality of earthworm *Lumbricus terrestris* in litter with higher concentrations of PE microplastics (<150 μm). In addition, we have evidenced that ≥1.5 % Bio-based microplastics (PLA mixed with PBAT, 250–1000 μm) significantly reduced root and shoot biomass of common bean (Meng et al., 2021). Dong et al. (2020) found that microplastics of polystyrene (PS) and polytetrafluoroethylene (PTFE) decreased rice biomass and root activity. Wang et al. (2020) found that 10 % PLA (100 to 154 mm) microplastics decreased maize biomass and leaf chlorophyll content. Microplastics can also affect soil microbial communities and nutrient status, for example, previous research by Liu et al. (2017) showed that 28 % (w/w) polypropylene (PP) microplastics could increase the activity of fluorescein diacetate hydrolase (FDAse) and phenol oxidase, thus stimulating the decomposition of soil dissolved organic matter and enhancing the accumulation of soil N-NO_3^-. Y. Yan et al. (2020) found that of 0.1 % and 1.0 % (w/w) polyvinyl chloride (PVC, <0.9 mm) microplastics showed no significant effects on the soil bacterial community, but significantly increased soil available P content. A study by Y. Qi et al. (2020b) found that starch-based microplastics (50–1000 μm) can significantly affect rhizosphere microbial communities and produce volatile compounds like dodecanal. Rhizosphere microbes are essential for soil nutrient cycling and respond rapidly to environmental changes (Cui et al., 2018; Fei et al., 2020; Zhu et al., 2014). Even considering these handful of studies, current data of the effects of microplastics on rhizosphere microbial communities are still scarce. This greatly impedes our understanding of the ecological effects of microplastics on the soil-plant systems.

Current study investigates the effects of conventional LDPE-MPs and Bio-MPs on rhizosphere bacterial communities by means of high throughput sequencing. Our previous findings showed that LDPE microplastics (LDPE-MPs) and biodegradable microplastics (Bio-MPs) stimulated the formation of common bean nodules for fixing nitrogen (Meng et al., 2021), soil available nitrogen content was not significantly affected by LDPE-MPs, while it was significantly reduced in 2.5 % Bio-MPs (Meng et al., 2022). Therefore, we hypothesized that (1) the presence of microplastics affects the composition and structure of the rhizosphere bacterial communities, (2) these effects vary according to the doses and types of microplastics and (3) both LDPE-MPs and Bio-MPs stimulate the growth of nitrogen fixation bacteria. These results will contribute to our understanding of the effects of microplastics on the soil microbiome.

2. Materials and methods

2.1. Soils, microplastics and common bean seed

The test soil was sandy soil collected from Unifarm, Wageningen University, the Netherlands (Supplementary material Table S1). Two types of microplastic particles (MPs) were selected for the current study: 1). low-density polyethylene (LDPE-MPs) and 2). biodegradable plastic (Bio-MPs). The parental industrial pellets of biodegradable plastic consisted of 85 % poly-butylene-adipate-*co*-terephthalate (PBAT), 10 % polylactic acid (PLA) and 5 % calcium carbonate. For both plastic types, the size categories of microplastics used in this experiment were 60 % 250–500 μm and 40 % 500–1000 μm. These two size categories were chosen based on Scheurer and Bigalke (2018) and Zhang and Liu (2018). The ratio was chosen to simulate the heterogeneity of sizes of MPs in terrestrial ecosystems. The MPs used in this research were showing arbitrary shapes (scanned by Laser Direct Infrared system, Agilent, US) (Supplementary Fig. S1). Additional information about the selected microplastics is provided in Meng et al. (2021). Common bean (*Phaseolus vulgaris* L., *P. vulgaris*), a Leguminosae crop, was selected as a model plant due to its sensitivity to changes in soil conditions, such as water deficiency and soil nitrogen availability (Chekanai et al., 2018; Fenta et al., 2019). Common bean (*Phaseolus vulgaris* L.) seeds were obtained from Unifarm, Wageningen University, the Netherlands.

2.2. Pot experiment design and soil sampling

The pot experiment took place from June 28th, 2019 until October 18th, 2019 in outdoor net houses (diameter 0.25 mm) at Unifarm, Wageningen University & Research (WUR), the Netherlands. LDPE-MPs and Bio-MPs were mixed into the soil at doses of 0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5 % (w/w by weight of dry soil). A control treatment (CON, pure soil) without MPs was also included. In total, 7 treatments with 8 replicates for each treatment were included in this study, resulting in 88 pots (Supplementary Fig. S2). Further details regarding the experiment and cultivation have been reported in Meng et al. (2021). Due to budget and time constrains, only three (0.5 %, 1.0 % and 2.5 % w/w) out of the five microplastic doses were selected for DNA extraction and analysis. Our previous research (Meng et al., 2022) revealed an accumulation of soil available nitrogen at concentrations ≤1.0 % LDPE-MPs and a decrease at concentrations ≥1.5 % LDPE-MPs. For Bio-MPs, significant responses of root growth were observed at concentrations ≥0.5 % Bio-MPs, significant responses of soil carbon and nitrogen cycling were observed at concentrations ≥1.5 % and were most significant at 2.5 % w/w. Therefore, MPs doses of 0.5 %, 1.0 % and 2.5 % w/w were selected to assess the effects of LDPE-MPs and Bio-MPs on the soil bacterial community. The treatments measured in current study and their abbreviations are shown in Table 1.

Rhizosphere and bulk soil samples were collected 46 days after seeding (D46), near the end of the vegetative stage when plant roots and leaves completed the early development stage. Rhizosphere samples were collected from soils that loosely adhered to the roots after gentle shaking. The collected soil samples were transferred to a Styrofoam box filled with ice and immediately stored at −80 °C for further analysis. Each bulk soil sample was comprised of 5 soil subsamples from each pot. The bulk soil samples were air-dried and passed through a 2 mm steel sieve and stored at 4 °C.

