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Effect of biodegradable films on microplastic distribution and microbial community composition in paddy soil

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KEYWORDS

Biodegradable plastic films, microplastics, paddy soil

HIGHLIGHTS

- Particle sizes, > 1.0, 1–0.25, 0.25–0.1 and 0.1–0.01 mm were recorded for soil with biodegradable plastic film (BF) mulch and without film mulch.
- BF only contributed to more microplastic (MP) abundance in the 0.25–0.1 mm size range relative to soil not mulched.
- Over 26 functional genes and 10 genera were identified in MP biodegradation.
- BF altered the composition and abundance of MP-degrading microbial communities in paddy soil.

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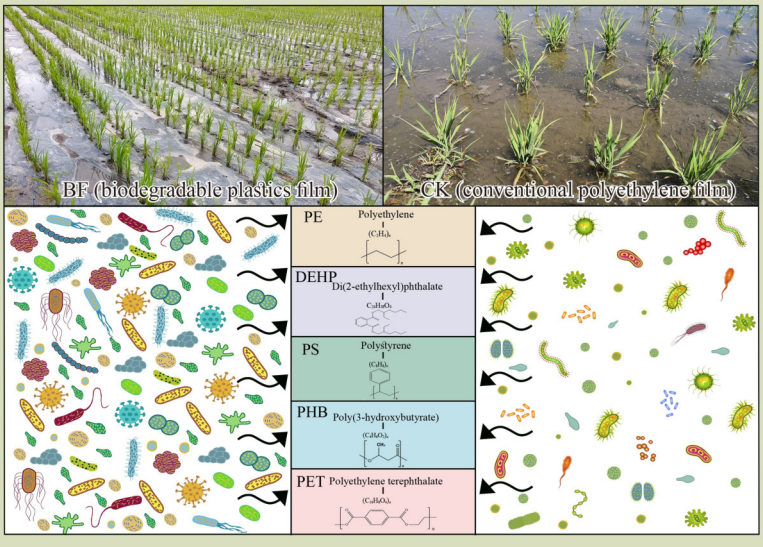
GRAPHICAL ABSTRACT



ABSTRACT

Biodegradable plastic film (BF) has been widely used in agriculture owing to concerns over microplastic (MP) contamination and its potential risks to

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agricultural sustainability. Elucidating the distribution of MP and the role of microbial communities in their biodegradation is crucial for evaluating the effectiveness of BF in paddy soil. In this study, soil samples were collected from typical paddies in southern China. The MP composition was analyzed by Fourier-transform infrared spectroscopy. Metagenomic sequencing was conducted to identify MP degradation genes and characterize microbial communities. The results revealed that BF-mulched soil had significantly higher MP abundance in the 0.25–0.1 mm size range than soil with a history of no film use as a comparator (CK) (P < 0.05). Similarly, BF had a significantly higher abundance of particular MP types than the CK (P < 0.05). Five main types of MP biodegradation pathways were identified in both BF and CK samples. Over 26 functional genes and 10 genera were associated with the biodegradation of the top five polymer types. However, only a subset of genes and genera significantly differed between BF and CK samples, particularly in the degradation of di(2-ethylhexyl) phthalate, polyethylene and other polymers (P < 0.05). At the functional level, similar genera contributed to MP degradation. However, the relative contributions of these genera varied depending on the polymer type. Overall, BF use led to more efficient MP degradation into simpler structures than in CK. Although the total MP content did not significantly differ between BF and CK samples, BF use had altered the composition and abundance of MP-degrading bacterial communities in the sampled paddy soil. This enhanced biodegradation efficiency under BF use further supports agricultural sustainability in paddy systems.

1 Introduction

Microplastics (MPs), being smaller than 5 mm, have emerged as significant environmental pollutants worldwide, particularly in agricultural soil[1]. The presence of MPs poses a threat to food chain safety[2], and the use of non-biodegradable plastics in agriculture has been identified as a major contributor to MP generation[3]. Studies have shown that MPs can alter the physical and chemical properties of soil, thereby disrupting soil microbial functions and compromising soil and plant health[4]. Therefore, biodegradable plastics have been considered a more sustainable alternative to non-biodegradable plastics. Research has indicated that biodegradable films degrade more readily than non-biodegradable films such as polyethylene or oxo-degradable modified polyethylene films[5]. However, biodegradable plastics can break down into microplastic particles, which are not fully degraded by microorganisms and may accumulate in soil, potentially affecting soil quality and plant health[6]. Biodegradable plastic films (BFs) are considered environmentally-friendly materials and can replace non-biodegradable plastic films without harming the environment. Although BFs may have potential adverse effects on agroecosystems (including crops, the microbiome and soil fauna[7]), they provide benefits similar to those of non-biodegradable plastic films, including soil warming, water conservation and increased yields. Additionally, these films are effectively degraded in soil[8], which reduces their contribution to MP pollution compared with non-biodegradable films. However, the degradation of biodegradable plastics can lead to changes in soil functional microorganisms or MP forms. Although numerous studies have investigated the hazards of MPs and their impacts on soil microorganisms[9], the effects of biodegradable plastics on MP-degrading microbes remain unclear across different soil types. In addition, the use of biodegradable films does not significantly increase MP accumulation in field soil compared with those without film mulching. Therefore, investigating the effect of biodegradable plastics on soil MP-degrading microorganisms is crucial for ensuring their sustainable use and promoting eco-friendly agricultural practices.

Different types of cultivated land affect the presence and degradation of MPs, with paddies being of particular concern owing to their close association with food security and agricultural development. Rice, one of the most significant cereal crops, has made paddies a focal point of global attention[10]. From 1970 to 2021, Asia accounted for over 90% of global rice production, with China ranking among the top producers[11]. This high productivity has been supported by various agronomic and engineering practices, including film mulching, fertilization, irrigation and ditch construction. Of these, film mulching has been widely adopted in paddies to conserve water and increase yields[12]. A meta-analysis conducted in China revealed that plastic film mulching increased rice yield by 12%, water use efficiency by 36% and nitrogen partial factor productivity by 12%[13]. Additionally, film mulching alters the emission patterns of greenhouse gases[14]. Although extensive research has highlighted the benefits of plastic film mulching in paddy rice production, the widespread presence of MPs in these environments poses significant challenges[15]. MPs can disrupt key functional microbial communities, such as denitrifiers and ammonia-oxidizing bacteria, potentially leading to nitrogen loss in paddies[16]. Also, a study has confirmed that MPs from paddies can be transported to nearby water bodies, contributing to marine plastic pollution[17]. Overall, the presence of MPs in paddy soils poses risks to food security and ecological health. Therefore, investigating the distribution of MPs and MP-degrading microorganisms in paddies is crucial.

