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Effects of biodegradable microplastics on soil microbial communities and activities: Insight from an ecological mesocosm experiment☆

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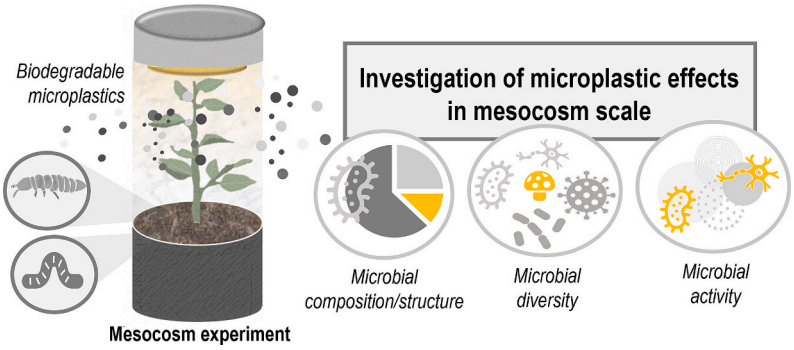
HIGHLIGHTS

- Alpha diversity of the bacteria and fungi increased with increasing PBAT-BD-MP concentration.
- Bacterial and fungal communities differed between the control and 0.8 % PBAT-BD-MP treatment.
- The increase in soil respiration correlated with increasing PBAT-BD-MP concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MP) are being released into the environment at an increasing rate, causing extensive pollution in soils and affecting biota and processes. Although the use of biodegradable plastic has increased, its effects on the soil microbial community are not yet well understood. A controlled mesocosm experiment was conducted to investigate the response of soil microbial communities to increasing amounts of starch-polybutylene adipate terephthalate MPs (PBAT-BD-MPs) added to the soil. The experiment included microbes, earthworms, springtails, and plants. The PBAT-BD-MPs were added to the soil column at doses ranging from 0 to 0.8 % *w*/w of soil dry mass, and the columns were incubated for 11 weeks under controlled climatic conditions. Bacterial and fungal amplicon sequencing was used to investigate the dose-dependent response of the soil microbial communities' alpha and beta diversity. The alpha diversity indices of the bacterial and fungal communities increased with increasing PBAT-BD-MP concentration. Bacterial richness was highest at the highest MP concentration (0.8 %). A similar trend was observed in the fungal community, with a significant increase in fungal richness as PBAT-BD-

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MP concentration increased. The alpha diversity of both bacterial and fungal communities significantly increased in MP treatments compared to the control treatment. At the highest MP concentration (0.8 %), the abundance of the bacterial phylum *Planctomycetes* showed a significant increase, while *Firmicutes* showed a significant decrease. The abundance of the fungal phyla *Ascomycota* and *Mortierellomycota* also significantly increased at the highest PBAT-BD-MP concentration compared to the control group. Alongside changes in the soil microbial community, we observed a rise in soil respiration as the concentration of PBAT-BD-MPs increased. Our three-month mesocosm study demonstrates that the introduction of biodegradable microplastics into the natural standard soil environment in realistic concentrations (0-0.025-0.05-0.2-0.8 %) and particle size distribution alters the soil bacterial and fungal community.

1. Introduction

Microplastics (MPs) are defined as synthetic polymer-based particles smaller than 5 mm in diameter and have become a great concern worldwide (Hartmann et al., 2019). The terrestrial environment is estimated to receive 4–23 fold more plastic waste than the ocean, and MP pollution is extensively found in soils (Horton et al., 2017; Xu et al., 2020). The median concentrations of MPs have been reported to be 1167 items kg^{-1} and 0.6 mg kg^{-1} (Büks and Kaupenjohann, 2020), and particularly, soils that receive sewage sludges containing high levels of MPs (6.75 %) and have a high risk of severe MP pollution (Fuller and Gautam, 2016). The concentration of MPs in the soil environment is expected to increase, and further research is needed to clarify their effects.

MPs affect soil ecosystems across different ecological levels, ranging from individual organisms to communities. Ecotoxicological experiments have been conducted using various soil-dwelling animals and plants, and several studies have reported negative effects on the reproduction of nematodes and springtails (Ju et al., 2019; Kim et al., 2020, 2024) or positive effects on plant growth (Lozano et al., 2021) at a wide range of effective concentrations depending on microplastic characteristics (e.g., size, shape, and a composition). The mechanisms underpinning MP effects differ from those of other typical pollutants (e.g., metals and organic chemicals), especially in terms of microbiology. For instance, microplastics can alter soil physical structure (de Souza Machado et al., 2018, 2019), and these changes could influence soil water retention capacity, evaporation, and water flow in the soil (Kim et al., 2021; Wan et al., 2019), potentially leading to effects that propagate to the microbial community (Rillig and Lehmann, 2020; Rillig et al., 2019). In addition, all plastics contain additional chemicals that give certain benefits during their useful life but can also be toxic to soil organisms (Kim et al., 2020; Rillig et al., 2021).