2.3. DNA extraction and bioinformation analysis and statistical analysis

Soil DNA was extracted from 2 g of rhizosphere soil sample using a lab-made protocol based on a phenol-chloroform-isoamylalcohol extraction (Harkes et al., 2019). Quality and quantity of the extracted DNA was measured with a Nanodrop and Qubit. DNA samples were then diluted to 1 ng/μl and used as a template for PCR amplification. The variable V4 region of the bacterial 16S rRNA gene was used as the target for the analyses of Illumina sequencing. A two-step PCR was performed according to (Harkes et al., 2019). First a targeted bacterial primer combination, extended with an Illumina read area and the appropriate adapter were used to produce primary amplicons (in triplicate). A second PCR was conducted on 40× diluted amplicons of PCR1 to attach the Illumina index and the Illumina sequencing adaptor. Products of PCR 1 and 2 were randomly checked on gel to ensure that amplification was successful. PCR1 was performed with the adapted version of primer 515F and 806R. All PCR2 products were pooled and sent for sequencing (Bioscience, Wageningen Research, Wageningen, The Netherlands) using the Illumina MiSeq Desktop Sequencer (2 * 250 nt paired-end sequencing) according to the standard protocols. A laboratory control was prepared to monitor for the contamination during the test.

The bacterial sequencing data obtained from the Illumina MiSeq platform were processed with the dada2 pipeline (v 1.18) in R (Callahan et al., 2016). Miseq forward reads were trimmed at 230 bp, reverse reads

Table 1
Abbreviations of microplastic treatments.

Treatment	Abbreviation
0.5 % w/w LDPE microplastic	0.5 % LDPE-MPs
1.0 % w/w LDPE microplastic	1.5 % LDPE-MPs
2.5 % w/w LDPE microplastic	2.5 % LDPE-MPs
0.5 % w/w biodegradable microplastic	0.5 % Bio-MPs
0.5 % w/w biodegradable microplastic	1.5 % Bio-MPs
0.5 % w/w biodegradable microplastic	2.5 % Bio-MPs

were trimmed at 200 bp. After trimming, reads with an error rate higher than the expected error rate of 1 (maxEE = 1) were discarded. Reads that matched against the phiX genome were discarded as well. The remaining reads were processed with the dada2 core sample inference algorithm using default settings. Forward and reverse reads were then merged, and an amplicon sequence variant table was produced. The chimeric sequences were removed with the built-in function in dada2 using the consensus method. Representative ASVs were assigned to a taxonomic classification using the IdTaxa method implemented in the DECIPHER package in R against the Silva SSU database (SSU_r138) (Quast et al., 2013; Wright, 2016). Low-abundance ASVs (abundance of <0.005 % in the total data set) were discarded prior to analysis. Samples were transformed to relative abundances for further analyses. Statistical and diversity analyses were performed using phyloseq (v1.34) and vegan (v2.5.7) in R (Dixon, 2003; McMurdie and Holmes, 2013).

To investigate the indicative taxa involved within each treatment, a linear discriminate analysis (LDA) effect size (LEfSe) was conducted using the microbiomeMarker package (v0.0.1) in R (Cao, 2020). We performed statistical analysis of the LEfSe of bacterial communities from kingdom to genus levels, results with an LDA score ≥ 3.5 were considered to be important contributors to the model (Fig. 4A and B, Supplementary Fig. S4 and Table S3).

2.4. Measurement of soil pH, EC, plant shoot and root biomass and root nodules

Soil pH and EC were determined by using a SenTix meter and a conductivity cell TetraCon 325 with a soil to-water ratio of 1:5. Plant shoot and root biomass, root nodules measurements were previously published in Meng et al. (2021). Here we present the data as supporting information in Supplementary file (Fig. S4).

Statistical analyses were performed by IBM SPSS Statistics 23. Plant root nodule indicator was normalized using arcsine square root transformation to avoid violating the underlying assumptions of normality. Comparisons between control and microplastic treatments were performed using one-way ANOVA. Post-hoc test (LSD) was only performed when parameters were significantly affected ($p < 0.05$) by the occurrence of MPs. Measured soil pH and EC were presented as "means ± standard deviations".

3. Results

3.1. Effects of microplastics on rhizosphere microbial community

A total of 980,000 bacterial sequences were obtained after passing quality filtering. The number of sequences reads per sample ranged from 12,000 to 947,00. In total, 10,474 OTUs were detected. α-Diversity (Chao 1, ACE, Shannon and Simpson) was used to analyze observable bacterial community complexity in each treatment (Fig. 1). Negative represent the laboratory control and it is significantly different from Bio-MPs and LDPE-MPs treatments. For each type of microplastic material, doses of 0.5 %, 1.0 % and 2.5 % were pooled together and compared by unpaired two-sample Wilcoxon test (Table 2). Overall, as compared to control, Bio-MPs and LDPE-MPs treatments led to significant higher α-diversity, while Bio-MPs and LDPE-MPs showed no significant difference as compared to each other. Specifically, highest species richness (Chao 1, ACE) and diversity (Shannon, Simpson) were observed in 1.0 % Bio-MPs and 0.5 % LDPE-MPs, respectively, and lowest in 2.5 % Bio-MPs and 2.5 % LDPE-MPs, respectively. 1.0 % Bio-MPs also showed the highest α-diversity among all the treatments (Fig. 1). In addition, a principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrix was used for comparing bacterial communities across treatments (β diversity analysis) (Fig. 2). The first two principal components explained 27.6 % of the observed community variance. Multivariate permutational ANOVA (PERMANOVA) was used to compare the microbial community structure (Table 3). The R^2 value indicates how much of the observed variance was explained by each individual variable. MPs dose explains most of the observed shifts in the bacterial community (22 %), while MP type explains only 8 %, the interaction between dose and type explains another 14 % of the observed variance (Table 3). Both weighted and unweighted UniFrac confirmed that MP dose was the most important factor, followed by the interaction of dose x type and MPs type ($p \leq 0.02$) (Supplementary Table S2).