Plastic film mulches in the soil undergo weathering owing to UV light exposure, soil microorganisms and mechanical fragmentation. Over time, these films become brittle and eventually break into smaller fragments[18]. The MP distribution is mainly determined by particle size and morphological features[19]. Environmental conditions significantly influence the weathering process of MPs. In addition, studies have confirmed that terrestrial and aquatic environments lead to different weathering processes[20]. Many large-sized MPs originate from residual plastic films owing to the short duration of their application and subsequent surface weathering. Various factors contribute to MP weathering. For example, solar radiation accelerates MP formation and high levels of UV radiation promote MP decomposition. Notably, regions at higher altitudes may promote the accumulation of smaller-sized MPs[21]. Higher temperatures accelerate the weathering rate of MPs[22]. Additional factors contributing to the weathering process include the physicochemical properties of the plastics[23], the crystallinity of oxidized plastic materials[24] and oxidation processes induced by light, chemicals or heat[25]. In aquatic environments, MPs undergo photoaging and may further degrade into nanoplastics under prolonged light exposure[26]. In paddy soils, which are characterized by alternating dry and wet conditions, MPs undergo different weathering processes. In addition, regional differences in environmental conditions can lead to varying patterns of MP weathering. Therefore, elucidating the particle size and morphological characteristics of MPs in paddy soils is crucial for assessing their weathering behavior and environmental impact. However, the specific functional microorganisms involved in MP degradation remain unknown. Further research is needed to explore whether the diversity and abundance of these functional microbes vary across different types of MPs, particularly in paddy soils with alternating dry and wet conditions.

Microbial degradation is the main mechanism through which MPs undergo biological weathering. Several strains of microorganisms capable of degrading plastics have been identified[27]. However, biodegradable mulch films consist of polymers and other components, and their degradation in agricultural environments is influenced by the resident microbial communities under fluctuating environmental conditions. These microbes may not contain the necessary polymer-degrading enzymes[28]. The efficiency of MP degradation depends on both the genera and the diversity of the microbial community[29]. For example, bacterial consortia, mainly from the phyla Actinobacteria and Firmicutes (isolated from the gut of earthworms), can degrade low-density polyethylene (LDPE) MPs[30]. Although bacterial consortia may provide promising solutions to enhance the biodegradation efficiency of MPs[29], the functioning of individual bacteria within these consortia remain unclear. Symbiotic and synergistic interactions among microorganisms are crucial for MP biodegradation[31]. Various genera involved in MP degradation include *Stenotrophomonas*, *Pseudomonas* and *Chryseobacterium*, which contribute to polyethylene (PE) degradation. Additionally, the larvae of the, so called, *superworms* involved in polystyrene (PS) degradation include *Zophobas atratus*, *Plesiophthalmus davidis* and *Tenebrio molitor*. Different types of MPs may be degraded by distinct or overlapping genera. We hypothesize that the specific microbial communities involved in paddy soil degradation are influenced by local environmental conditions. Identifying these genera could provide valuable insights into the MP degradation process in paddy soils.

This study aimed to evaluate the particle sizes, morphological characteristics and abundance of MPs under BF and standard, non-biodegradable PE film use in paddy soils. In addition, the study assessed the characteristics of MP biodegradation, quantified the abundance and contribution of MP-degrading microorganisms, and examined the correlation between bacterial taxa and specific MP types.

2 Materials and methods

2.1 Sample collection

Soil samples were collected from paddies in Suzhou, Jiangsu Province, China, at the Kunshan Fengchan Fang Agricultural Professional Cooperative (31°16′ N, 120°83′ E). Between 24 and 25 October 2024, two sampling areas were selected and categorized as BF and comparator (CK) based on the film mulching method, biodegradable films has been applied for more than three years, and the latter had a history of no plastic film mulching. In each sampling area, surface soil from the top 0–15 cm was collected using a stainless steel sampler. One field plot was selected, which comprised three sampling areas. Each area contained five sampling points, which were combined to form a single sample. Three samples were collected for each film-use context (BF and CK) (Fig. 1). Each sample was then split in two subsamples: one for analyzing soil physical and chemical properties and MP characteristics, and the other for microbiological analysis.

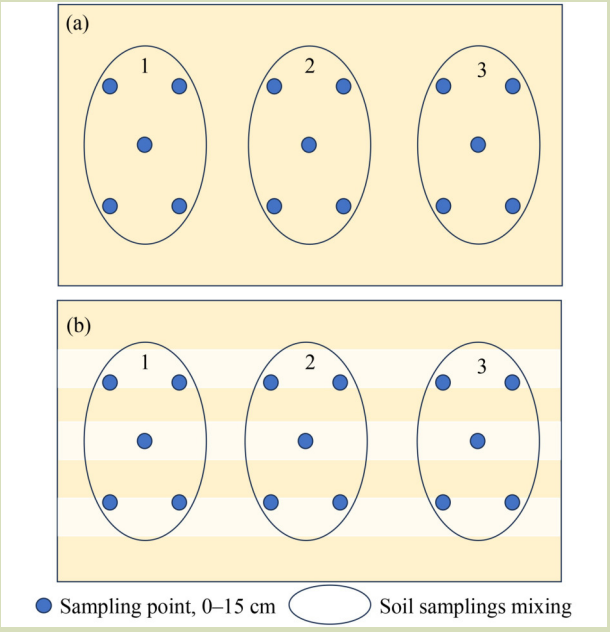

Fig. 1 Soil sample collection method. (a) No film mulch (CK); (b) biodegradable plastics film mulch.

2.2 Analytical methods

2.2.1 Soil physical and chemical property analysis

Key soil parameters were analyzed by standard techniques. pH was determined using a glass electrode meter (Mettler Toledo, Columbus, OH, USA) in 1:2.5 soil:water suspensions. Soil organic carbon content was quantified by dichromate oxidation, while total nitrogen was measured through the Kjeldahl method. Potassium and phosphorus concentrations were determined by flame photometry and molybdenum-antimony colorimetry, respectively.

2.2.2 MP detection

MPs were isolated from soil using optimized protocols[16,32]. The soil samples were first fractionated through stainless steel sieves with mesh sizes of 1, 0.25 and 0.1 mm, with particles smaller than the detection limit (0.01 mm) excluded from the analysis. For density separation, 10 g of soil samples were mixed with 100 mL of saturated NaCl solution (1.2 g·mL^{-1}), magnetically stirred for 10 min, and allowed to settle overnight. This flotation process was repeated three times to enhance separation. High-density MPs were further isolated using NaI solution (1.7 g·mL^{-1}).