MPs can form a distinct microhabitat by enriching the microbial taxa that can degrade plastics or plasticizers, and these changes in the dominant taxa shift the structures and functionings of the microbial community (Chen et al., 2020; Li et al., 2021; Ren et al., 2020). These effects may vary depending on concentrations, characteristics (e.g., polymer structure, size, shape), and other soil environmental factors. Agricultural activities (e.g., manure and fertilization), contamination levels of other chemical pollutants (e.g., pesticides, antibiotics, and metals), and different source communities (e.g., rhizosphere) can be correlated with a variation of MP effects on the soil microbial community (Ding et al., 2021; Li et al., 2021; Potrykus et al., 2021; Wang et al., 2021; Xie et al., 2021; Zhang et al., 2019; Zhu et al., 2021). Although much more global data is needed to predict the impacts of MPs at environmentally relevant concentrations (Aralappanavar et al., 2024), several studies have reported that significant changes (positive or negative) in microbial community diversity can occur at 0.2 % (Feng et al., 2022; Li et al., 2022) or ≥1 % (Hu et al., 2022; Li et al., 2022; Shi et al., 2022; Wang et al., 2022). Small-scale or microcosm experiments have been predominantly conducted (approx. 83 % of total reported articles; Table S1) to understand the impacts of MPs since this approach can improve the repeatability and reproducibility of the results. However, mesocosm experiments that resemble more realistic conditions need to be conducted to investigate the unexplored mechanism in the actual multitrophic environment.

Given the evidence that the effects of MPs on the soil microbial community highly depend on the surrounding environmental and anthropogenic conditions, MP research needs to reflect these variables. In this study, we conducted a mesocosm experiment that was programmed to create realistic ecological conditions consisting of soil, vegetation, and soil dwelling animals. We chose biodegradable (BD) microplastics (polybutylene adipate terephthalate, PBAT, PBAT-BD-MPs) originating from commonly used mulching film for investigating the effects of MPs in a mesocosm scale experiment. Effects of MPs on bacterial and fungal communities were characterized by amplicon sequencing analysis (16S and ITS) and microbial activity analyzed as carbon (C) mineralization to carbon dioxide (CO_2). We hypothesized that the PBAT-BD-MPs would influence the soil microbial community and activity in a dose-dependent manner.

2. Materials and methods

2.1. Test soil and polybutylene adipate terephthalate microplastics

We used standard natural soil (Lufa 2.2, Lufa Speyer, Germany) in the experiment with properties (as given by the supplier): sandy loam, 1.8 ± 0.6 % of organic C (mean ± SD), pH (0.01 M $CaCl_2$) of 5.6 ± 0.3, and water holding capacity (WHC) of 43 ± 5 %. The soil was dried for 48 h at 40 °C prior to the experiment.

BD-MPs were obtained from cryomilling a commercially available PBAT-starch blend based mulching film (M-BIOIT-15-black-A0 in Hurley et al., 2024). The size distribution of PBAT-BD-MPs used in this study is presented in Table S2 and corresponds with observations from natural agricultural soils (Ranneklev et al., 2019). Here the average particle size was 131 μm and the median grain-size (D50) was 67 μm. Four concentrations of PBAT- BD-MPs were mixed in with the soil using a concrete mixer (Promischer PM145L) at 24–29 rpm for five minutes to final concentrations of 0.025 %, 0.05 %, 0.2 % and 0.8 % (w/w) of soil dry mass. These levels represent background conditions without added MPs, present-day MP concentrations (0.025–0.05 % w/w) found in soil, and future high exposure concentrations (0.2–0.8 % w/w of soil dry mass) to illustrate realistic levels of severely contaminated agricultural soil. To ensure the same amount of disturbance, also the control (0 %) soil was mixed equally.

2.2. Soil mesocosm experiment and sampling

High-density polyethylene (HDPE) cylinders (16.6 cm Ø), filled with PBAT-BD-MP-spiked or control soil to 12 cm depth (vol 2.6 L), were inserted into the CLIMECS system (CLImatic Manipulation of ECosystem Samples, CLIMECS, Amsterdam, The Netherlands; Franken, 2019). The columns were tapped on the ground five times to pack the soil in the columns. Columns were left to stabilize for a two-week period before the start of the experiment. Eight replicate columns were prepared for each MP concentration including the control without MPs.

Two weeks before the start of the experiment, lettuce (*Lactuca sativa*) was sown into pressed peat boxes (vol 0.06 L) filled with the

experimental soil and allowed to germinate for ten days. The pre-germinated lettuce seedlings were transferred into the columns before the introduction of the animals. Simultaneously, cress (*Lepidium sativum*) was sown directly into the columns as a shelter for the springtails, four days prior to the introduction of the animals. Organic cress and lettuce seeds were obtained from the Dutch Garden Seeds company (Volendam, the Netherlands). After 20 days all the cress was cut down and left on top as mulch. The introduction of the animals is henceforth referred to as week zero. At weeks four and eight two lettuce plants were removed from each column. In the final sampling after 11 weeks the last two lettuce plants were harvested. The adverse effects on lettuce growth and stress in responses to increasing soil PBAT-BD-MP are discussed in Adamczyk et al. (2024).

Earthworms *Aporrectodea caliginosa* and *Lumbricus rubellus* were obtained from Prodigga (Caumont-sur-Durance, France) and Lasebo (Nijkerkerveen, The Netherlands), respectively. The three species of springtails used in the mesocosm experiment: *Sinella curviseta*, *Heteromurus nitidus* and *Protaphorura fimata*, were taken from cultures kept at the Vrije Universiteit Amsterdam. In total four earthworms and 750 springtails were added to each soil column. Responses of soil dwelling animals and soil properties to PBAT-BD-MP will be presented in a separate publication.

The experimental conditions had a light/dark regime of 14 and 10 h, respectively and the soil temperature at soil depth of 0.5 cm was set to 18 °C during the day and 15 °C during the night, deeper soil horizons were kept constantly around 15 °C. The daylight hours and temperatures chosen were based on average growing seasons in northwestern Europe. Soil water content was maintained at 22 %, corresponding to 50 % WHC, by watering twice a week with 20 mL of demineralized water, as well as bringing the columns back to their original weight within a two-week interval.