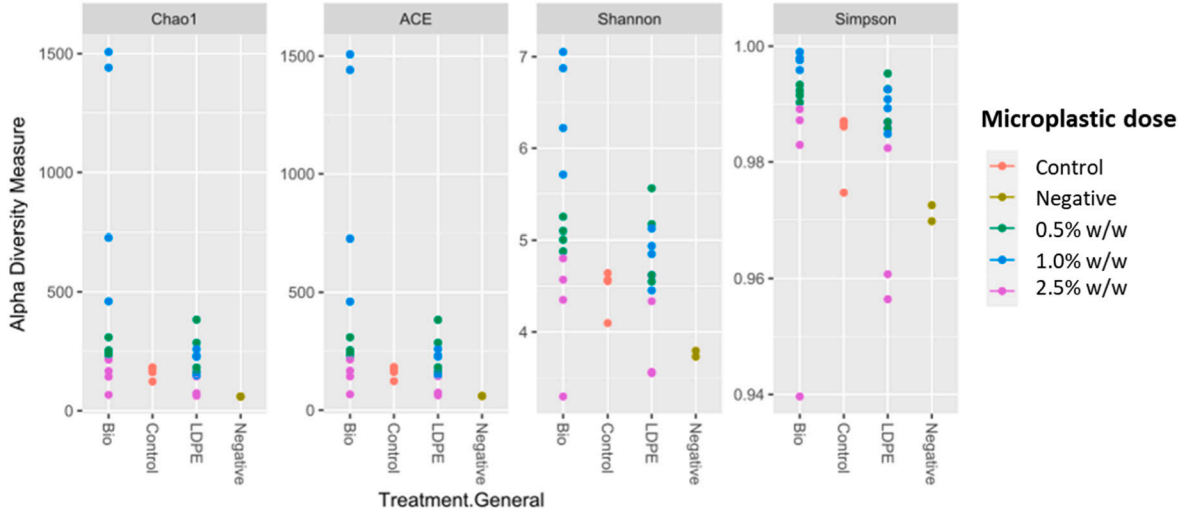


Fig. 1. Alpha diversity of bacteria, Chao 1, ACE, Shannon, Simpson index. Chao1 and ACE indices reflect the number and richness of the bacteria in samples. The greater the Chao 1 and/or ACE index, the higher the expected species richness of the microbiota. Shannon and Simpson indices reflect the diversity of the bacteria in samples, the greater the Shannon and Simpson index, the higher the diversity of the microbiota. Negative refer as the laboratory quality control.

Table 2
Statistic test of α-diversity among Bio-MPs, LDPE-MPs and control. Significant differences were test by unpaired two-sample Wilcoxon test (wilcox.test function in R).

Indicators	Treatment	Control	LDPE-MPs	Negative
Chao1	Bio-MPs	**0.003**	0.15	0.025
	Control		**0.043**	0.056
	LDPE-MPs			0.03
ACE	Bio-MPs	**0.003**	0.15	0.025
	Control		**0.043**	0.056
	LDPE-MPs			0.03
Shannon	Bio-MPs	**0.002**	0.15	0.025
	Control		**0.033**	0.22
	LDPE-MPs			0.03
Simpson	Bio-MPs	**0.001**	0.11	0.025
	Control		**0.043**	0.5
	LDPE-MPs			0.03

Note. Control indicates treatments without microplastic addition; LDPE-MPs indicates all the LDPE-MPs treatments; Bio-MPs indicates all the Bio-MPs treatments. Negative indicates a laboratory quality control. Differences are considered significant if $p < 0.05$ (bold letters).

3.2. Effects of microplastics on bacterial community composition

The rhizosphere bacterial communities at the phylum level were dominated by *Proteobateria*, *Actinobacteria*, *Gemmatimonadetes* and *Acidobacteriota* (Supplementary Fig. S3). The relative abundance at the family level is illustrated in Fig. 3. Compared to the control, the family *Comamonadaceae* was observed to be more abundant in all LDPE-MPs and Bio-MPs treatments. The family *Micrococcaceae* was more abundant in both 2.5 % Bio-MPs and 2.5 % LDPE treatments, while the family *Rhizobiaceae* exhibited higher relative abundance in 2.5 % LDPE-MPs and lower abundance in 2.5 % Bio-MPs than in the control. The family *Xanthobacteraceae* was mostly abundant in 2.5 % LDPE-MPs and least abundant in 1.0 % Bio-MPs. The family *Sphingomonadaceae* was least abundant in 2.5 % LDPE-MPs.

LEfSe analysis (LDA cutoff value ≥ 3.5) illustrated the taxon-specific differences in the rhizosphere bacterial communities between the control treatment and different types of microplastics (Fig. 4A and B). The pairwise comparison showed that more bacterial taxa were detected by LEfSe as important contributors for the difference between the control and the Bio-MPs (Fig. 4A) than between the control and the LDPE-MPs (Fig. 4B). Specifically, the comparison between the control and the Bio-MPs showed that the indicative taxa for the control soils were *Pseudolabrys*, *Devosiaceae*, *Chitinophaga*, *Micropepsis* and *Nitrososphaeraceae*. For 0.5 % Bio-MPs, the genera *Pedomicrobium*, *Pseudomonas* and *A21b* were considered indicative. For 1.0 % Bio-MPs, the families *Chitinophagaceae* and *Beijerinckiaceae* were considered indicative. Finally, for 2.5 % Bio-MPs, the families *Comamonadaceae*, *Hydrogenophaga*, *Bradyrhizobium*, *Pseudarthrobacter*, *Ramlibacter* and *Cupriavidus* were considered indicative. The comparison between the control and LDPE-MPs (Fig. 4B) showed that the genus *Micropepsis* was an indicative taxon in control soils. For 0.5 % LDPE-MPs, the family *Planococcaceae* and phylum *Myxococcota* were considered indicative. For 1.0 % LDPE-MPs, the class *Polyangia* was considered indicative and in 2.5 % LDPE-MPs, the families *Xanthobacteraceae*, *Nocardiaceae* and *Methyloligellaceae* were considered indicative.