The collected supernatant was vacuum-filtered through 0.45 μm membranes, followed by organic matter digestion using 30% H_2O_2 for 72 h at room temperature. After secondary filtration, MPs were examined under a microscope (Olympus CX31, 40× magnification, Japan) and characterized by Attenuated total reflectance-Fourier-transform infrared spectroscopy (ATR-FTIR; Nicolet iS5, Thermo Fisher, Waltham, MA, USA) and μ-FTIR (Spotlight 400, PerkinElmer, Waltham, MA, USA)[17,33]. Strict anti-contamination measures were implemented, including the exclusive use of non-plastic equipment, precleaning with distilled water and covering all samples with aluminum foil during processing.

2.3 Metagenomic analysis

Genomic DNA was extracted from soil samples using the Mag-

Bind Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. DNA concentration and purity were assessed using a fluorometer (TBS-380, Molecular Devices, San Jose, CA, USA) and a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA), respectively. DNA integrity was confirmed by 1% agarose gel electrophoresis.

For library preparation, purified DNA was fragmented to ~400 bp using a Covaris M220-focused ultrasonicator (Covaris, Inc. Woburn, Massachusetts, USA). Paired-end libraries were constructed using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA) and Illumina-compatible adapters were ligated by blunt-end ligation. Final library quality control involved analyzing the fragment size distribution and quantification.

Sequencing was conducted on an Illumina NovaSeq 6000 platform using NovaSeq 6000 S4 Reagent Kits (300 cycles) according to the manufacturer's protocol (Illumina Inc., San Diego, CA, USA).

2.4 Sequence processing and genome assembly

Raw sequencing data were processed using the standardized bioinformatics pipeline on the Majorbio Cloud Platform. Initial quality control involved adapter trimming and removal of low-quality reads using fastp version 0.20.0[34]. The stringent thresholds applied included a minimum read length of 50 bp, a quality score cutoff of 20, and the exclusion of reads containing undetermined bases. This preprocessing step ensured that only high-quality sequences were retained for downstream analysis.

2.5 Functional genomic characterization

Gene prediction was conducted using a dual-algorithm approach with Prodigal[35] and MetaGene[36]. The resulting ORFs (open reading frames) were translated into amino acid sequences according to NCBI translation standards (Genetic Codes Database ID: SG1).

To reduce redundancy, a comprehensive gene catalog was constructed using CD-HIT version 4.6.1[37] with clustering parameters set to 90% sequence identity across 90% of the shorter sequence. To quantify gene abundance, the quality-filtered reads were mapped to the non-redundant gene catalog using SOAPaligner version 2.21[38] with a 95% identity threshold.

For functional annotation, we implemented a multi-tiered approach: (1) taxonomic classification against the NCBI NR database, (2) functional categorization using the Cluster of Orthologous Groups by eggNOG, and (3) metabolic pathway mapping by KEGG (Kyoto Encyclopedia of Genes and Genomes). All annotations were performed using Diamond version 0.8.35[39] with stringent alignment parameters (e-value $\leqslant 1 \times 10^{-5}$). This comprehensive strategy enabled the simultaneous assessment of microbial community composition and metabolic potential.

2.6 Statistical analysis

Data analysis was conducted using Microsoft Excel 2021. Graphs were generated using Origin (version 9.0; OriginLab Corporation, MA, USA) and/or R version 4.4.2. Statistical differences between BF and CK samples were assessed in R with a significance threshold of $P < 0.05$.

3 Results

3.1 MP characteristics

3.1.1 Types of MPs

Two particle size categories (> 1 mm and < 1 mm) were used to analyze the types of MPs in BF and CK (Fig. 2). In the size category larger than 1 mm, CK contained poly (methylphenylsiloxane) and polyester (terephthalate and isophthalate), while BFs contained poly(ethylene:vinyl chloride) and poly(methylphenylsiloxane). Notable differences in MP types were observed between BF and CK, particularly in the size range smaller than 1 mm. In BF, the MP types larger than 1 mm included poly(ethylene:vinyl chloride), low-density PE, PE, and poly(methylphenylsiloxane). The MP types smaller than 1 mm included low-density PE, atactic polypropylene, poly(ethylene:propylene:diene), poly(styrene:4-vinylpyridine) and polyester (terephthalate and isophthalate).

3.1.2 MP abundance

Regarding the particle sizes of MPs, the abundance of MPs with particle sizes of 1–0.25 mm was significantly higher than that of

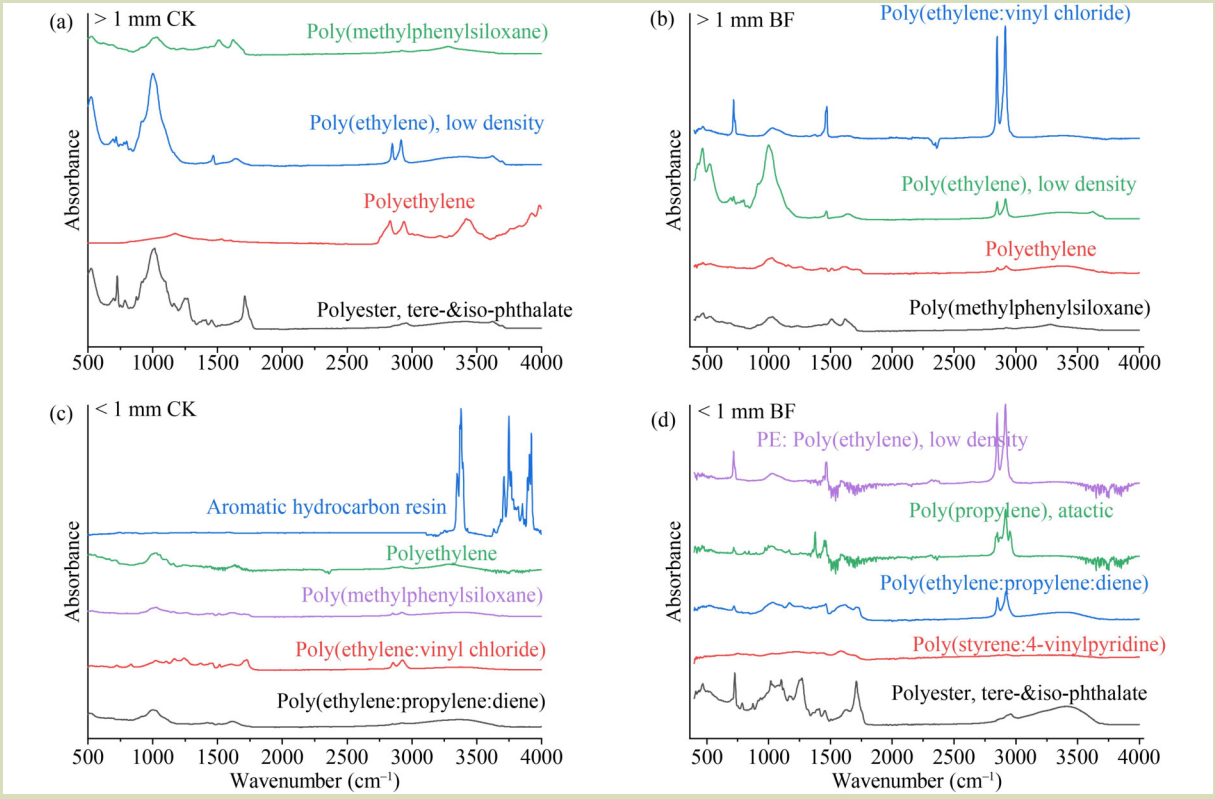

Fig. 2 Types of microplastic (MP) in soil based on FTIR. MP types larger than 1 mm in CK (a) and BF (b) samples, respectively. MP types smaller than 1 mm in CK (c) and BF (d) samples, respectively.