Soil was sampled from the whole column depth, after the animals and plants were removed at the end of the 11-week experiment. Two replicates of 2 mL of homogenized but not sieved soil were sampled for microbial community analyses. Soil was stored frozen (−80 °C) until DNA extraction. About 200 g of homogenized soil samples were collected and stored under aerobic conditions in the dark, at 5 °C and 75 % relative humidity before being shipped for C mineralization analysis.

2.3. DNA extraction and library preparation for analysis of soil microbial community

Isolation of microbial genomic DNA from approximately 250 mg soil samples was done with the DNeasy PowerSoil Pro kit (Qiagen) using the QIAcube Connect (Qiagen) according to the manufacturer's instructions. Prior to lysis, samples were incubated for 10 min at 65 °C after adding the C1-lysis buffer followed with disruption by 15 min horizontal mixing with Vortex-Genie 2 (Scientific Industries, Inc.) and lastly homogenized in FastPrep (MP Biomedicals) for 30s with 4.5 m s^{-1} speed. DNA was eluted to 100 μL elution buffer. Quality and quantity of DNA were examined by a Qubit 4.0 fluorometer (Invitrogen, USA).

The bacterial V4-V5 region of the 16S rRNA gene was amplified using primers 515F-926R (Parada et al., 2016; Quince et al., 2011), and the fungal ITS genomic region was amplified using the fITS7 and the ITS4 primers (Ihrmark et al., 2012; White et al., 1990). The PCR cycles were as follows: a denaturation step for 3 min at 95 °C (bacteria and fungi), 30 (bacteria) and 27 (fungi) cycles of denaturation for 20 s at 95 (bacteria) and 98 °C, annealing for 30 s at 53 °C (bacteria) and 54 °C (fungi), and elongation for 30 s at 72 °C (bacteria and fungi), and a final elongation step of 5 min at 72 °C. PCRs were performed in a 25 μL volume containing 1× KAPA HiFi buffer, 0.2 mM of each dNTP, 0.5 U of KAPA HiFi polymerase (Kapa Biosystems, Woburn, MA, USA), 0.3 μM of each primer, and DNA template. Each PCR product was purified using magnetic beads (GC Biotech, Alphen aan den Rijn, The Netherlands) and used in a second PCR step with primers containing the sequencing adaptors and an 8 nt long index sequence for multiplex sequencing. After the second purification, the amplicon library was sequenced on an Illumina MiSeq 2000 platform (Illumina Inc., San Diego, CA, USA) at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv, Berlin, Germany) using 2 × 300-bp paired-end sequencing. The raw amplicon sequencing data for bacteria and fungi have been deposited in the NCBI SRA bioproject PRJNA1150082. We used amplicon sequence variant (ASV) richness and inferred a total bacterial 3155 ASVs and fungal 1506 ASVs in our samples. Lufa 2.2 soil contains low bacteria richness below 200 operational taxonomic unit (Silva et al., 2022), and this seems to be in accordance with our data. We obtained denoised, chimera-free, non-singleton bacterial and fungal ASVs using DADA2 (Callahan et al., 2016). Sequencing reads of the bacterial community were unable to be merged due to the lack of enough overlapping sequences. Thus, we used only the forward reads (R1) in the bacterial analysis (Siddiqui et al., 2022). The bacterial taxonomy of each ASV was assigned using the Silva reference database (ver. 132) (Quast et al., 2013), and the fungal taxonomic annotation was performed using the UNITE dataset (Abarenkov et al., 2023).

2.4. Soil microbial activity - carbon mineralization

The samples were moistened to 40 % WHCmax and stored in the fridge at 4 ± 2 °C until analysis (ISO, 2023). The maximum duration of the storage period at 4 °C was 8 weeks, so well within the maximum duration for storing microbial samples under refrigeration of three months according to International Organization for Standardization (ISO, 2007). For soil pH assessment, duplicate samples of six grams of soil from each soil column, at approximately 50 % WHC, were mixed with 24 mL of 0.01 M $CaCl_2$ and shaken at 200 rpm for two hours. After allowing samples to settle overnight, pH was measured using a pH meter (WTW pH 7710).

A subsample of soils was analyzed for microbial activity i.e., C mineralization: four samples from each control (0 %), 0.05 % and 0.8 % of PBAT-BD-MPs treatments were selected and analyzed in 3–4 sub-replicates. Microbial activity was characterized by basal respiration (BR) and substrate-induced respiration (SIR) according to ISO (2002) and ISO (1997), respectively. The soil samples were preincubated for seven days in the dark at 21 ± 2 °C to stabilize the soil environment (Hund-Rinke and Simon, 2008). The equivalents of 10 g dry soil weight (gdw) were placed in the infusion bottles (150 mL) for BR measurement. The soil was moistened to 60 % WHC and kept in the closed jar for two days to stabilize. After two days, the infusion bottles were aerated, hermetically sealed, and kept in the dark at 21 ± 2 °C for 24 h. The amount of carbon dioxide (CO_2) was measured on an Aquilent GC 6850 (manual injection, column GasPRO, mobile phase He, detector TCD, SW Clarity). Each sub-replicate was measured two times. The data were expressed as μg CO_2-C gdw^{-1} h^{-1}. The measurement of SIR was conducted similarly to BR, only 5 mg glucose per gdw^{-1} was added to each aerated infusion bottle at the beginning of the measurement. The bottles were then hermetically sealed, and the CO_2 was measured after 0, 3, and 6 h. SIR was calculated using the linear regression and expressed as μg CO_2-C gdw^{-1} h^{-1}.