In addition, another LEfSe analysis was performed by comparing all the treatments together (LDA > 3.5, Supplementary Table S3, Fig. S4). According to the results, control and 0.5 % LDPE-MPs showed no indicative taxa. For 1.0 % LDPE-MPs, families *TRA3–20*, *Methylophilaceae*, and *Nitrosomonadaceae* were considered indicative. For 2.5 % LDPE-MPs, families *Nocardiaceae*, *A21b*, *Reyranellaceae*, *Nitrososphaeraceae*, *Rhizobiaceae* and *Xanthobacteraceae* were considered indicative. For 0.5 % Bio-MPs, family *Pseudomonadaceae* was considered indicative. For 1.0 % Bio-MPs, family *Chitinophagales* and phylum *Bacteroidota* were considered indicative. For 2.5 % Bio-MPs, family *Comamonadaceae* and its belonging order *Burkholderiales* were considered indicative.

3.3. Effects of microplastics on plant growth and soil chemical properties

Soil pH and EC were significantly affected by the addition of microplastics (Table 4). For all the treatments, an increase in pH was detected as compared to control treatment. For soil EC, only 1.0 % LDPE-MPs was significantly lower than control, other treatments showed no significant differences.

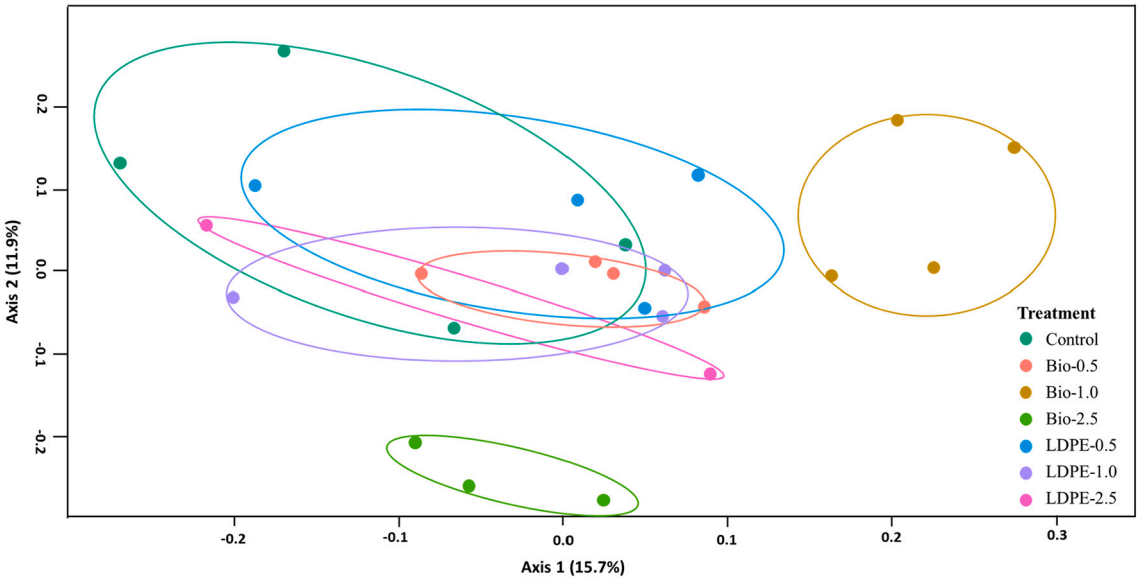


Fig. 2. Principal coordinate analysis of the rhizosphere microbial communities. Control indicate control treatment without microplastic addition; LDPE-0.5, LDPE-1.0 and LDPE-2.5 indicated LDPE-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight); Bio-0.5, Bio -1.0 and Bio -2.5 indicated Bio-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight).

Table 3
Results of PERMANOVA based on Bray-Curtis dissimilarity distances.

Factors	F	R^2	p
Microplastic dose	30.504	0.22182	***0.001***
Microplastic type	24.175	0.0879	***0.001***
Dose: Type	19.926	0.1449	***0.002***
Residuals		0.54539	

Note: Differences are considered significant if $p < 0.05$ (bold and italic letters).