other sizes ($P < 0.05$). Particularly, CK and BF samples contained 1173 ± 350 and 1747 ± 348 particles kg^{-1}, respectively. Significant differences were observed between particles larger than 1 mm (0.25–0.1 mm) and those smaller than 0.1 mm in BF. However, no significant difference in particle size was observed in the CK group. BF samples had a significantly higher abundance of MPs in the 0.25–0.1 mm size range than CK ($P < 0.05$), with 800 ± 193 and 260 ± 158.7 particles kg^{-1}, respectively (Fig. 3(a)).

MPs were categorized into three types based on their morphological characteristics. BF samples had a significantly higher abundance of MPs (particularly film MPs) than CK ($P < 0.05$), with 1587 ± 242 and 747 ± 264 particles kg^{-1}, respectively. In BF, the abundances of film and fiber MPs were higher than those of fragment MPs. Conversely, in CK, the abundance of fiber MPs exceeded both film and fragment MPs. Additionally, CK had a significantly higher abundance of film MPs compared with fragment MPs. Both BF and CK and had low abundances of fragment MPs, 413 ± 145 and 207 ± 110 particles kg^{-1}, respectively (Fig. 3(b)).

3.2 MP biodegrading diversity

The α and β microbial diversity were analyzed using the Shannon index and principal coordinate analysis (PCoA) (Fig. 4). For both contexts, the type of plastic film did not affect the Shannon index in paddy soil, with no significant difference observed between BF and CK (Fig. 4(a)). However, PCoA analysis revealed a significant difference in the structure of MP-degrading microorganisms between BF and CK (Fig. 4(b)).

3.3 MP biodegrading abundance and contribution

The analysis of the selected gene set for MP biodegradation markers from the metagenomic data of this study was performed using a functional composition stacking chart (Fig. 5). The top 11 MP biodegrading markers in the BF

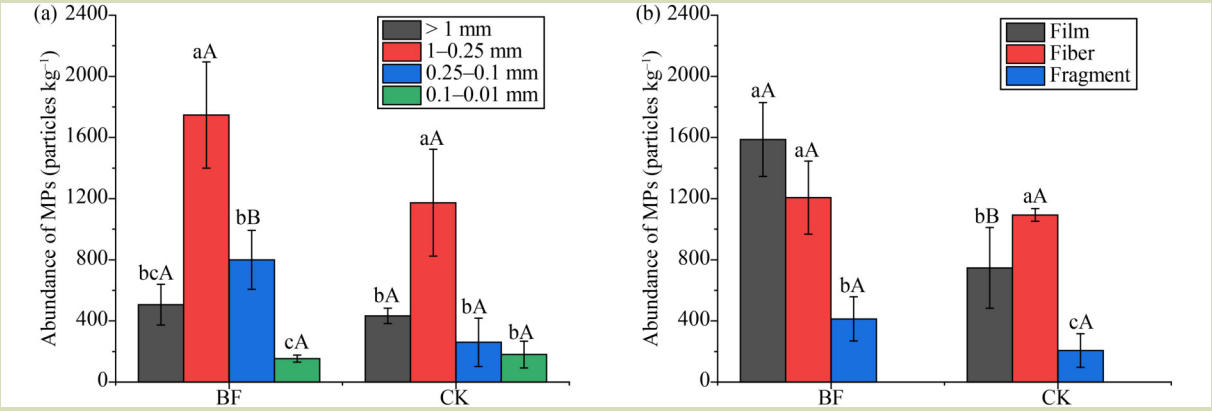

Fig. 3 Microplastic (MP) abundance in soil in BF and CK contexts. (a) Abundance of MPs with different particle sizes; (b) abundance of MPs with different shapes. Lowercase letters (a, b, c) indicate significant differences among different types of MPs within the same samples. Uppercase letters (A, B) denote significant differences for the same type of MPs between different samples ($P < 0.05$, Tukey's HSD test).

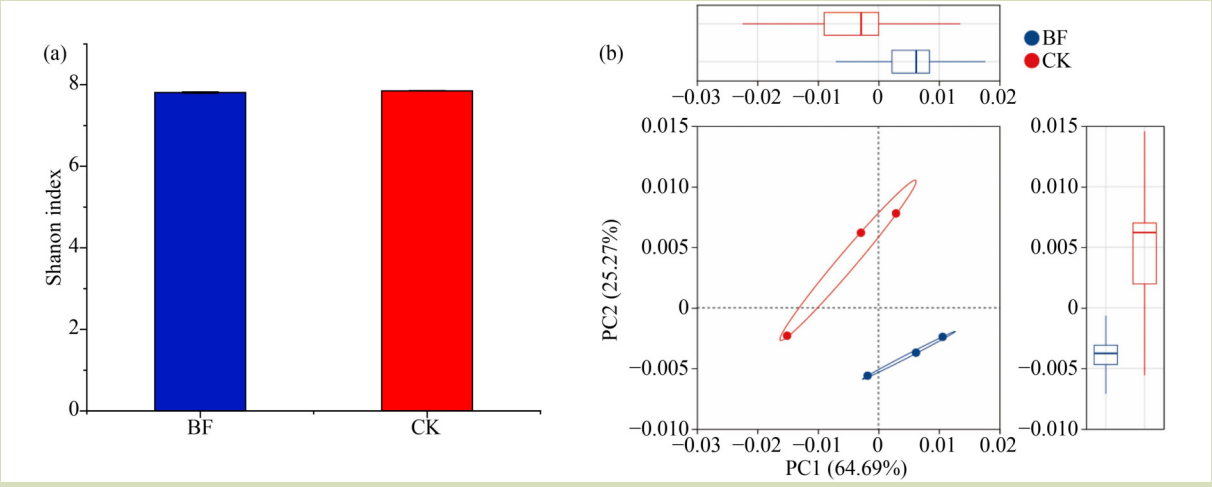

Fig. 4 Microplastic (MP) biodegradation diversity and structure. (a) Shannon index of MP biodegradation; (b) PCoA of MP biodegradation based on Bray-Curtis distances.

samples were similar to those of CK. The marker abundance in these samples (quantified as reads/transcript kb/million mapped reads, RPKM) exceeded 400. The top five functional groups in both BF and CK were PS-biodegradation, DEHP-biodegradation (di(2-ethylhexyl) phthalate), PE-biodegradation, PE-PS-PHB-biodegradation (poly(3-hydroxybutyrate)) and PET-biodegradation (polyethylene terephthalate). The abundance of these functional groups was 6111, 5492, 2505, 2136, and 1910 in BF, and 6290, 5173, 2654, 2180, and 1933 in CK, respectively.

