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Credit authorship contribution statement

Yue Sun: Performing the experiments, investigation, analysis, data collection, methodology, and writing the draft manuscript.

Sabry M. Shaheen: Scientific concept, coordination, experimental guiding, writing, editing, proof reading for the entire manuscript.

Esmat F. Ali: Review, editing, and proof reading.

Hamada Abdelrahman: Review, editing, and proof reading.

Binoy Sarkar: Revising, editing, and proof reading of the entire manuscript.

Hocheol Song: Revising, editing, and proof reading of the entire manuscript.

Jörg Rinklebe: Revising, editing, and proof reading of the entire manuscript.

Xiuna Ren: Investigation, analysis, and data collection.

Zengqiang Zhang: Scientific Concept, Foundation, Revising, editing, and proof reading of the entire manuscript.

Quan Wang: Supervision, Project administration, conceptualization, research idea, experimental guiding, technical facilities, foundation, review, editing and corresponding author.

Enhancing microplastics biodegradation during composting using livestock manure biochar

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Abstract

Biodegradation of microplastics (MPs) in contaminated biowastes has received big scientific attention during the past few years. The aim here is to study the impacts of livestock manure biochar (LMBC) on the biodegradation of polyhydroxyalkanoate microplastics (PHA-MPs) during composting, which have not yet been verified. LMBC (10% wt/wt) and PHA-MPs (0.5% wt/wt) were added to a mixture of pristine cow manure and sawdust for composting, whereas a mixture without LMBC served as the control (CK). The maximum degradation rate of PHA-MPs (22–31%) was observed in the thermophilic composting stage in both mixtures. LMBC addition significantly (P<0.05) promoted PHA-MPs degradation and increased the carbon loss and oxygen loading of PHA-MPs compared to CK. Adding LMBC accelerated the cleavage of C-H bonds and oxidation of PHA-MPs, and increased the O-H, C=O and C-O functional groups on MPs. Also, LMBC addition increased the relative abundance of dominant microorganisms (*Firmicutes*, *Proteobacteria*, *Deinococcus-Thermus*, *Bacteroidetes*, *Ascomycota* and *Basidiomycota*) and promoted the enrichment of MP-degrading microbial biomarkers (e.g., *Bacillus*, *Thermobacillus*, *Luteimonas*, *Chryseolinea*, *Aspergillus* and *Mycothermus*). LMBC addition further increased the complexity and connectivity between dominant microbial biomarkers and PHA-MPs degradation characteristics, strengthened their positive relationship, thereby accelerated PHA-MPs biodegradation, and mitigated the potential environmental and human health risk. These findings provide a reference point for reducing PHA-MPs in compost and safe recycling of MPs contaminated organic wastes. However, these results should be validated with other composting matrices and conditions.

***Keywords*:** Biowastes, Biodegradable plastics, Microbial community, Biodegradation, Environmental remediation.

1. Introduction

Petroleum-based plastics have seen widespread usage in recent decades, with a global annual production reaching up to 368 million tons in 2019 (Plastics Europe, 2020). It is estimated that 12 billion tons of plastic waste will be discarded in natural environments or landfills by 2050 (Geyer et al., 2017). The huge volume of plastic products does not only increases greenhouse gas emissions at various stages of plastic production and usage, but also causes white pollution due to the degradation resistance of plastics (Kasar et al., 2020; Liu et al., 2022).

Plastic waste could be broken into small particles such as microplastics (MPs; <5 mm) and nanoplastics (<100 μm) through physical wear, ultraviolet radiation, thermal oxidation and microbial effects, which can impart damaging impacts to the ecosystem and living beings (Guo et al., 2020; Jiang et al., 2022; Sarkar et al., 2021). In addition, the additives released from MPs (such as salt, metal(loid)s and plasticizers) can be harmful to the environment and human health (Khandare et al., 2021; Duan et al., 2021; Yu et al., 2021). These potential negative consequences of plastic use have driven the scientific community to look into sustainable (re)cycling and management of plastics (Chen et al., 2020).

Biodegradable plastics, as an environmentally friendly alternative to petroleum-based plastics, can alleviate the environmental and disposal problems of plastics (Zimmermann et al., 2020; Qin et al., 2021). Biodegradable plastics including polyhydroxyalkanoate (PHA), poly(propiolactone) (PPL), and poly (L-lactic acid) (PLA) have been widely used in agricultural films, compost bags, polyester fabrics, package materials and other biodegradable resins (Lambert and Wagner, 2017; Urbanek et al., 2020).

Compared to petroleum-based plastics, biodegradable plastics (often termed bioplastics, such as PHA, polybutylene succinate (PBS) and Polycaprolactone (PCL)) are more easily converted into microbial biomass, and/or degraded to H_2O, CO_2 and CH_4 by microorganisms (Lambert and Wagner, 2017). Moreover, in the last two decades, the application of bioplastics has been increasing worldwide (Atiwesh et al., 2021; Cucina and Nisi et al., 2021), especially since the European Union's new directive to ban the use of single-use plastics in 2019 (European Parliament, 2019). Therefore, bioplastics have already replaced petroleum-based plastics in many developed countries and it's becoming a trend due to bioplastics better biodegradability and application performance than petroleum-based plastics (Cucina and Nisi et al., 2021; Dilkes-Hoffman et al., 2019). However, bioplastics can be degraded slowly in natural environments (e.g., soil, coastal water, and river), and MPs would be released during the degradation process (Volova et al., 2007; Volova et al., 2010; Sintim et al., 2019; Qin et al., 2021).