2.5. Statistical analysis

All statistical analyses and visualizations for microbial communities were carried out in OriginPro software and R software, ver. 4.2.3 (https://www.r-pr oject.org). Significant differences in alpha diversity indices (Chao1, Invsimpson. Shannon, and Richness) and relative abundances (%) of top ten (bacteria) and nine (fungi) phyla, respectively, were assessed by ANOVA with Tukey and Bonferroni's multiple range tests at $p < 0.05$. Beta diversity was visualized using Bray-Curtis distances, and the significance was calculated through non-parametric multivariate analysis of variance test (ADONIS2) and permutational multivariate analysis of variance (PERMANOVA) in the VEGAN package of R using 9999 permutations. Non-metric multidimensional scaling

(NMDS, function metaMDS) ordination plots were used to visualize the microbial community profiles based on Bray-Curtis distances. Significant differences in the Bray-Curtis distances of each treatment were assessed by ANOVA Bonferroni's multiple range tests at $p < 0.05$.

Statistical significance of differences in microbial activity between the control (0 %), low-medium 0.05 %, and high concentration (0.8 %) of PBAT-BD-MP treatments, and soil pH between the control and MP treatments were tested by analysis of variance (ANOVA) followed by the Dunnett test at $p < 0.05$ (control (0 %) as the reference level). All analyses were performed in Statistica for Windows version 9.1 (StatSoft, Inc. 2010).

3. Results

3.1. Effects of microplastics on soil microbial communities

The rarefaction curves of samples reached saturation levels in each treatment (Fig. S1), indicating that the sequencing depths were sufficient to cover the bacterial and fungal diversity although the number of ASVs in our study was relatively low. The alpha diversity indices (Chao1, Invsimpson, Shannon, and Richness) of both bacterial and fungal communities are shown in Fig. 1. The alpha diversity indices of the bacterial community showed an increasing trend with increasing PBAT-BD-MP concentration, and the Chao1 index was significantly higher at the highest concentration (0.8 %) as compared to the control group (Fig. 1A-D). A similar phenomenon was observed in the fungal community, and we found significant changes in the Invsimpson index even at the lowest concentration (0.025 %) (Fig. 1E-H).

The dominant bacterial phyla in the control group were *Proteobacteria* (31.8 %), *Firmicutes* (27.0 %), *Actinobacteriota* (14.8 %), *Verrucomicrobia* (11.8 %), *Acidobacteriota* (7.3 %), *Bacteroidota* (3.4 %), *Planctomycetes* (2.5 %), and *Choroflexi* (1.3 %) (Fig. 2A). The relative abundances of most phyla showed an increasing trend with the increase of PBAT-BD-MP concentration, and *Planctomycetes* showed a significant increase at the highest concentration (0.8 %). However, the relative abundance of *Firmicutes* was significantly reduced across all MP concentrations (0.025 to 0.8 %).

The dominant fungal phyla in the control group were *Ascomycota* (51.3 %), *Basidiomycota* (29.0 %), *Mortierellomycota* (8.3 %), Chytridiomycota (6.2 %), *Rozellomycota* (2.0 %), *Glomeromycota* (1.6 %), and *Mucoromycota* (1.5 %) (Fig. 2B). A similar phenomenon to the bacterial community was observed in the fungal community, and the