According to the selected gene set of MP biodegradation markers from the metagenomic data, a functional composition heatmap analysis was performed (Fig. 5) at the gene level. Within the same MP biodegradation function, not all genes had high abundance, particularly in DEHP-biodegradation, PBAT-biodegradation, PE-PS-PHB-biodegradation, PET-biodegradation and PS-biodegradation, in both BF and CK samples. In DEHP-biodegradation, the gene abundance of *pcaD*, *paaH*, *pcaC*, *bcrB*, *bcrC*, *ligI* and *pobA* was higher in BF than in CK. However, CK had a greater abundance of *dmpD* and *dmpB*.

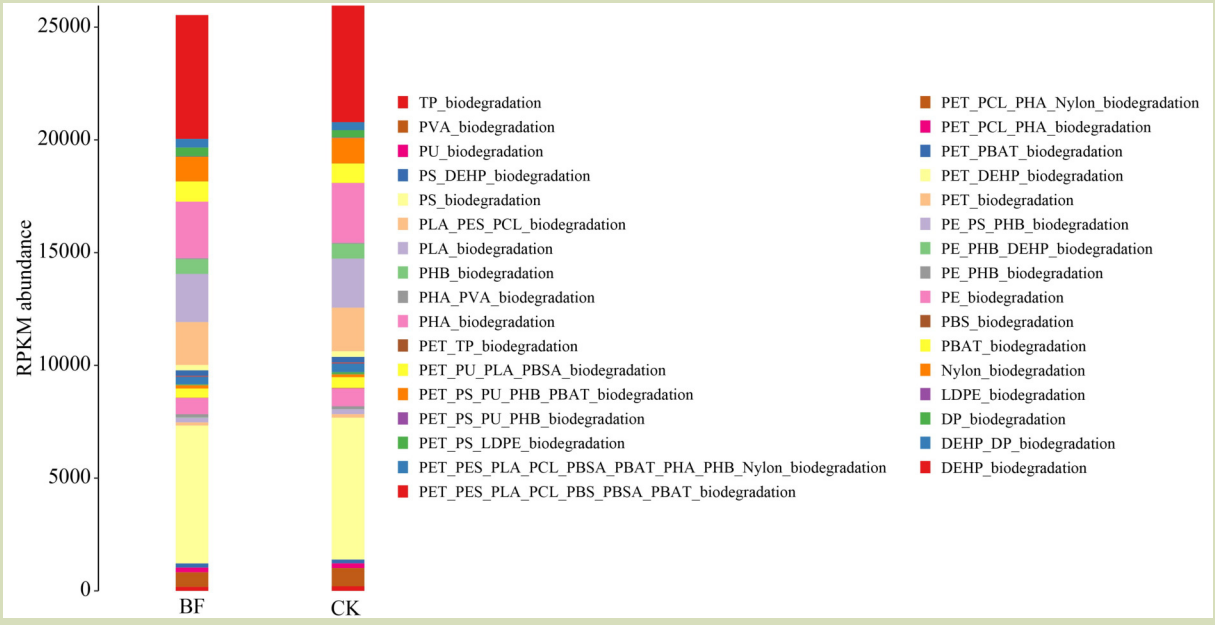

Fig. 5 Abundance (RPKM) of various microplastic biodegradation markers: PET, polyethylene terephthalate; PE, polyethylene; PES, polyethylene succinate; PS, polystyrene; PHA poly(3-hydroxyalkanoates); PHB, poly(3-hydroxybutyrate); PHO, poly(*ß*-hydroxyoctanoate), PLA, polylactic acid; PU, polyurethane; PVA, polyvinyl alcohol; TP, terephthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; LDPE, low-density polyethylene; nylon; polyamide; PBAT, poly(butylene adipate-co-terephthalate); PBS, polybutylene succinate; PBSA, poly(butylene succinate-co-adipate); and PCL, polycaprolactone.

A significant difference in gene abundance was observed between BF and CK (Fig. 6). Notably, various genes had greater positive correlations in BF than in CK. However, not all MP degradation genes had significant differences between BF and CK (for the relevant plastic codes see Fig. 5). For DEHP-biodegradation, the abundance of only *pcaC*, *bcrC* and *ligl* significantly differed between BF and CK ($P < 0.05$). In PE-biodegradation, the abundance of *alkR* significantly varied between BF and CK ($P < 0.05$). For PE-PS-PHB-biodegradation, the abundance of *paaF* and HSD17B10 significantly differed between BF and CK ($P < 0.05$). For PET-biodegradation, the abundance of ALDH significantly varied between BF and CK ($P < 0.05$). In contrast, the abundance of the five genes involved in PS-biodegradation did not significantly differ between BF and CK.

According to the selected gene set of MP biodegradation markers from the metagenomic data and the corresponding genera data, the contribution of genera to specific functions was analyzed. The results are given as a stacked bar graph (Fig. 7). The analysis was performed at the function level, focusing on the contribution of genera with the top five horizontal abundance to each function. The findings reveal that *Nocardioides* and *Anaeromyxobacter* were mainly involved in PS biodegradation wheras *Bradyrhizobium* was mainly associated with DEHP biodegradation. *Pseudolabrys* and *Nocardioides* were mainly involved in PE-PS-PHB biodegradation. In BF, *Bradyrhizobium* and *Anaerolinea* were associated with PE biodegradation. In CK, *Mycobacterium* and *Nocardioides* contributed to PE biodegradation. In PET biodegradation in BF, *Nocardioides,* and *Bradyrhizobium* were involved in BF, and *Nocardioides* and *Mycobacterium* in CK. For example, *Pseudolabrys* accounted for ~5% and 3% of DEHP_biodegradation in BFs and CK, respectively. *Bradyrhizobium* contributed ~5% to PE biodegradation in BFs, while *Nocardioides* accounted for ~7% of PE biodegradation in CK. *Pseudolabrys* represented ~5% in PE_PS_PHB biodegradation in BFs, while *Nocardioides* contributed ~9% to PE_PS_PHB biodegradation in CK. *Nocardioides* contributed about 5% and 3% of PET biodegradation in BF and CK, respectively. Additionally, *Nocardioides* accounted for 5% and 9% of PS biodegradation in BF and CK, respectively.