The high temperature and active microbes during composting may facilitate the bioplastics degradation (Gui et al., 2021; Sun et al., 2021). Fukushima et al. (2009) found that PLA plastics were degraded significantly after 17 weeks of composting. Also, Sintim et al. (2020) observed that biodegradable plastic films (PHA and PLA) were 85–99% degraded based on surface area measurements after 18 weeks of composting. However, MPs could be formed following the degradation of (bio)plastics during the composting process (Chen et al., 2020; Gui et al., 2021). Sintim et al. (2019) demonstrated that micro- and nanoplastics were released from biodegradable plastic mulches (PHA and PLA) during composting over 18 weeks. Yet, the current literature has a dearth of information on the degree of MPs generation during composting of various biodegradable plastics, associated degradation rate and mechanisms, environmental longevity of generated MPs, and their ecotoxicological impacts. Our previous study indicated that only 29% of PHA microplastics were degraded during composting over 60 d, and the abundance of *Firmicutes* and *Proteobacteria* phyla of bacteria were positively correlated with the degradation of PHA (Sun et al., 2021). Plastic mulching and application of the microplastic-containing compost in agriculture could increase MPs concentration in soil. MPs, with small volume, strong hydrophobicity and large specific surface area, can enrich toxic pollutants from the surrounding environment (Vedolin et al., 2018; Zhou et al., 2019), leading to the accumulation of pollutants in soil, and consequently increasing the risk of the ecosystem (Vithanage et al., 2021). During the composting process, the MPs degradation was positively related to the composting condition (e.g., temperature, enzyme and microbial community, etc.) (Ali et al., 2021; Gui et al., 2021). However, the literature about the improvement of MPs degradation during composting is still limited. Only one reference indicated that utilization of hyperthermophilic composting technology to improve the temperature and microbial activity of composting could improve the MPs degradation in sewage sludge (Chen et al., 2020). Thus, there is an urgent need to optimize the composting conditions and explore more available modifications/additions to promote full degradation of biodegradable MPs before returning the composted product to the soil.

Biochar is a promising environmental friendly amendment can immobilize pollutants and enhance microbial activities in compost, soils, and water (Ali et at., 2020; Azeem et al., 2022; Bolan et al., 2022; Farid et al., 2022; Shaheen et al., 2022). Recently, some studies reported that biochar addition (such as cornstalk biochar, manure biochar, bamboo biochar, hard-wood biochar) could have a positive effect on composting organic wastes where biochar serves as an exogenous additive, and about 10% biochar addition (wt/wt) was suggested to obtain the best outcome (Chen et al., 2017; Farid et al., 2022; Zhang et al., 2021). During the composting process, biochar addition could promote the relative abundance of *Firmicutes* and *Proteobacteria*, and consequently prolong the thermophilic phase of composting (Tokiwa et al., 2009; Zainudin et al., 2020). Additionally, the large specific surface area and porous structure of biochar are beneficial characteristics that enhance aeration and microbial activities in the composting matrix (Zainudin et al., 2020). Previous studies indicated that the degradation of MPs was closely related to high temperature, moisture and oxygen contents, and enhanced microbial activity (Bahl et al, 2020). Thus, the addition of biochar could be an effective way to promote MPs degradation during the composting of biodegradable plastics (Zhang et al., 2019).

Herein, we hypothesized that biochar addition could facilitate MPs degradation by altering the physicochemical characteristics and microbial community succession of composting process, and thereby reducing MPs concentration in biowastes and mitigating the potential environmental risks. This study aims to examine the effect of livestock manure biochar (LMBC) on the degradation of PHA-MPs during the composting process with the following specific objectives: i) explore the degradation characteristics (including the degradation rate, size distribution, surface morphology, elemental analysis and functional groups) of PHA-MPs in response to LMBC addition during composting; ii) unravel the possible mechanism of LMBC for facilitating PHA-MPs degradation through the succession of microbial community composition (bacteria and fungi) and alternation of network patterns of microbial community during the composting process.

2. Materials and methods

2.1. Composting experiments

The raw materials including fresh cow manure and sawdust were collected from a local farm and wood-processing factory in Yangling, Shaanxi, China. The PHA plastic and LMBC (pyrolysis of 550 ^{o}C for 2 h) were obtained from Shenzhen Fuxin Plastic Raw Material Co., Ltd. and Yixing Biotechnology Co. Ltd., China, respectively. The PHA plastics were treated with liquid nitrogen and then crushed by a grinder into microparticles (<2 mm).

LMBC was characterized by carbon 64.52%, nitrogen 1.77%, moisture 3.57%, pH 7.67, electrical conductivity (EC) 1683.7 μS/cm (pH and EC measured in distilled water at ratio of 1:10, w/v), and specific surface area (SSA) 4.36 m^2 g^{-1}. The properties of the cow manure and sawdust were shown as follows: i) the carbon concentrations of cow manure and sawdust were 41.94% and 48.24%, respectively; ii) the nitrogen concentrations of cow manure and sawdust were 2.31% and 0.23%; iii) the pH values of cow manure and sawdust were 8.53 and 7.17 (Sun et al., 2020).