4. Discussion

4.1. Microplastics changed the common bean rhizosphere bacterial community

Microplastic-induced dynamics on microbial diversity have been reported earlier and the observed shifts in microbial community composition were highly varied. In terms of Bio-based microplastics, Zhou et al. (2021) found that the addition of poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBA) increased soil microbial α-diversity, while Wang et al. (2020) and Yang et al. (2021) observed no significant effects of up to 10 % PLA-MPs on the α-diversity of the soil arbuscular mycorrhizal fungal community. The impacts on the soil microbial community from PE-MPs also varied from positive (Ren et al., 2020), insignificant (Huang et al., 2019; Wang et al., 2020) to negative (Fei et al., 2020; Gao et al., 2021). In this study, the α-diversity of rhizosphere bacterial communities was found to be enhanced at lower doses of MPs (0.5 % LDPE-MPs and 1.0 % Bio-MPs), while it was decreased at the highest dose (2.5 % LDPE-MPs and 2.5 % Bio-MPs) (Fig. 1 and Table 2). Several mechanisms might explain the changes in soil bacterial α-diversity. Firstly, the surface of microplastics, also known as the "plastisphere" (Jiang et al., 2018; Zettler et al., 2013), can provide novel and distinct habitats for soil microorganisms (M. Zhang et al., 2019). Xie et al. (2021) stated that the plastisphere of microplastics can provide an inclusive and compatible niche for a wide variety of rhizosphere microbes to colonize, resulting in more diverse microbial communities. On the other hand, an excessive amount of microplastics can exhibit selective effects on the indigenous bacteria, which are more compatible with microplastic surfaces (Y. Qi et al., 2020b; Ren et al., 2020; Xie et al., 2021), thus leading to the decrease of microbial α-diversity in 2.5 % dose microplastic treatments. Secondly, it has been reported that carbon supplied by Bio-MPs (such as PBAT, PLA and PHA) can be utilized by microorganisms to gain biomass and energy (Urtuvia et al., 2014; Zumstein et al., 2018). However, by gaining access to this bioavailable-C source, these microorganisms might outcompete other microorganisms that are unable to metabolize this carbon source (Dini-Andreote et al., 2015; Rüthi et al., 2020), thus lowering microbial diversity at 2.5 % Bio-MPs.