The genera contributing to specific biodegradation functions varied between BK and CK, with distinct patterns observed in the top five MP biodegradation groups (Fig. 8). Different

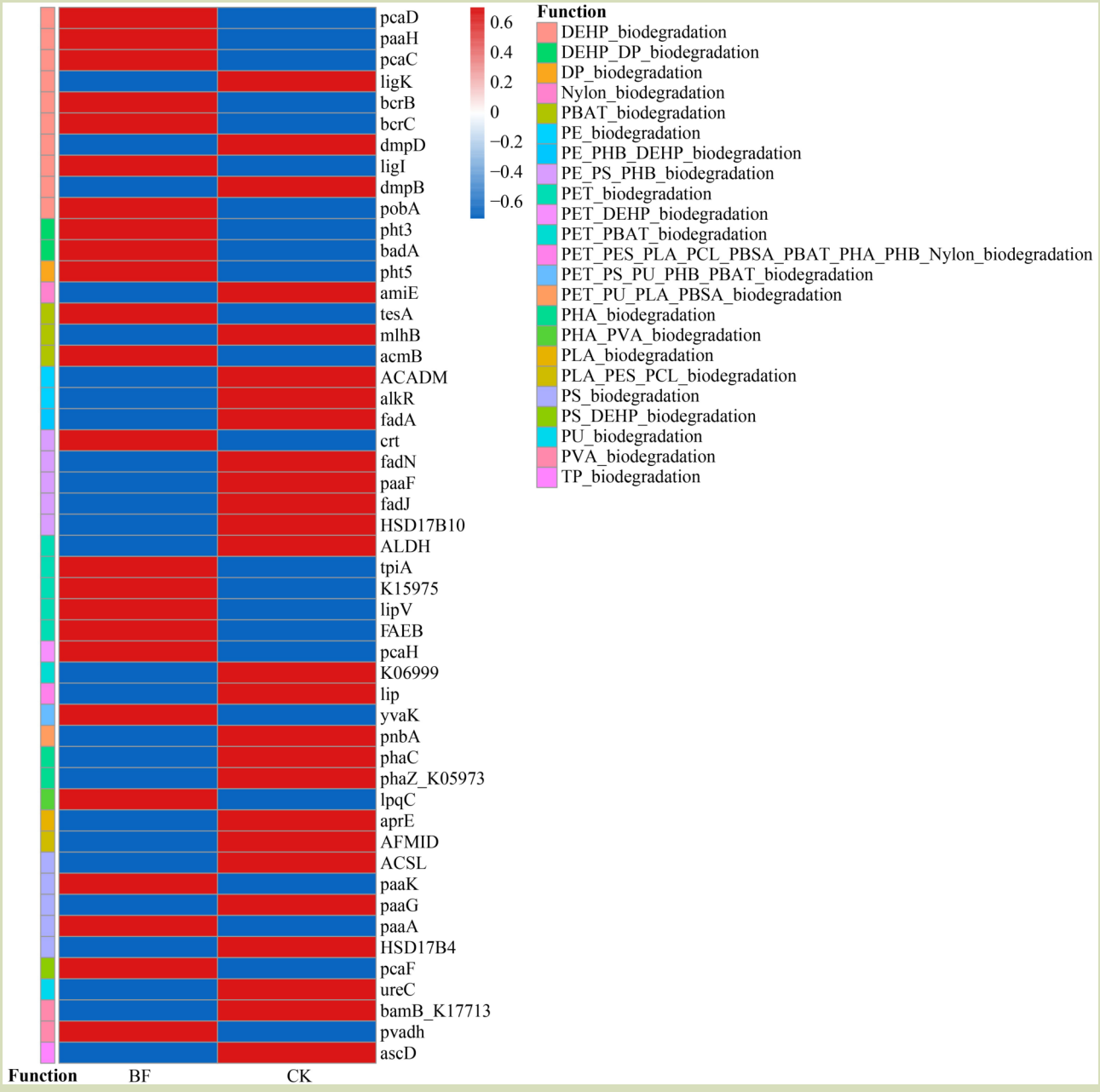

Fig. 6 Heatmap of biodegradation functions of microplastics: PET, PE, PES, PS, PHA, PHB, PHO, PLA, PU, PVA, TP, DBP, DEHP, LDPE, polyamide, PBAT, PBS, PBSA and PCL (see Fig. 5 for the definitions of the plastic codes).

genera were associated with the biodegradation of various MPs, such as *Bradyrhizobium* in PET biodegradation and *Sphingomonas*, *Pseudolabrys*, *Mycobacterium*, and *Aromatoleum* in PS biodegradation. The differences in bacterial composition were particularly pronounced in DEHP biodegradation, with *Bradyrhizobium* having a significant role.

4 Discussion

The distribution of MPs in paddy soil in BF and CK was analyzed using scanning electron microscopy and FTIR. The results reveal that BF use altered the MP composition, as indicated by different absorption peaks in the FTIR spectra