The composting experiment included two mixtures: (1) a control mixture containing cow manure (14.63 kg), sawdust (5.37 kg) and PHA-MPs (40 g; 0.5% wt/wt) (<2 mm), and (2) a treatment mixture (T1) containing the ingredients of the control plus LMBC (10%; wt/wt). The number of PHA-MPs added in the compost mixtures was within the concentration of MPs in organic wastes, according to previous work (Zhang and Chen, 2020). The loading of LMBC was based on earlier findings, as Chen et al. (2017) indicated that higher temperature and longer thermophilic duration during the composting process were achieved by adding 10% LMBC as compared to the control without any LMBC addition.

The moisture content and C/N ratio of the initial mixtures (control, T1) were adjusted to ~60% and ~25, respectively (Wang et al., 2017). After thorough mixing, the compost mixtures were individually placed into a 60 L composting reactor for 60 d. A schematic diagram of the composting reactor was presented elsewhere (Sun et al., 2020). During the composting process, 500 g homogeneous compost samples were collected from the control and T1 mixtures on days 0, 7, 28 and 60, respectively. The collected samples were divided into two portions; one portion was stored at a −80 ºC refrigerator for biological analyses, whereas the other portion was air-dried for the extraction of MPs.

2.2. Extraction and characterization of MPs

2.2.1 MPs extraction

To extract PHA-MPs from compost samples, 20 g compost sample in triplicates was mixed with up to 300 mL of a saturated sodium chloride solution (1.2 g mL^{-1}; Ding et al., 2019) in glass beakers, and steadily stirred for 15 min. The mixture was subsequently allowed to settle for 2 h, and the supernatant layer was recovered through a vacuum pump and filtered through a 37 μm filter membrane. This extraction process was repeated three times to completely extract MPs from individual compost samples. To remove and reduce organic materials, the extract on the filter membrane was rinsed in a glass beaker with H_2O_2 solution (30%) overnight at room temperature, where the glass beaker was covered with clean watch glass all the time to avoid contamination (Ren et al., 2020). Subsequently, 200 mL distilled water was poured into the mixture after digestion with H_2O_2, and vacuum-filtrated through a glass fiber membrane with a pore size of 0.8 μm (GF/F, 50 mm Ø, Whatman). All glass fiber membranes were dried at 50 ℃ for 3d, and then placed in clean glass petri dishes for further analysis (Li et al., 2018).

2.2.2 Microplastics characterization

The degradation characteristics of MPs were revealed by determining the decrease of MPs abundance, surface morphological change of MPs, carbon loss and oxygen loading of MPs, and breakage of bonds and formation of new functional groups (Gu, 2003; Ali et al., 2021). The FE-SEM, EDX and ATR-FTIR were used to analyze the degradation characteristics of MPs in both the mixtures during composting.

The abundance of PHA-MPs was detected using a stereomicroscope (CX23, Olympus, Japan) and Nano Measurer 1.2 software (Sun et al., 2021). For morphological characterization, the extracted PHA-MPs were sputtered and coated with an approximately 10 nm platinum layer, and then transferred to a conductive carbon strip. The morphology of PHA-MPs was examined by an FE-SEM (Geminisem 500, Zeiss, Germany) at 200× magnification. The acceleration voltage of SEM was set to 15 kV, and the resolution of the secondary electronic image was 0.5 nm. The elemental composition (carbon and oxygen) of PHA-MPs was analyzed by EDX. The variation in functional groups of PHA-MPs during composting was studied using FTIR in ATR mode (Nicolet 8700, Thermo Fisher Scientific, USA) with a scanning range and resolution of 400–4000 cm^{-1} and 2 cm^{-1}, respectively.

2.3. Microbiological analysis of compost

The microbial community in the compost samples collected on days 0, 7, 28, 60 of composting were analyzed at a specific commercial company in Beijing, China. Each sample had three replications. The detailed procedure for DNA extraction was reported elsewhere (Sun et al., 2021). The 16S rDNA gene was amplified in the V3-V4 hypervariable region of bacteria with the primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). The 18S rDNA gene was amplified in the ITS1–5F region with the primers ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). Subsequently, TruSeq® DNA PCR-free Sample Preparation Kit (Illumina, USA) was used to construct bacterial and fungal libraries for all samples, and the recovered amplified products were sequenced using Illumina NovaSeq6000.

To study the species composition, operational taxonomic units (OTUs) were clustered for all samples, and then species annotation was made for the OTUs sequence. According to the results of species annotation, the top 10 microorganisms with the largest abundance at the genus level were selected to intuitively compare the differences in the relative abundance of microorganisms in composts with and without LMBC addition. Principal coordinate analysis (PCoA) and Linear discriminant effect size (LEFSe) analysis were used to evaluate the Beta Diversity of the microorganisms. Differences in microbial community structure were shown by PCoA based on the Bicular-Jaccard method. The LEFSe analysis was used to estimate the average relative abundance differences of microbial species that degraded PHA-MPs during composting with and without LMBC, and also to identify the strains with a significant difference. The relationship between microbial communities and PHA degradation characteristics, including degradation rate, carbon loss and oxygen loading of PHA-MPs, was demonstrated by co-occurring network model.