The results of β-diversity showed that 1.0 % Bio-MPs and 2.5 % Bio-MPs treatments were clearly separated from other treatments, including control, 0.5 % Bio-MPs and all LDPE-MPs treatments, which were clustered together. Bio-MPs and LDPE-MPs used in current research are featured in distinct physical and chemical properties. Bio-MPs contains heteroatomic biopolymers that are edible and biodegradable for microorganisms (Guerrini et al., 2017; Madhavan Nampoothiri et al., 2010), while LDPE is a petroleum-derived hydrocarbon that has stable C-C bones and is almost non-degradable in soil (Briassoulis et al., 2004; Steinmetz et al., 2016). Hence, Bio-MPs showed more effects on soil biological process than LDPE-MPs, while LDPE-MPs tend to induce the changes in soil physical properties (Rillig et al., 2021; Zhou et al., 2021). This difference might be account for the divergence of the rhizosphere microbial community structure in LDPE-MPs treatments and (1.0 % and 2.5 %) Bio-MPs, while for 0.5 % Bio-MPs, dosage might be too low to induce changes in soil bacterial communities, hence was clustered with other treatments. PERMANOVA results showed that the dose of microplastics contributed the most to the changes in bacterial community composition, followed by the interaction of the type and dose of microplastics and finally the type of microplastics. This result emphasized that the accumulation of microplastics in soil, either Bio-MPs or LDPE-MP, after reaching a certain level, might ultimately pose environmental threats to the soil-plant systems. To date, studies on the effects of different types and doses of microplastics on the rhizosphere microbial community are still scarce. In order to make stronger

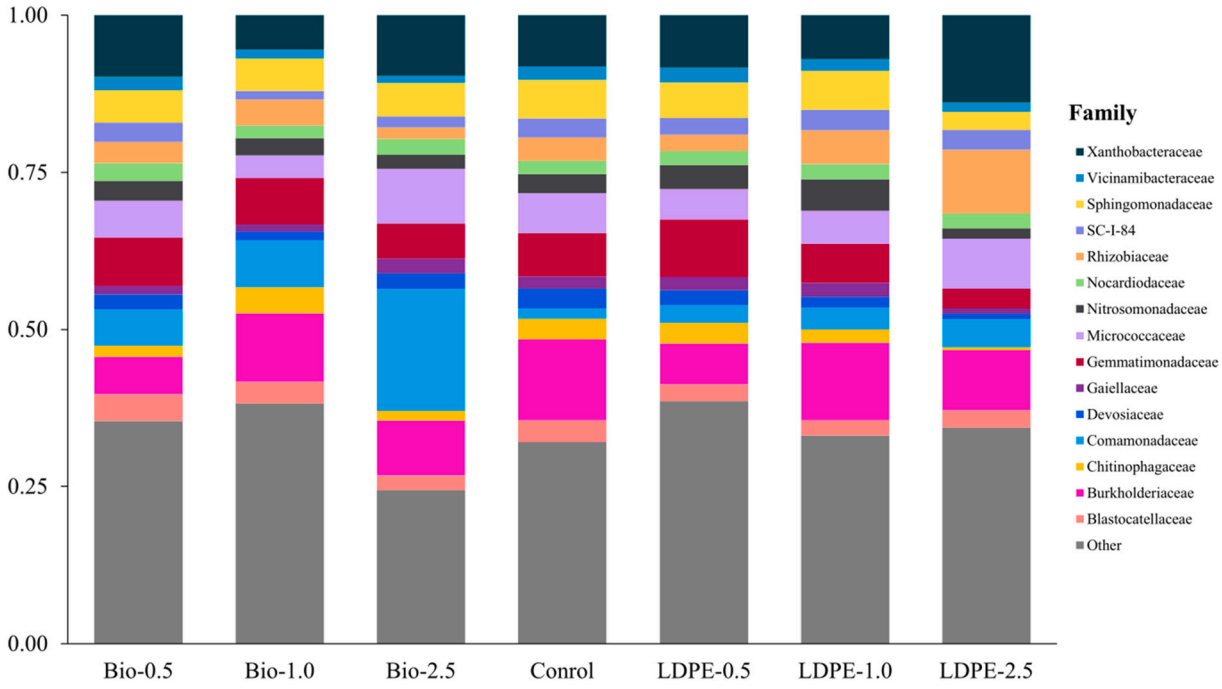
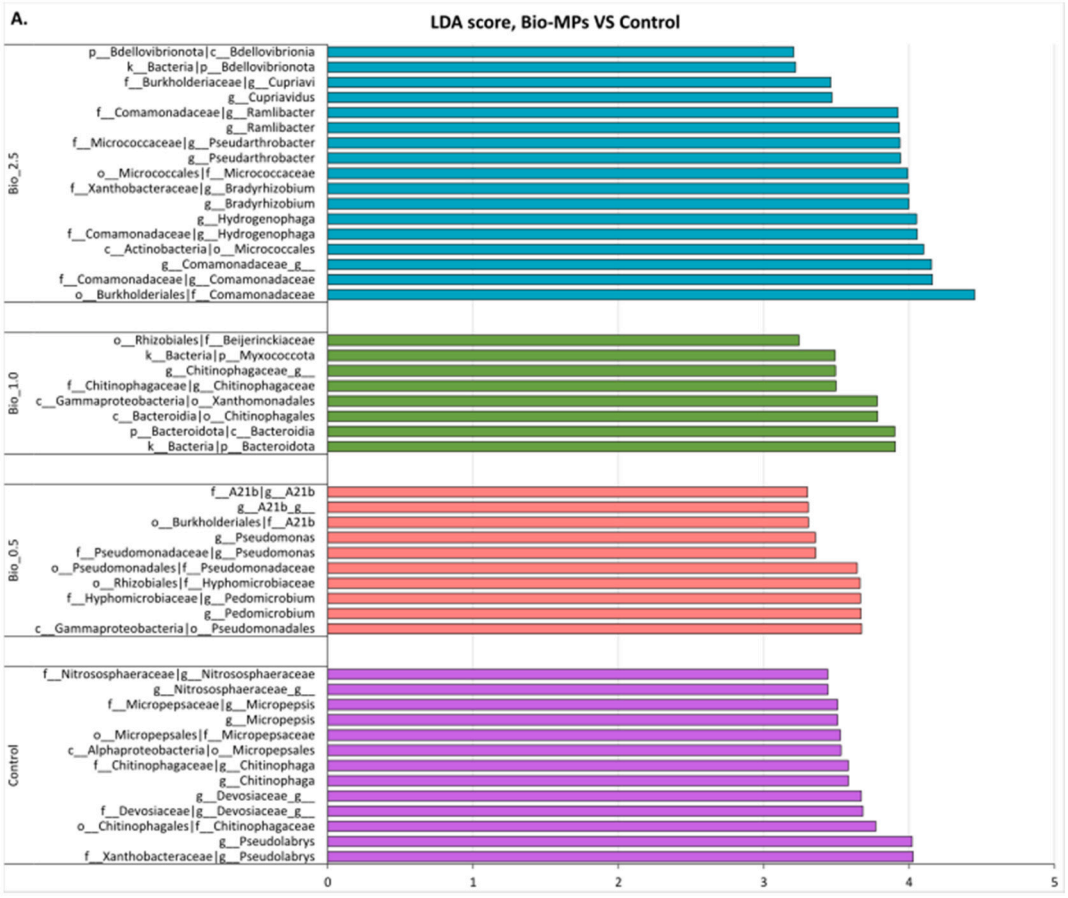