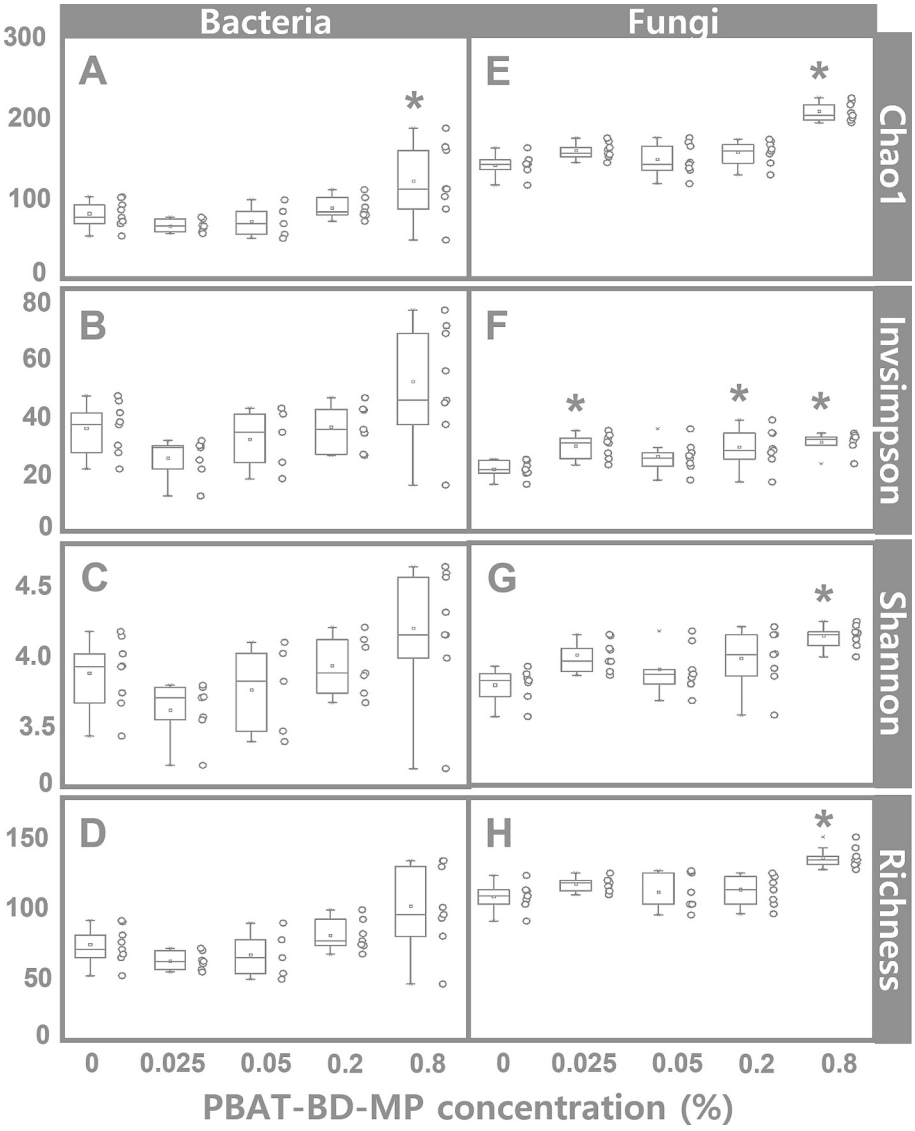

Fig. 1. Effects of polybutylene adipate terephthalate microplastics (PBAT-BD-MP) on alpha diversity indices (Chao1, Invsimpson, Shannon, and Richness) of (A-D) bacterial and (E-H) fungal communities in each PBAT-BD-MP concentration treatment (control (0 %) without any MP, 0.025, 0.05, 0.2, and 0.8 %). The asterisks (*) indicate significant differences compared to the control group ($p < 0.05$).

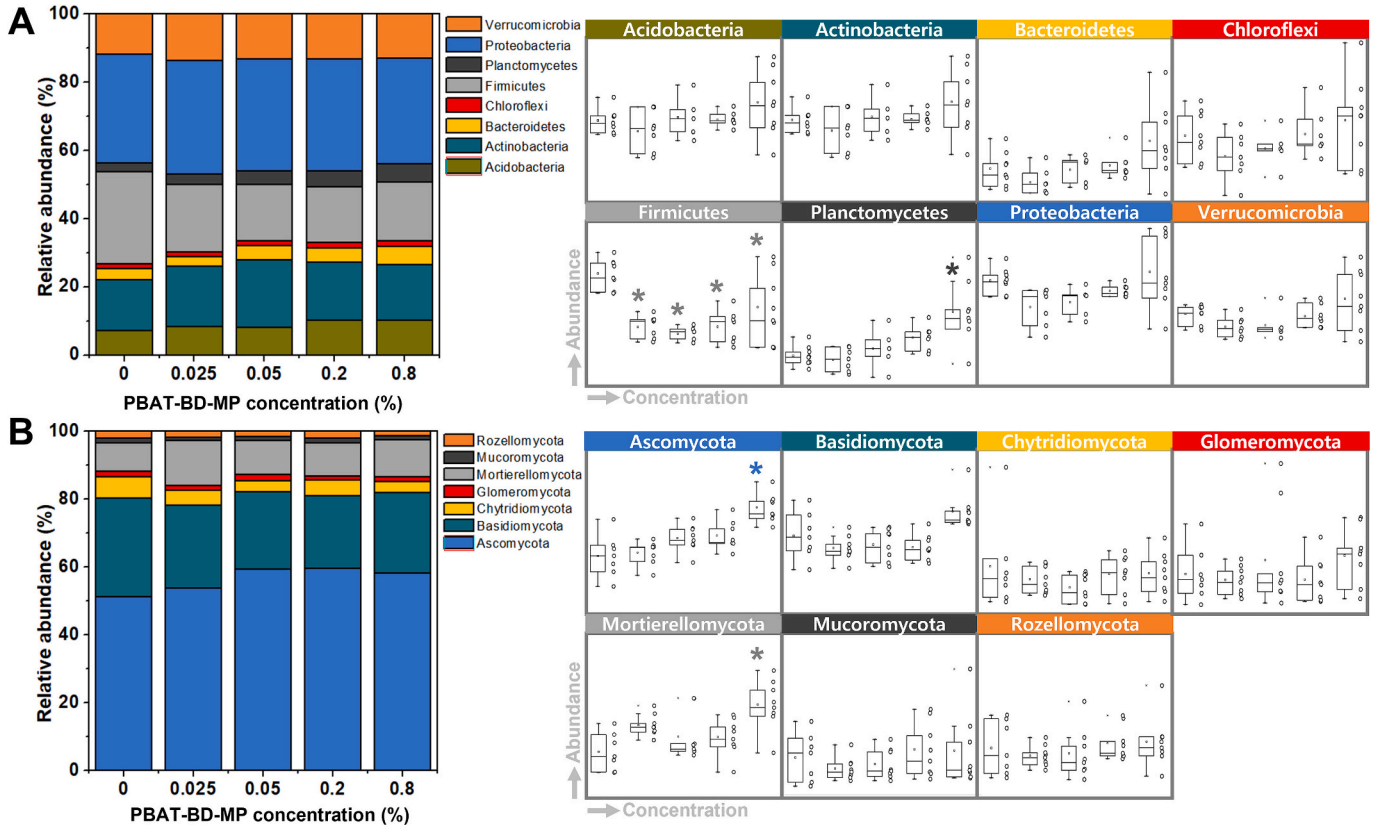

Fig. 2. Relative abundance of (A) bacterial and (B) fungal phyla in each PBAT-BD-MP concentration treatment (0, 0.025, 0.05, 0.2, and 0.8 %). The abundance of each phylum was shown in the right panel, and the asterisks (*) indicate significant differences compared to the control group ($p < 0.05$).

relative abundance of *Ascomycota* and *Mortierellomycota* significantly increased at the highest PBAT-BD-MP concentration, as compared to the control group.