2.4. Data analysis

All analyses, including extraction and characterization of MPs and microbial analysis of composts, were based on three replications. The data of PHA-MPs abundance and FTIR were visualized using GraphPad Prism 8 (GraphPad Software Inc., USA). One-way analysis of variance (ANOVA) was conducted using SPSS software based on three replicated measurements. Redundancy analysis (RDA) was performed using CANOCO (version 4.5) to evaluate the correlation between microbial community composition, composting temperature and degradation characteristics of PHA-MPs. To analyze the connection between microbial communities and degradation characteristics of PHA-MPs, Cytoscape_3.7.2 was used to draw co-occurring network model.

3. Results and discussion

3.1. Biodegradation of PHA-MPs during composting

During the composting process, the proportion of PHA-MPs (%) in the control and T1 mixtures both presented a declining trend (Fig. 1a), which was consistent with other works that reported the degradation of MPs during hyper-thermophilic composting of sewage sludge (Chen et al., 2020). Over the entire composting period, the highest degradation rate (22–31%) of PHA-MPs in both mixtures was observed at the thermophilic (0–7 d; Fig. 1a) stage likely due to the high temperature and superior relative abundance of bacterial and fungal community at this phase (as discussed later in section 3.3; Chen et al., 2020). At the end of composting, PHA-MPs proportion persisting in the control and T1 was 71% and 50%, respectively, where the final degradation rate of PHA-MPs in the T1 mixture was significantly ($p<0.05$) higher than that of the control, indicating that LMBC addition promoted PHA-MPs degradation during the composting process. The difference in PHA-MPs degradation was likely because LMBC addition promoted the temperature increase, extended the thermophilic period, and improved the relative abundance of beneficial microbial species (Section 3.3 and Fig. S1; Chen et al., 2017). The higher PHA-MPs degradation rate observed in T1 could be beneficial for reducing the soil MP pollution caused by the application of compost.

The size distribution (0–2000 μm) of PHA-MPs in both mixtures is shown in Fig. 1b,c. During the composting process, the particle size distribution of PHA-MPs in both mixtures changed, with different degrees in different mixtures. For the control, the abundance of 0–200 μm PHA-MPs decreased ($p<0.05$) at the thermophilic (0–7 d) stage, and then tended to be stable until the end of the experiment (Table S1). The abundance of 200-400 μm PHA-MPs in the control firstly increased ($p<0.05$), and then continuously decreased ($p>0.05$) during the composting process. The abundance of the PHA-MPs of other diameters (400–600, 600–800, 800–1000 and 1000–2000 μm) in the control mixture fluctuated during the composting process, possibly due to the degradation of large MPs and consequent formation of smaller MPs (Gui et al., 2021). The abundance of PHA-MPs in the diameter of 0–200, 600–800 and 1000–2000 μm in the LMBC amended mixture showed a declining trend ($p<0.05$), while PHA-MPs particles of the diameter of 200–400, 400–600 and 800–1000 μm initially increased and then decreased during the composting process. At the end of composting, the abundance of PHA-MPs in the control (14,050 particles kg^{-1} dry weight) was significantly ($p<0.05$) higher than that of the T1 mixture (9,950 particles

kg^{-1} dry weight), indicating that LMBC addition accelerated MPs degradation. In general, almost all particle sizes of MPs in the LMBC amended mixture were lower ($p<0.05$) than those of control, except for the 200–600 and 800–1000 μm size. These results implied that LMBC addition could promote different sizes of PHA-MPs degradation differently.

3.2 Characterization of PHA-MPs

During the composting process, the surface morphology and elemental composition of the PHA-MPs in both mixtures encountered changes (Fig. 2). The surface morphology of the initial PHA-MPs was relatively smooth without cracks in both mixtures, but gradually became rougher with the progress of composting period, which was consistent with the finding of Gui et al. (2021) during the composting of rural domestic waste. Linear cracks appeared in the PHA-MPs of the control mixture, while there was a large number of holes and voids in addition to the linear cracks in the PHA-MPs of the T1 mixture (Fig. 2), indicating that the roughness of PHA-MPs in the T1 mixture was higher than that of the control. The notable roughness of PHA-MPs observed in the LMBC added mixture was probably because LMBC optimized the abiotic (e.g., temperature, moisture and oxygen) and biotic (e.g., bacteria and fungi) composting conditions, and thus accelerated MPs weathering and degradation (Fig. S1 and Fig. 2; Ali et al., 2021). In addition, the degradation rate of MPs could be positively correlated to their surface roughness (Ali et al., 2021). The surface of PHA-MPs in the LMBC amended mixture was rougher than that of the control (Fig. 2), which could increase the surface area of MPs and provide more habitats, i.e., surface attachment sites, for microorganisms to adhere and consequently degrade the particles at an increased rate (Wright et al., 2020; Ali et al., 2021; Wang et al., 2021a).