Fig. 3. Relative abundance of bacterial families in the community structure of each treatment. Control indicate control treatment without microplastic addition; LDPE-0.5, LDPE-1.0 and LDPE-2.5 indicated LDPE-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight); Bio-0.5, Bio -1.0 and Bio -2.5 indicated Bio-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight).



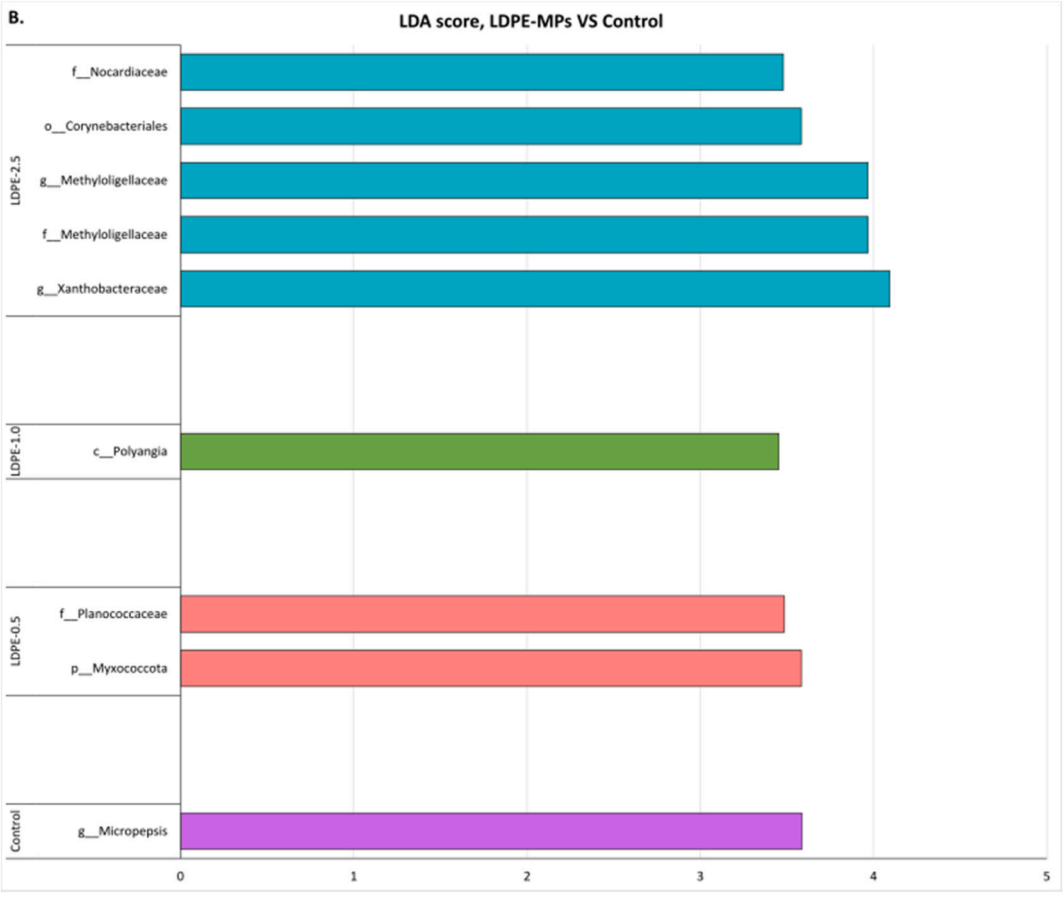


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Fig. 4. A) LEfSe analysis identifying active taxa (LDA score > 3.5) of rhizosphere bacterial communities resulting from biodegradable microplastic treatments. Control indicate control treatment without microplastic addition; LDPE-0.5, LDPE-1.0 and LDPE-2.5 indicated LDPE-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight); Bio-0.5, Bio -1.0 and Bio -2.5 indicated Bio-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight).
B). LEfSe analysis identifying active taxa (LDA score > 3.5) of rhizosphere bacterial communities resulting from LDPE microplastic treatments. Control indicate control treatment without microplastic addition; LDPE-0.5, LDPE-1.0 and LDPE-2.5 indicated LDPE-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight); Bio-0.5, Bio -1.0 and Bio -2.5 indicated Bio-MPs contaminate level of 0.5 %, 1.0 % and 2.5 % (weight percentages of MPs to dry soil weight).

statements, more detailed research is hence needed.

4.2. Microbial taxa affected by microplastic dose and type

The relative abundance of bacteria at the family level varied with microplastic type and dose. For instance, LDPE-MPs are featured of stable C-C bones and are resistant to degradation (Shen and Worrell, 2014). After entering in soil, it tends to improve the soil aeration, allowed more air diffusion and oxygen supply (Y. Qi et al., 2020a; G.S. Zhang et al., 2019), which in turn benefits the growth of aerobic bacteria such as *Comamonadaceae* (all LDPE-MPs treatments), *Xanthobacteraceae*, *Micrococcaceae* and *Rhizobiaceae* (2.5 % LDPE-MPs). By comparing all the treatments together. LEfSe showed that family *Nitrosomonadaceae* and *Nitrososphaeraceae* were indicative in LDPE-MPs treatments. Families *Nitrosomonadaceae* and *Nitrososphaeraceae* are well known for oxidizing ammonia into nitrite (Prosser et al., 2014; Seneca et al., 2020). This also verified that the addition of LDPE-MPs can stimulate more oxygen diffusion in the soil.

Bio-MPs showed higher relative abundances of *Comamonadaceae* (in all treatments) and *Micrococcaceae* (2.5 % Bio-MPs). Family *Comamonadaceae* has been observed to thrive in starch-based plastic treated soil (Y. Qi et al., 2020b) and PHBV-MPs treated soil (Zhou et al., 2021). *Comamonadaceae* is known to harbor hydrocarbon decomposers and play a role in the decomposition of various organic compounds (Kerster et al., 2006; Nuccio et al., 2013; Willems, 2014), including also biodegradable materials (PHBV and PLA) (Khan et al., 2002; Takahashi et al., 2011; Xu et al., 2018). Bio-MPs used in our experiment offered edible carbon source to the microbial communities, thus it's no surprise that family *Comamonadaceae* showed higher relative abundance in all Bio-MPs treatments. Our LefSe results of comparing all the treatments together also showed that *Comamonadaceae* was indicative in 2.5 % Bio-MPs, which provided the most C-substrate (Supplementary Table S3 and Fig.S4). *Micrococcaceae* is the primary decomposer of bean plant residues and is positively related to soluble carbon content (Monreal et al., 2018; Ortiz-Cornejo et al., 2017; Schellenberger et al., 2010). Our previous research revealed that 2.5 % Bio-MPs led to higher root decay in the common bean (Meng et al., 2021) and higher DOC (Meng et al., 2022), which might account for the increase in the relative abundance of the family *Micrococcaceae* in the 2.5 % Bio-MPs treatment.

Additionally, the genus *Hydrogenophaga* and *Ramlibacter* (Family Comamonadaceae) was stimulated by 2.5 % Bio-MPs treatment. A previous study by Bandopadhyay et al. (2020) found that the genus *Hydrogenophaga* was enriched from PLA-containing plastics. Chen et al. (2019) found that *Ramlibacter* positively responded to PLA-MPs. Members of *Ramlibacter* have been reported to have the catabolic potential of utilizing complex organic fractions like hydroxybenzoate, 3-hydroxybenzoate, and D-melibiose as sole carbon sources (Wang et al., 2012; Y. Yan et al., 2020). Members of *Hydrogenophaga* are characterized by the ability to oxidize hydrogen (Fagervold et al., 2014; Willems, 2014) and have also been reported to prefer using carboxylic acids as a growth substrate (Magic-Knezev et al., 2009). Hence, our findings, combined with previous findings, suggest that biodegradable materials as a carbon source for the rhizosphere bacterial communities and can affect rhizosphere bacterial composition.

In our previous paper (Meng et al., 2021), we assumed that 2.5 % Bio-MPs treatment might have higher relative abundance of N_2 fixating bacteria due to the highest root nodule numbers per gram of dry root (Supplementary Fig. S4C). This hypothesis is partly verified. Our LEfSe analysis revealed that the genus *Bradyrhizobium* was an indicative taxa in 2.5 % Bio-MPs. *Bradyrhizobium* was also observed thrived in a starch based microplastic experiment by Y. Qi et al. (2020b). *Bradyrhizobium* is one of the main nitrogen fixating genera that is capable of forming symbiotic nodules that develop in legumes (Avontuur et al., 2019; Ormeno-Orrillo and Martinez-Romero, 2019). However, *Rhizobiaceae*, another aerobic bacterial family that also involved in soil symbiotic nitrogen fixation processes (Carareto Alves et al., 2014; S. Yan et al., 2020), showed a lower relative abundance of *Rhizobiaceae* in 2.5 % Bio-MPs. To date, very few studies have reported the effects of microplastics on the relative abundance of the family *Rhizobiaceae*, therefore, we cannot draw clear conclusion between rhizosphere nitrogen fixation bacteria and common bean root nodulation under the microplastic pollution. More attention is needed in order to provide more in-depth understanding of microplastic pollution in soil-plant systems.