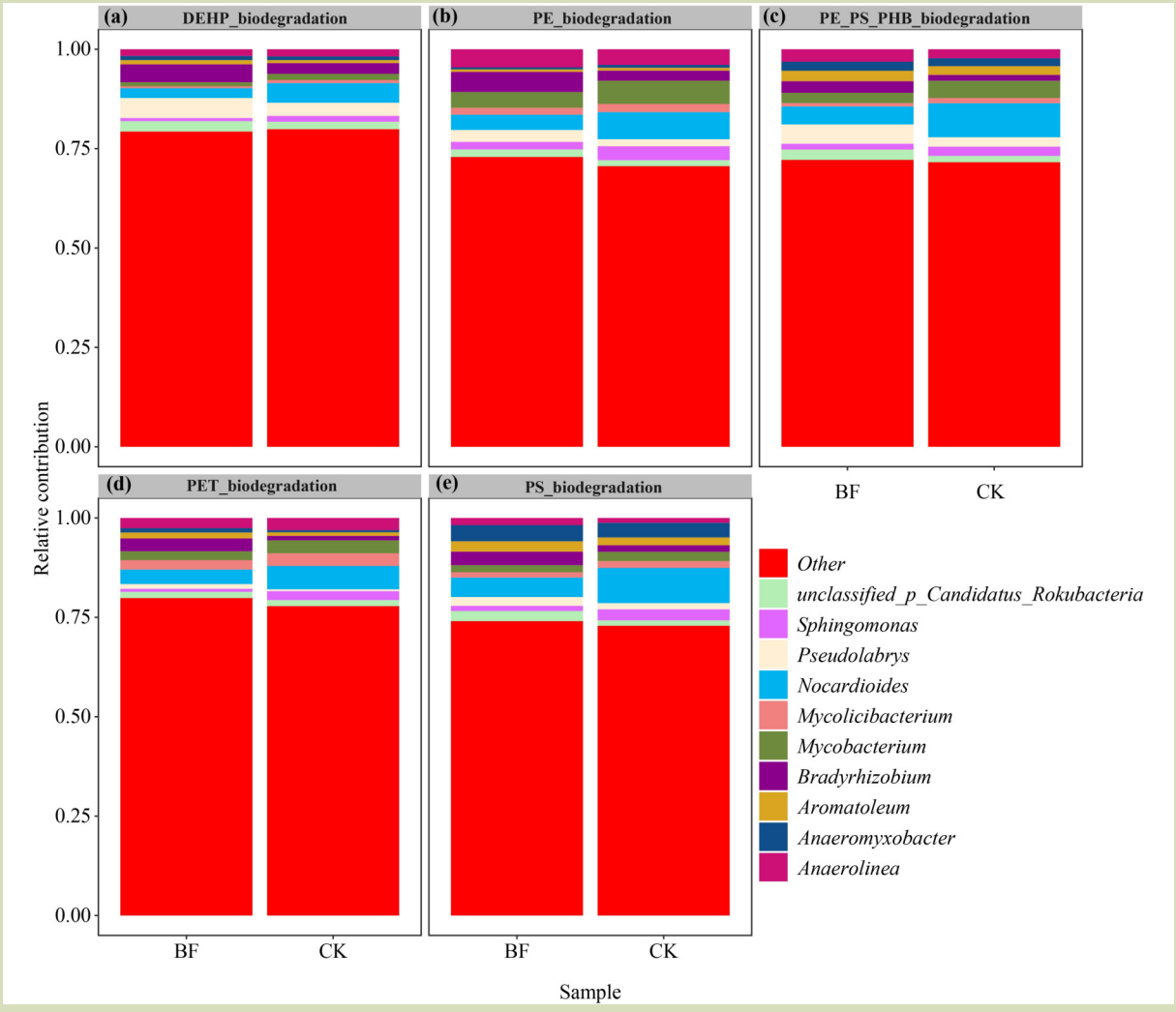

Fig. 7 Relative contributions of different genera to the top five microplastic degradation groups: (a–e) PET, PS, DEHP, PE, PES, and PHB (see Fig. 5 for the definitions of the plastic codes).

(Fig. 2). Notably, biodegradable plastics produced more MPs than conventional PE plastics, consistent with the findings of Wang et al.[40]. In the present study, BF use mainly influenced the composition, size and morphological characteristics of MPs. The MP composition differed between BF and CK. In BF, MPs larger than 1 mm included poly(ethylene:vinyl chloride), LDPE, PE, and poly(methylphenylsiloxane) (Fig. 2). Conversely in CK, MPs larger than 1 mm included poly(methylphenylsiloxane), LDPE, PE and polyester terephthalate. A significant difference in MP types was observed between BF and CK for particles smaller than 1 mm (Fig. 2). Atmospheric deposition can contribute to the accumulation of small MPs in soil[41]. MPs in irrigation water are a major source of MPs in soil[42], particularly those smaller than 1 mm. According to these criteria, both BF and CK may have similar MP compositions. However, MPs with particle sizes less than 1 mm and greater than 1 mm had different compositions in CK and BFs. Notably, BFs contributed to the occurrence of MP types in the less than 1 mm size range.

Regardless of whether BFs were used or no mulch was applied, MPs in the 0.25–0.1 mm size range had the highest abundance, and not all MP sizes significantly differed between BF and CK. In contrast, over 50% of MPs from conventional plastic films

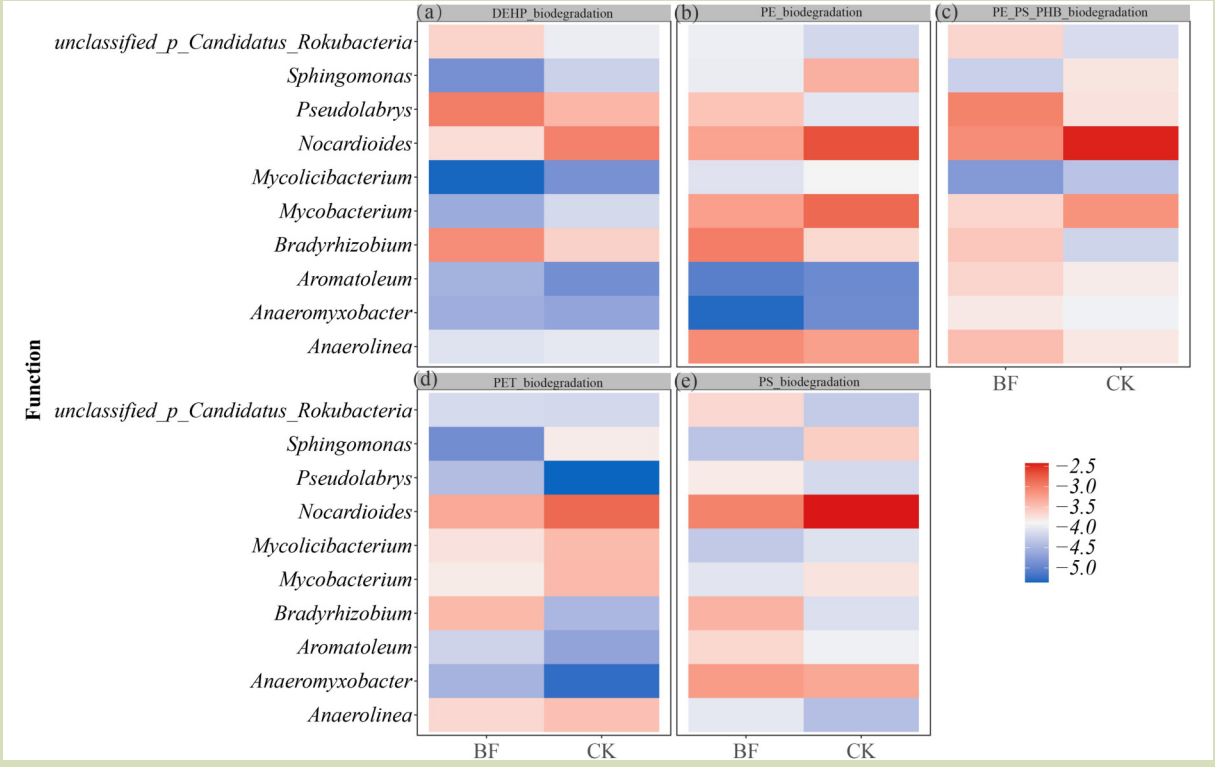

Fig. 8 Relative contributions of different genera to the top five microplastic degradation groups: (a–e) DEHP, PE, PE-PS-PHB, PET and PS (see Fig. 5 for the definitions of the plastic codes).