During the composting process, the carbon content of the PHA-MPs in both mixtures continuously decreased, while the oxygen content of the PHA-MPs increased (Fig. 2). The decrease in the carbon content of the PHA-MPs might be related to the degradation of MPs by microorganisms using those particles as a carbon source (Gu, 2003; Shah et al., 2008), whereas the increase of oxygen content of the

PHA-MPs was likely due to the oxidation of MPs by abiotic and biotic effects (Pathak and Navneet et al., 2017; Ali et al., 2021). During the composting process, carbon loss of the PHA-MPs in the control and T1 mixtures was 21–26% at the initial phase (0-7 d), 6–9% at the intermediate (7–28 d) phase, and 2–17% at the final phase (28–60 d). The oxygen content increment of the PHA-MPs in the control and T1 mixtures for the same phases was 18–25%, 5–9%, and 6–16%, respectively. The highest carbon loss and oxygen loading of PHA-MPs in both mixtures occurred at the thermophilic stage (0–7 d), which agreed with the results of PHA-MPs degradation performance (Fig. 1). At the thermophilic phase of composting, high temperature and high relative abundance of thermophilic microorganisms would be beneficial for PHA-MPs degradation (Bahl et al., 2020), which was supported by the redundancy analysis in this study (Fig. S2). At the end of composting, carbon loss of the PHA-MPs in the control and T1 mixtures was 30 and 51%, respectively, which was consistent with the results of oxygen loading. The RDA results indicated that the PHA-MPs degradation rate had positive correlations with carbon loss and oxygen increment of MPs (Fig. S2), implying that LMBC addition improved PHA degradation and subsequently reduced the abundance of MPs in the final compost (Fig. 1).

The functional groups of the PHA-MPs in both mixtures provided additional details on the MPs degradation process (Fig. 3; Table S2). The band intensity of PHA-MPs functional groups in both mixtures was altered, whilst new functional groups appeared with the progress of the composting period. Similarly, Sintim et al. (2019) found that the functional groups of plastic particles increased with the extent of composting time. The C-O functional group at 955 cm^{-1} of the PHA-MPs in the T1 mixture began to appear on the 7th d of composting, while in the control mixture it appeared on the 60th d. These results indicated that LMBC addition accelerated the oxidation of PHA-MPs, which was likely due to the biochar addition facilitating oxygen diffusion and microbial activities in the system (Wang et al., 2021b). At the end of composting, the band intensity of CH_2 and CH at 1400 cm^{-1}, 2848 cm^{-1} and 2997 cm^{-1} of PHA-MPs in both mixtures was decreased, and that of C-O, C-O-C and O-H at 955 cm^{-1}, 1104 cm^{-1}, 1260 cm^{-1} and 3454 cm^{-1} was increased. These results implied that the cleavage of C-H bonds and oxidation of PHA-MPs took place, which was consistent with the results of EDS analysis (Fig. 2). Additionally, the band intensity of C=O and O-H bonds at 1740 cm^{-1} and 3454 cm^{-1} of PHA-MPs in the T1 mixture increased, while the band intensity of CH_2 and CH bonds at 2848 cm^{-1} and 2997 cm^{-1} in the T1 mixture decreased as compared to those of the control, indicating that LMBC addition promoted the cleavage of C-H bonds and oxidation of PHA-MPs. Gui et al. (2021) found that the increase of C=O, C-O and O-H groups improved the hydrophilicity of MPs, which would be beneficial for microbial colonization to degrade MPs (Wright et al., 2020). However, the environmental risks of composted MPs should be further studied due to the surface morphology and functional groups of MPs changing after composting.

3.3. Microbial community structure analysis

3.3.1 Effect of LMBC addition on bacterial community

In this work, the changes in the top 10 bacteria at the genus level were studied (Fig. 4a) where the four dominant bacteria of the initial material included *Romboutsia*, *Paeniclostridium*, *Luteimonas* and *Pusillimonas*. With progress in composting, the structure of the bacterial community changed significantly, which was consistent with the results of Liu et al. (2021) during the co-composting of sewage sludge and corn cob biochar. There was a significant difference in the relative abundance of bacteria at the genus level between the control and T1 mixture during composting. At the thermophilic phase of composting (on day 7), *Thermobacillus* and *Bacillus* were the dominant bacteria in both mixtures. The relative abundance of *Thermobacillus* and *Bacillus* in the T1 mixture was significantly ($p<0.05$) increased by 34% and 72%, respectively, as compared to the control. *Thermobacillus* and *Bacillus* belonging to the phyla *Firmicutes* are typical thermophilic bacteria (Wang et al., 2021c), and an increase in the abundance of these bacteria could promote the temperature increase during composting, and consequently enhanced the MPs degradation (Fig. 1; Fig. S1; Fig. S2; Chen et al., 2020). Moreover, Lambert and Wagner (2017) and Bahl et al. (2020) also reported that *Bacillus* could degrade biodegradable aliphatic polyester plastics (e.g., PHA, PCL and PPL). During the cooling phase of composting (28 d), *Pusillimonas* and *Moheibacter* were the predominant bacteria in the control mixture, whereas *Luteimonas* and *Truepera* were predominant bacteria in the T1 mixture (Krishnan et al., 2017; Wang et al., 2021c). The RDA results (Fig. S2) showed that *Pusillimonas*, *Moheibacter*, *Luteimonas* and *Truepera* were positively correlated with the degradation rate, carbon loss and oxygen loading of PHA-MPs. Moreover, *Luteimonas* and *Truepera* in the T1 mixtures were more correlated with PHA-MPs degradation characteristics than in the control (Fig. S2), implying that the addition of LMBC could promote the activities of these microorganisms to degrade MPs (Figs. 1–3). At the mature phase of composting (60 d), the dominant bacteria in the control and T1 mixtures were also distinguishable, where *Pusillimonas*, *Truepera* and *Moheibacter* were dominant in the control and *Luteimonas*, *Truepera* and *Chryseolinea* belonging to the phyla *Proteobacteria*, *Deinococcus-Thermus* and *Bacteroidoota* were dominant in the T1 mixture (Wang et al., 2021c; Krishnan et al., 2017). The relative abundance of *Luteimonas* and *Chryseolinea* in the T1 mixture was enhanced by 1175 and 3550%, respectively, as compared to the control. *Chryseolinea* is a primary decomposer of the high-molecular-weight polymer during composting (Wang et al., 2021c), and LMBC addition increased its relative abundance, which was favorable for PHA-MPs degradation (Fig. S2), thereby contributing to a higher degradation rate of PHA-MPs in the T1 mixture during the mature phase of composting.