NMDS ordination based on Bray-Curtis distances showed that bacterial and fungal communities were distinct between the MP concentrations (0 to 0.8 %) at the ASV level (Fig. 3A and B). Each data point represents an individual sample of each MP concentration or control. Clustering by PBAT-BD-MP concentration revealed distinct bacterial ($R^2 = 0.149$; $p = 0.004$) and fungal ($R^2 = 0.164$; $p = 0.002$) communities. The Bray-Curtis distance estimates of bacterial and fungal communities were significantly different in PBAT-BD-MP treatments compared to the control (Fig. 3C and D).

3.2. Effects of microplastics on microbial activity and carbon mineralization and pH

C mineralization measured as BR did not significantly differ between the control (0 %) and the low-medium concentration of 0.05 % of PBAT-BD-MPs ($p > 0.05$, Table 1). A significant increase compared to the control was found at the high (0.8 %) PBAT-BD-MP concentration ($p < 0.05$). SIR showed a significant decrease compared to the control at 0.05 % PBAT-BD-MPs ($p < 0.05$), and a significant increase at the 0.8 % PBAT-BD-MP concentration ($p < 0.05$). The homogeneity of microbial activity (BR, SIR) in the replicates was verified because the sub-replicates were sampled randomly. The coefficients of variance among sub-replicates were 2.6 % to 17.2 %, except for SIR in one replicate of the control soil, where the coefficient of variance was 20.9 %.

The control soil pH was slightly but statistically significantly different from all other PBAT-BD-MP concentrations ($p < 0.01$): soil pH was 0.6 units higher in the high MP (0.8 %) treatment when compared to the control group (Table 1).

4. Discussion

4.1. Microbial diversity is affected by the polybutylene adipate terephthalate microplastics in a dose-dependent manner

Supporting our hypothesis, we observed an increase of alpha diversity of both bacterial and fungal communities depending on PBAT-BD-MP concentrations. Biodegradable plastics have been considered as a replacement for other conventional plastic materials since they are expected to be naturally mineralized (Lambert and Wagner, 2017). However, they can be more vulnerable to degradation forces, probably leading to the production and accumulation of smaller-sized MPs with repeated application and more severe environmental impacts (Fojt et al., 2020; Qin et al., 2021).

The relative abundance of the bacterial community depended on BD-MP concentrations. *Planctomycetes*, which play a major part in the nitrogen (N) cycle (Fuerst and Sagulenko, 2011), showed higher abundance at the highest PBAT-BD-MP concentration compared to the control. *Firmicutes* play a role in carbohydrate metabolism and plant growth promotion (Hashmi et al., 2020), and their abundance was significantly reduced in all PBAT-BD-MP treatments. BD-MPs and their degradation intermediates can be utilized as a C source by heterotrophic microorganisms (Hu et al., 2022; Zhou et al., 2021), thus the related microbial communities such as *Proteobacteria* and *Actinobacteria* have been described to be potentially affected by BD-MPs (Li et al., 2022; Shi et al., 2022; Sun et al., 2022b). Although our results partly confirm that BD-MPs can provide an unsuitable environment for the growth of *Firmicutes* (Li et al., 2022), the major phyla in our study (*Firmicutes* and *Planctomycetes*) have been less highlighted in previous studies. According to previous studies, BD-MPs decrease the abundance of *Actinobacteria* (Shi et al., 2022), *Armatimonadetes*, *Dependentiae*, and *Verrucomicrobia* (Feng et al., 2022), but also increase those of