used in south-west paddy soils in China were in the 1–3 mm size range[19]. This indicates that the size distribution of MPs in paddy soils is altered by the use of BFs. Specifically, BFs mainly affected the abundance of MPs with particle sizes between 0.25 and 0.1 mm. BF had a significantly higher abundance of MPs in this size range compared to CK ($P < 0.05$), 800 ± 193 vs 260 ± 159 particles kg^{-1}, respectively. In BF, significant differences were observed in MP abundance across different particle size groups (Fig. 3(a)). The morphological characteristics were used to characterize the degradation process[43–45]. In the present study, MPs in BF had transitioned toward smaller forms. The film and fiber MPs were the predominant MP forms in BF and CK, respectively. This indicates a significantly higher abundance of film MPs in BF than in CK ($P < 0.05$), with 1586.7 ± 241.9 particles kg^{-1} in BFs and 746.7 ± 264.01 particles kg^{-1} in CK (Fig. 3(b)). Earlier research has shown that MP fibers were the dominant type of MPs in soil without film mulch[43], consistent with the findings of this study. Additionally, the present results indicate that BFs induce changes in the dominant MP shapes in paddy soil. However, no significant differences were observed in the abundance of fiber and fragment-shaped MPs between BF and CK samples, confirming the environmentally-friendly nature of BFs. In conclusion, BFs significantly influence the particle size, shape and composition of MPs, while CK contains MPs from other sources, such as atmospheric deposition and/or irrigation water.

The particle size and shape observed in this study reflects the process of BF degradation, which is influenced by several factors. Among these factors, microbial activity is likely to be a major contributor[28]. Unlike pure polymer systems, biodegradable mulch films require the action of resident soil microorganisms to degrade polymers under heterogeneous field conditions. Microbial consortia and enzymatic pathways are crucial for MP biodegradation[46], with different soil microbes and enzymes impacting various polymer types. Although polymers, such as PET, PS, PE, PVC and PP (polypropylene) are commonly found in soils[47,48], this study identified the top five polymer types as DEHP, PE, PE-PS-PHB, PET and PS. These differences can be attributed to the

influence of BFs. The degradation process of these polymers typically occurs in stages, such as depolymerization, fragmentation, assimilation and mineralization. Particularly, PE polymers eventually break down into simpler forms[49]. Over 20 bacterial genera have been identified as capable of degrading PE[46], and microbes such as *Bacillus algicola* and *Pseudomonas juntendi* can degrade PET[50]. These microbial capabilities extend to other polymers, including PS, PHB, DEHP and PES. The present study further examined the genes involved in polymer biodegradation in BF and CK samples. The results revealed differences in gene abundance between the two contexts. Over 26 DNA sequences associated with the biodegradation of the top five polymer types were identified (Fig. 6). At the functional level, the contributions of genera were similar across polymer types. However, the specific contribution ratios varied. For example, *Pseudolabrys* contributed ~5% in BF and *Nocardioides* contributed ~9% to PE-PS-PHB biodegradation in CK (Fig. 7). Different soil microbes had varying degrees of effect on MP degradation depending on the polymer type.

These analyses indicate that microbial degradation of BFs leads changes in the shape (polymorphic transformations) and size of MPs. The biodegradation rate of BFs is influenced by the type of filler used in the composite polymer matrix. Studies have shown that oat hull and silkworm exuvia composites biodegrade faster than sugarcane bagasse composites. This difference is likely attributed to the depletion and/or use of nutrients from the soil, and the immobilization of different MPs by the biodegrading microbiota[9]. Additionally, the same BFs may degrade at different rates depending on the geographic location. Field studies have shown that starch-based composite films degraded more rapidly in Hebei Province than in Xinjiang, highlighting the influence of climatic factors on the biodegradation processes[51]. Further research has indicated that fragments from biodegradable plastic formulations can completely degrade within 32 days. The degradation rate could potentially decrease to less than 24 days with the incorporation of *Bacillus subtilis* into BFs[52]. A field study on the degradation of PBAT films has indicated that more MPs were detected in BFs after three years of mulching[53]. In the present study, we systematically characterized the microbial taxa responsible for degrading different MP types and quantified both their abundance profiles and functional contributions to the degradation processes. BFs were substantially degraded within a single rice growth period. The genera contributing to specific functions typically comprised an *unclassified_p_Candidatus_Rokubacteria*, *Sphingomonas*, *Pseudolabrys*, *Nocardioides*, *Mycolicibacterium*, *Mycobacterium*, *Bradyrhizobium*, *Anaeromyxobacter*, and *Anaerolinea* (Figs. 7 and 8). The degradation process was facilitated by the assimilation of matrix-embedded nutrients in BFs by these genera, which enhanced the degradation.

Overall, the quantification and enhancement of MP biodegradation efficiency confirmed that BFs can provide a sustainable solution for MP pollution. BFs degrade more easily into simpler forms. Although the total content of MPs did not reveal a significant difference between BFs and CK, BFs altered the composition, particle size and shape characteristics of MPs. Additionally, BFs influenced the biodegradation functions of MPs in paddy soil.

5 Conclusions

The particle size and shape of MPs in paddy soil were influenced by the type of plastic film used. BFs degraded easily into simpler forms. Although no significant difference in the total MP content was observed between BF and CK, BF use resulted in different absorption peaks in the MP spectra. Additionally, the abundance of genes involved in the biodegradation process differed between BF and CK. Over 26 DNA sequences were related to the biodegradation of the top five polymer types. The abundance of *alkR* gene involved in PE biodegradation significantly differed between BF and CK ($P < 0.05$). Differences were found in the abundance of *paaF* and *HSD17B10* involved in PE-PS-PHB biodegradation and *ALDH* abundance associated with PET biodegradation. In addition, bacterial taxa had different correlations with specific MP types, such as *Bradyrhizobium* for PET biodegradation and *Sphingomonas*, *Pseudolabrys*, *Mycobacterium*, and *Aromatoleum* for PS biodegradation. Overall, BFs can serve as an environmentally friendly material for widespread use in rice production.

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Compliance with ethics guidelines

Liyin Ren, Wei Zhu, Aihua Zhang, Junling Cai, Lei Xu, Kai Wang, Xuejun Liu, and Rui Jiang declare that they have no conflict of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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