3.3.2 Effect of LMBC addition on fungal community

The relative abundance of the top 10 fungi at the genus level in both mixtures is shown in Fig. 4b. With progress in composting, the fungal community composition in both mixtures changed ($p<0.05$). In the initial materials (0 d), the relative abundance of *Tausonia* was the highest, while other fungi genera represented only small fractions. At the intermediate phase of composting (7–28 d), *Aspergillus*, *Entyloma*, *Trichoderma* and *Penicillium* belonging to the phyla *Ascomycota* and *Basidiomycota* were the major fungi genera in both mixtures, as also reported earlier (Zhou et al., 2022). The relative abundance of *Aspergillus*, *Entyloma*, *Trichoderma* and *Penicillium* in the T1 mixture was increased by 778–4938%, 80–4281%, 342–55% and 70–88%, respectively, compared to the control. Previous studies indicated that fungi including *Aspergillus*, *Penicillium* and *Trichoderma* could break ester bonds of polyester plastics (such as PCL, polyhydroxybutyrate (PHB), polyurethane (PU) and PHA) by releasing acetyl xylan esterase (Pathak and Navneet, 2017; Bahl et al., 2020). An increase in the abundance of these dominant fungi explained the higher degradation rate of PHA-MPs in the T1 mixture than in the control (Fig. 1a).

At the end of composting (60 d), *Mycothermus* belonging to the phyla *Ascomycota* was the most dominant fungi genera in both mixtures, which was consistent with the results which found that *Mycothermus* was primary abundant during peach sawdust composting (Guo et al., 2021) and during cattle manure-maize straw and biochar composting (Bello et al., 2021). *Mycothermus* could secrete extracellular enzymes (e.g., amylases, cellulases, xylanases) and break down polymeric materials into oligomers and monomers (Wang et al., 2020). Ali et al. (2021) reported that fungi including *Mycothermus* were able to degrade plastics through secreting extracellular enzymes. Compared to the control, the relative abundance of *Mycothermus* in the T1 mixture was increased by 1390%, indicating that LMBC addition improved the abundance of *Mycothermus*, which was beneficial for degrading PHA-MPs. Additionally, the RDA results (Fig. S2) revealed that *Mycothermus* in both mixtures had positive effects on the degradation rate, carbon loss and oxygen loading of PHA-MPs. *Mycothermus* in the T1 mixture had a more positive effect on the above-mentioned parameters than that of the control, suggesting that LMBC addition enhanced PHA-MPs decomposition by *Mycothermus*, and consequently led to a higher degradation rate of PHA-MPs in the T1 mixture than in the control. During the whole composting process, the dominant fungi genera *Fusarium*, *Trichoderma*, *Thermomyces*, *Issatchenkia*, *Aspergillus* and *Penicillium* in the control were positively correlated with the PHA-MPs degradation characteristics in all stages of composting, while *Mycothermus*, *Acaulium*, *Fusarium*, *Entyloma*, *Issatchenkia* and *Aspergillus* were positively correlated with PHA-MPs degradation charicteristics in the T1 mixture (Fig.S2), indicating that LMBC addition changed the preponderant fungi for degrading PHA-MPs, thereby influencing the degradation rate of MPs.

3.3.3 Effect of LMBC addition on microbial community structure

The PCoA helped to monitor the microbial community structure during the composting process (Fig. S3). The PCoA showed significant differences ($p<0.05$) in microbial communities at various stages of composting in the individual mixture. Microbial community structures of the control and T1 mixtures were significantly ($p<0.05$) different, except for the fungal community on 28 d, which indicated that LMBC addition had an impact on the microbial community structure during the composting process. Previous studies also reported that biochar addition could influence the bacterial community structure during sewage sludge composting (Du et al., 2019) and distilled grain waste composting (Wang et al., 2021b). However, Bello et al. (2020) demonstrated that biochar addition had no obvious effect on the structure of the bacterial community during cattle manure and maize straw composting. The differences observed in these results were associated with the composting conditions and raw materials (Du et al., 2019). During the composting process, microorganisms particularly bacteria and fungi would play vital roles in decomposing MPs (Bahl et al., 2020). Hence, the variable degradation performance of PHA-MPs in the two mixtures and at different composting stages (Figs. 1–3) could be attributed to the difference in microbial community structure.