4.3. Implications and changes in the rhizosphere bacterial community

According to LEfSe (Fig. 4), the pair comparison of Bio-MPs VS Control induced more distinct taxa than LDPE-MPs VS Control, indicating that Bio-MPs exerted stronger effects than LDPE-MPs on rhizosphere microbial communities. One possible explanation might be ascribed to the significant higher pH of Bio-MPs than LDPE-MPs. Soil pH is a crucial factor that association with microbial activities, soil nutrient dynamics and SOC decomposition (Sheng and Zhu, 2018; Zhalnina et al., 2015). Hence, in combination with previous research, it is clear that microplastic pollution can alter the microbial communities directly or indirectly through affecting the physicochemical processes in the soil. The changed microbial communities might also threat the nutrient dynamics, i.e., members of the family *Comamonadaceae* can mineralize organic forms of sulfate into inorganic forms, thus inhibiting the nitrification process in sediments and soils systems (Ouyang et al., 2019; Schmalenberger et al., 2008). The families *Comamonadaceae* and *Micrococcaceae* also played crucial roles in the denitrification process (Huang et al., 2014; Khan and Hiraishi, 2002; Takahashi et al., 2011), thus, it should note that changes in soil microbial composition may pose potential legacy effects on soil quality (Chen et al., 2019; Meng et al., 2022). Besides, some of the taxa that were stimulated by microplastic pollution are also associated with pathogenic bacteria. For example, the family *Nocardiaceae* was observed to be the biomarker taxa in the 2.5 % LDPE-MPs treatment in our study (Fig. 4B). Previous research by Huang et al. (2019) also observed that the relative abundance of the family *Nocardiaceae* was enriched by LDPE-MPs. The family *Nocardiaceae* is known to harbor causal pathogens of suppurative and granulomatous diseases of humans and animals, it should be noted that not all members of the family *Nocardiaceae* are pathogenic (Goodfellow, 1996; Goodfellow, 1998; Goodfellow and Maldonado, 2006). However, considering the transport of microplastics in food web has already been found, more in-depth evaluation thus required since microplastics may act as a vector for transporting opportunistic pathogens (Zhu et al., 2022).

5. Conclusion

The observed results of current study verified our first two hypotheses, the existence of LDPE-MPs and Bio-MPs (PLA + PBAT) can affect common bean rhizosphere bacterial community structure and

Table 4
Soil pH and electrical conductivity (EC) values measured for the bulk soil samples in each treatment. Differences are considered significant if $p < 0.05$. Post-hoc test was only performed when parameters were significantly affected by the occurrence of MPs. Lowercase letters (a, b, c, d and e) within the same column indicate significant differences among different microplastic treatments.

Treatment	pH	EC
Control	6.02 ± 0.03e	187 ± 33.0a
LDPE-0.5	6.23 ± 0.05bc	161 ± 28.4a
LDPE-1.0	6.23 ± 0.05b	107 ± 39.0b
LDPE-2.5	6.16 ± 0.05d	143 ± 37.0ab
Bio-0.5	6.13 ± 0.02d	144.8 ± 31.5ab
Bio-1.0	6.18 ± 0.02cd	173.3 ± 27.3a
Bio-2.5	6.33 ± 0.03a	186.8 ± 37.0a

composition, and their effects vary with the polymer type and dose. PERMANOVA results revealed that the dose of microplastics contributed most to the changes in microbial community composition as compared to the type of MPs and their interaction. α-Diversity (richness and diversity) was significantly improved after exposure to 1.0 % Bio-MPs and 0.5 % LDPE-MPs while decreased in the treatments of 2.5 % Bio-MPs and 2.5 % LDPE-MPs, which might be attributed to the selective effects of the two types of polymers. β-Diversity showed that rhizosphere microbial communities exposed to 1.0 % and 2.5 % Bio-MPs were clearly separated from other treatments. LEfSe showed that the addition of Bio-MPs induced more distinct taxa in the rhizosphere than LDPE, indicating Bio-MPs exerted stronger effects on our rhizosphere bacterial communities. The observed shifts in the relative abundance of the families *Comamonadaceae*, *Rhizobiaceae* and *Micrococcaceae* varied with microplastic type and dose, these families play important roles in soil organic matter decomposition and nutrient cycling, implying that increasing microplastic contamination in soil might have profound effects on soil nutrient cycling. The higher LDA value of family *Comamonadaceae* in 2.5 % Bio-MPs suggests that PLA-PBAT microplastics may be acting as an organic C substrate for rhizosphere microbial communities. Our third hypothesis assumed that LDPE-MPs and Bio-MPs can stimulate the growth of nitrogen fixation bacteria, however, this is not entirely true since 2.5 % Bio-MPs significantly decrease the relative abundance of family *Rhizobiaceae*, as such, the mechanisms behind soil nitrification process and microplastic pollution remained unsolved. Therefore, further research is needed to uncover the ecological effects microplastics on soil-plant systems. By relating to our previous findings, the work presented here systematically illustrates that microplastics, when reaching at certain level, are capable of induce alteration of soil biological processes, these effects can finally affect soil nutrient dynamics and plant growth. This is indispensable for future efforts to assess risks of microplastic pollution in terrestrial ecosystems.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Violette Geissen reports financial support was provided by European Commission.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104649.

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