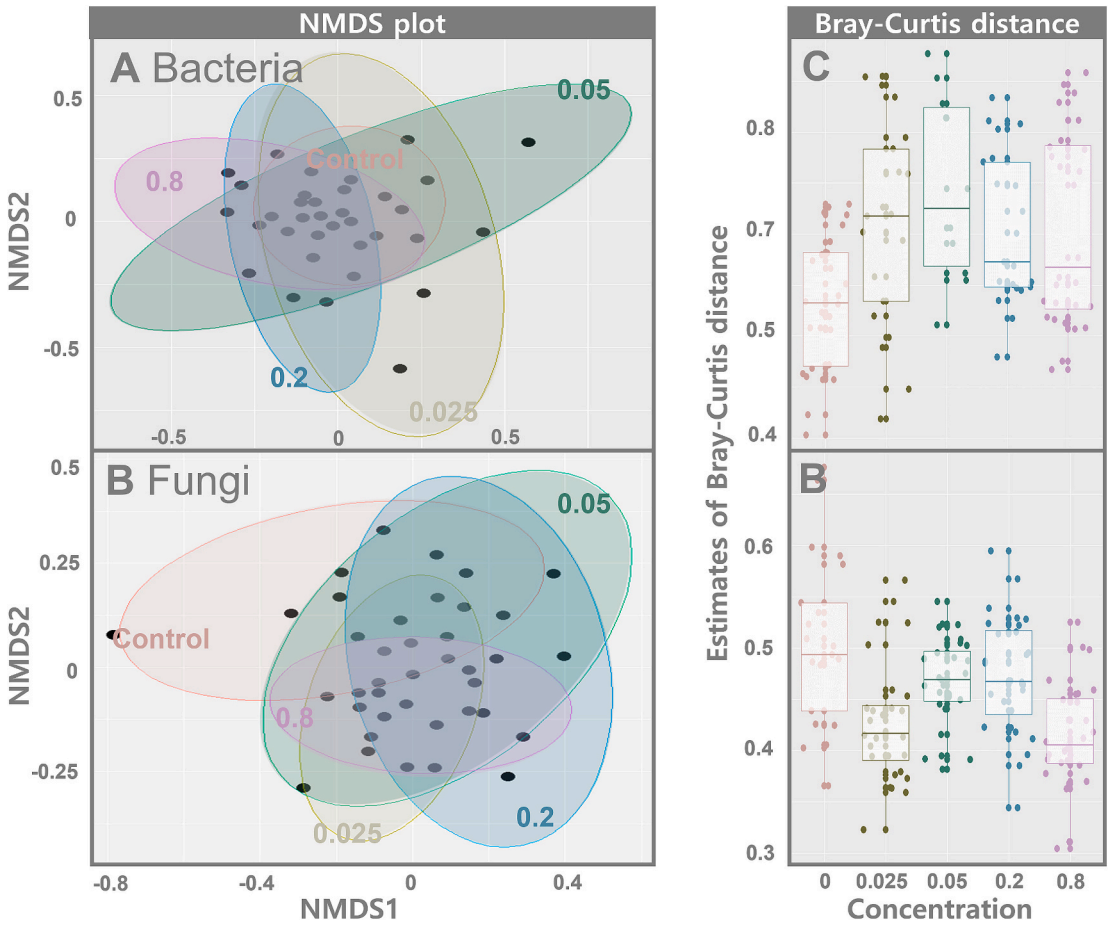

Fig. 3. Non-metric multidimensional scaling (NMDS) visualizations and Bray–Curtis metric distances in each PBAT-BD-MP concentration treatment (0, 0.025, 0.05, 0.2, and 0.8 %): (A and C) bacterial and (B and D) fungal communities the asterisks (*) indicate significant differences compared to the control group ($p < 0.05$).

Table 1
Soil pH and microbial activity measured in samples spiked with polybutylene adipate terephthalate microplastics (PBAT-BD-MPs) in mesocosms. Results are presented as averages ± standard deviation. Asterisks (*) show significant differences from the control samples (Dunnett's test).

Method		PBAT-BD-MPs treatment, added in per gram of Lufa 2.2 soil				
		Control (0 %)	0.025 %	0.050 %	0.200 %	0.800 %
pH ($n = 8$)	1:4 soil:0.01 M CaCl	5.09 ± 0.025	5.02 ± 0.032**	5.02 ± 0.035**	5.61 ± 0.074***	5.68 ± 0.055***
C-mineralization ($n = 4$)	Basal respiration BR (μg CO_2-C gdw^{-1} h^{-1})	0.497 ± 0.065	NA	0.489 ± 0.026	NA	0.749 ± 0.095*
	Substrate-induced respiration SIR (μg CO_2-C gdw^{-1} h^{-1})	7.78 ± 0.856	NA	6.54 ± 0.279*	NA	12.5 ± 1.31*

Asterisks (*) show significant differences from the control samples (Dunnett test, *P < 0.05, **P < 0.01, ***P < 0.001).
NA not assessed.

Actinobacteria (Sun et al., 2022b), *Bdellovibrionota*, *Gemmatimonadota*, *Myxococcota*, *Patescibacteria* (Li et al., 2022), *Proteobacteria* (Li et al., 2022; Sun et al., 2022b), and *Nitrospirae* (Hu et al., 2022). PBAT-BD-MPs can alter soil bacteria community more manifoldly compared to conventional plastic types such as polyethylene (Shi et al., 2022), low-density polyethylene, polystyrene (Sun et al., 2022b), polyvinyl chloride, and polyethylene terephthalate (Sun et al., 2022a). In our study, the PBAT-BD-MP had a diverging effect on the bacterial communities. The significant changes (positive or negative) in bacteria community diversity can occur at a PBAT-BD-MP concentration of 0.2 % (Feng et al., 2022; Li et al., 2022) or ≥1 % (Hu et al., 2022; Shi et al., 2022; Wang et al., 2022).

The fungal community displayed significantly higher diversity even at the lowest PBAT-BD-MP concentration of 0.025 % (250 mg kg^{-1}) in comparison to the control. The increase in *Ascomycota* and *Mortierellomycota* was dose dependent, and these phyla were abundant especially in the high concentration treatments. Due to the limited number of studies (approx. 4 % of total reported articles; Table S1), it is still unclear whether the PBAT-BD-MPs positively or negatively influence soil fungal diversity. Here, the high PBAT-BD-MP concentration had a homogenizing effect on fungal communities. Qi et al. (2022) observed no significant effects of starch-based BD-MPs on the fungal community at 1 % of particle concentration. Given the fact that these previous studies have been conducted as small-scale laboratory and pot plant experiments, our results imply that the true extent of BD-MP effects might be over- or underestimated, and our mesocosm approach with more realistic growing conditions showed that microbial communities could exhibit more sensitive responses to MP exposure.