3.4. Linear discriminant effect size analysis

The LEfSe analysis helped to identify the unique microbial taxa as biomarkers, and characterize the difference between the control and T1 mixtures during the composting process. Linear discriminant analysis (LDA) scores of all biomarkers higher than four ($p<0.05$) revealed the difference in the abundance of bacterial community with biological significance (Zhao et al., 2021). Bacterial biomarkers of 78 taxa with different abundance were detected during different composting stages (Fig. 5a; Table S3).

The bacterial biomarkers of initial (0 d), thermophilic (0–7 d), cooling (7–28 d) and maturity (28–60 d) stages were 22, 13 (Control=3; T1=10), 16 (Control=9; T1=7), and 27 (Control=15; T1=12), respectively. The number of biomarkers in the T1 mixture at the thermophilic stage increased ($p<0.05$) compared to the control, while at the cooling and mature stages it was slightly ($p>0.05$) lower than that of the control. These bacterial biomarkers were different at each stage of composting, while all biomarkers in both mixtures belonged to the phyla *Firmicutes*, *Chloroflexi*, *Deinococcus*-*Thermus*, *Proteobacteria*, *Bacteroidetes* and *Gemmatimonadetes*, among which *Firmicutes* and *Proteobacteria* were the dominant bacteria for degrading MPs. This observation was consistent with the findings of Tokiwa et al. (2009), who also showed that *Firmicutes* and *Proteobacteria* could degrade MPs.

At the thermophilic stage of composting, three taxa including *Bacillaceae* (phylum), *Sinibacillus* (genus) and *Oceanobacillus* (genus) were enriched in the control, while 10 taxa, predominantly *Firmicutes* (phylum), *Bacillus* (genus) and *Thermobacillus* (genus), were enriched in the T1 mixture. Similar to the results in 3.3.1, *Bacillus* and *Thermobacillus* are the dominant bacteria in MPs degradation, thus LMBC addition promoted the PHA-MPs degradation at the thermophilic phase of composting (Figs. S2; Fig. 1a).

At the cooling stage (7–28 d), nine taxa were distinctly enriched in the control, predominantly with *Bacteroidetes*, *Bacteroidia* and *Moheibacter* (from phylum to genus level). Seven taxa were overtly enriched in the T1 mixture, predominantly having *Proteobacteria*, *Luteimonas* and *Limnochordaceae* (from phylum to genus level). These results showed that LMBC addition promoted the enrichment of *Proteobacteria* and *Luteimonas* which were the dominant bacteria for degrading MPs (such as PHA, PCL and PBS; Table. S3; Tokiwa et al., 2009). At the maturity stage of composting (28–60 d), 15 groups of bacteria were enriched in the control, predominantly with *Trueperaceae*, *Truepera* and *Deinococcus*-*Thermus* (from phylum to genus level). Twelve groups of bacteria were distinctly enriched in the T1 mixture predominantly with *Chryseolinea*, *Acidimicrobiia* and *Actinomarinales*, among which *Truepera* and *Chryseolinea* were the most dominant bacteria for degrading PHA-MPs (as described in section 3.3.1). These results confirmed that LMBC addition changed the dominant bacterial biomarkers for degrading PHA-MPs. Compared with the control, LMBC addition enriched more dominant bacterial biomarkers (such as *Firmicutes*, *Proteobacteria*, *Bacillus*, *Thermobacillus*, *Luteimonas* and *Chryseolinea*) for degrading MPs, thus improving the degradation rate of PHA-MPs in the T1 mixture (Figs. 1–3; Tokiwa et al., 2009; Lambert and Wagner, 2017), which was consistent with the results of RDA.

Fungal biomarkers of 14 taxa with different abundances were detected during various composting stages (Fig. 5b; Table S4). The fungal biomarkers of thermophilic (0–7 d), cooling (7–28 d) and maturity (28–60 d) stages were 8 (Control=2; T1=6), 1 (Control=0; T1=1), and 5 (Control=0; T1=5), respectively, which were different from the results of bacterial biomarkers. The number of biomarkers in the T1 mixture at each stage was higher than that of control, indicating LMBC addition increased the number of fungal biomarker, which was in line with the findings of Liu et al. (2021). These fungal biomarkers were different at each stage of composting, while all biomarkers in both mixtures belonged to the phyla *Ascomycota* and *Basidiomycota*.