4.2. Polybutylene adipate terephthalate microplastics accelerates carbon mineralization

Our study showed the soil C mineralization, measured as CO_2 production, increased with increasing PBAT-BD-MP concentration. This has been observed before using similar PBAT concentrations compared to ours (Rauscher et al., 2023). The authors concluded that the DB plastic was consumed by bacteria known to degrade polyesters and other DB-MPs, such as members of the *Caulobacteraceae* and *Comamonadaceae* which increased in their study due to PBAT addition. Our study could not verify the increase of these two families. The PBAT-BD-MPs were most likely C sources themselves and/or released additives and small molecular compounds, thus contributing to the district changes of the microbial community (especially bacteria) (Li et al., 2023). Using isotope-sensitive analytical equipment, Zumstein et al. (2018), found that the ^{13}C from PBAT was not only converted into CO_2 as a result of microbial respiration but was also incorporated into microbial biomass (hyphae and single cells) at the surface of the polymer. But also, two other mechanisms can explain or partly explain the increase in CO_2 production of our experiment. The evolved CO_2 can also stem from the soil organic matter since PBAT is known to induce a priming effect (Huo et al., 2024) and this can serve as a second explanation. If so, then the use of PBAT could contribute to an increased CO_2 flux from soil into the atmosphere which is an unwanted effect of greenhouse gas production and mitigating global warming (Inubushi et al., 2022). A third mechanism increasing CO_2 production is the 0.6 unit rise in soil pH, which was observed between the control and the highest PBAT-BD-MP concentration. In acidic soils, like the Lufa soil used in the experiment, a treatment induced increase in soil pH can be accompanied by increased microbial growth rates (Jokinen et al., 2006) and activity such as CO_2 production (Perkiömäki and Fritze, 2002). Li et al. (2022) reported no change in pH during a four-month small scale incubation with similar concentrations of PBAT that we used in our mesocosm. Moreover, we are not aware of any other studies where PBAT addition to soil and its impact on the pH is reported. However, the effects on soil pH have been reported from multiple polymer types and MP shapes, but the survey did not include PBAT (Zhao et al., 2021). Soil pH increased due to MP fragments addition after the initial decrease in the first days of incubation (Zhao et al., 2021).

4.3. Effects of MPs should be further studied under realistic conditions

In this CLIMECS system mesocosm study, we found distinct results that allow assessing the effects of PBAT-BD-MPs at the microbial community levels. The influences of trophic interactions in soil microbial communities can emerge from the decomposition of organic matter by each trophic level organism. Primary resources (e.g., plant litter or chitin) can be different depending on the trophic interactions, and these changes affect many features of microbial community assembly (Gralka et al., 2020). In addition, several previous studies highlighted that the consumptive (or non-consumptive) effects on above- and under-ground systems directly or indirectly mediate soil microbial communities by regulating the C retention time or input of labile C (Lucas et al., 2020). In this study, we found that the phylum *Firmicutes*, which plays an important role in plant growth promotion (Hashmi et al., 2020), showed a lower abundance in the PBAT-BD-MP treatments than the control group, and these results correlate with the decreased chlorophyll concentrations, increased oxidative stress and the activated plant defense mechanisms of lettuce in the same mesocosm experiment (Adamczyk et al., 2024). Furthermore, the springtail species used are known to consume bacteria and fungi and thus may be affected by the microbial community changes. The springtails, however, seemed not affected by the PBAT-BD-MP levels tested (van Loon et al. unpublished). Efforts to understand the interactions with other soil biota (earthworms) or soil disease suppressiveness (*Fusarium culmorum*) have been pursued in previous studies (Lu et al., 2023; Qi et al., 2022). Earthworm inoculation can contribute to the diversity and stability of the soil bacterial community, and accelerate the aging of MPs (Lu et al., 2023). While the small-scale based experiments can improve the repeatability and reproducibility of the results in highly controlled conditions, the actual environment contains unpredictable factors such as high biodiversity, anthropogenic activities, and meteorological conditions than can be accounted for in a mesocosm. Several studies elucidate the alterations of MP effects on microbial communities in rhizosphere soil, cultivating with lettuce (*Lactuca sativa*) (Zeb et al., 2022), maize (*Zea mays*) (Lian et al., 2021; Fu et al., 2022), beans (Kim et al., 2023; Lian et al., 2022; Meng et al., 2023), pepper (*Capsicum annuum*) (Ran et al., 2023), reed (*Phragmites australis*) (Tian et al., 2023), rice (*Oryza sativa*) (Dong et al., 2021; Liu et al., 2021), and wheat (*Triticum aestivum*) (Guo et al., 2022). However, the results of these studies are still collectively unclear due to the limited data. For instance, some studies found that MPs show more distinct effects on microbial communities in the rhizosphere soils (in which beans are grown) compared to bulk soils (Kim et al., 2023; Lian et al., 2022; Meng et al., 2023), but these effects can vary depending on the MP characteristics or the crop species and cultivars (Lian et al., 2021, 2022). Considering the interactive effects of different biota, mesocosm and field scale experiments containing multiple factors and biota are needed to more deeply understand the effects of MP pollution on soil life.

5. Conclusion

We observed notable changes in bacterial and fungal community diversity and structure at lower starch polybutylene adipate terephthalate MP (PBAT-BD-MP) concentrations than previously reported. The results suggest that the PBAT-BD-MP-affected microbiome functions differently, reflected in increased soil CO_2 release with potential climate impacts. Whether this mesocosm result holds in field trials is a challenge for future research.

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CRediT authorship contribution statement

Shin Woong Kim: Writing – original draft, Methodology, Formal analysis, Data curation. **Klára Šmídová:** Writing – original draft, Methodology, Data curation. **Sam van Loon:** Writing – review & editing, Investigation, Conceptualization. **Cornelis A.M. van Gestel:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Matthias C. Rillig:** Writing – review & editing, Funding acquisition, Conceptualization. **Hannu Fritze:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Sannakajsa Velmala:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization.

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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

Data will be available upon publication; raw sequence data are deposited in the sequence read archive SRA of the NCBI database under PRJNA1150082, and other used data will become available via Zenodo https://doi.org/10.5281/zenodo.10715716.

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