At the thermophilic stage of composting, *Ascomycota* (phylum) and *Tremellomycetes* (class) were enriched in the control mixture, while six taxa, predominantly *Aspergillaceae* (family), *Aspergillus* (genus) and *Basidiomycota* (phylum), were enriched in the T1 mixture. Maeda et al. (2005) found that *Aspergillus* was able to produce cutinase to decompose biodegradable plastics and utilize the polymer as a carbon source. In this study, the RDA also demonstrated that *Aspergillus* in the T1 mixture was positively correlated with the degradation rate of PHA-MPs, which suggested that LMBC addition was favorable for the enrichment of *Aspergillus*, and consequently promoting the degradation of PHA-MPs. During the cooling and mature stages, no fungal biomarker was detected in the control, while six taxa were enriched in the T1 mixture predominantly with *Aspergillus-fumigatus* (Species), *Mycothermus* (genus) and *Mycothermus-thermophilus* (Species). The biomarkers (e.g., *Aspergillus* and *Mycothermus*) in the T1 mixture had positive effects on PHA-MPs degradation rate (Fig. S2), where LMBC addition enriched these dominant fungal biomarkers, promoting PHA-MPs degradation in the mature stage (Fig. 1a).

3.5. Microbial community network analysis (co-occurrence network)

The co-occurrence pattern at the genus level illustrated the relationship between microbial community and degradation characteristics of PHA-MPs in the control and T1 mixtures (Fig. 6). The co-occurrence network pattern of microbial community and key topological features of the network at the genus level showed obvious differences between the control and T1 mixture (Fig. 6; Table. 1). For the bacterial co-occurrence networks, the total edges in the LMBC amended mixture (1,158) were more than those in the control (856), showing that the structure of the network pattern in the T1 mixture was more complex than that in the control (Bello et al., 2020). The central coefficient values of the three degradation characteristics (carbon loss, oxygen loading and degradation rate of PHA-MPs) in both mixtures were the largest among all nodes. The numbers of bacterial nodes connecting these three nodes in the T1 mixture increased by 38%, 49% and 27%, respectively, compared to the control, indicating that LMBC addition enhanced the commensalism and/or mutualism relationship between bacteria and PHA-MPs degradation characteristics, and consequently boosted the degradation rate of PHA-MPs (Figs.1–3; Deng et al., 2021). Compared to the control, the values of average clustering coefficient and average path length of the LMBC amended mixture were increased by 36.5% and 7.6%, meaning that adding biochar promoted the close relationship between bacteria and MPs degradation (Ye et al., 2016). In this study, the dominant bacteria for degrading PHA-MPs (e.g., *Thermobacillus*, *Bacillus*, *Luteimonas* and *Chryseolinea*) in the T1 mixture had a more positive correlation with PHA-MPs degradation characteristics than those in control (Fig. S2). Therefore, it could be concluded that LMBC changed the network pattern of bacterial community and PHA-MPs degradation by increasing the complexity and connectivity of microorganisms.

Compared to the control, the co-occurrence network of the fugal community at the genus level in the T1 mixture was more complex and well-connected compared to the control mixture. The values of total edges, average clustering coefficient and average path length in the LMBC amended mixture were increased by 96.7%, 26.5% and 7.9%, respectively, indicating that LMBC addition promoted the close relationship between the fungal community and PHA-MPs degradation too. Moreover, the positive correlation between fungi and degradation characteristics of PHA-MPs in the T1 mixture was significantly higher than that in the control. The preponderant fungi for degrading PHA-MPs (e.g., *Aspergillus* and *Mycothermus*) in the T1 mixture had a higher positive interaction with PHA-MPs degradation characteristics than the control, further confirming that LMBC addition accelerated the biodegradation of PHA-MPs (Figs. 1–3).

4. Conclusions and environmental implications

The addition of LMBC promoted the degradation rate, carbon loss, and oxygen loading of PHA-MPs compared to the control mixture (without LMBC addition) during the composting process. LMBC addition accelerated the surface roughness, cleaved the C-H bonds of PHA-MPs, and enhanced the hydrophilicity of MPs via increasing the oxygen-containing surface functional groups. Moreover, LMBC addition changed the composition, structure and biomarkers of the microbial community to promote PHA-MPs degradation. LMBC addition also enhanced the commensalism and mutualism relationships between microorganisms and PHA-MPs degradation characteristics, and thus contributed to a higher degradation rate of PHA-MPs than in the control treatment. Our findings are of crucial significance for reducing MPs' abundance in organic waste and mitigating the potential environmental and human health risks of MPs. However, it is essential to further optimize the composting process improving MPs degradation efficiency and more attention should also be paid to the interaction between composted MPs and pollutants. More studies about the impact of biochars produced at different temperatures on the MPs biodegradation during composting process and also in amended soils are required.

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Table 1. Network of key topological features for composting microbial communities in the control and T1 mixture

Network indices	**Bacteria**		**Fungi**	
	Control	**T1**	**Control**	**T1**
Total nodes	30	30	30	30
Total edges	856	1158	241	474
Average clustering coefficient	0.425	0.580	0.468	0.592
Average path length	4.582	4.928	4.957	5.348

Control: mixture containing 0.5% PHA microplastics, T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust.

Figure Captions

Fig. 1. Degradation rate and size distribution of polyhydroxyalkanoate (PHA) microplastics during the composting process. (a) PHA microplastic % represented the percentage of PHA microplastics content present in the compost, degraded PHA % represented the percentage of degraded PHA microplastics in the compost; (b) represented the size distribution of PHA microplastics in the control mixture during the composting process and (c) represented the size distribution of PHA microplastics in the T1 mixture during the composting process. Control: mixture containing 0.5% PHA microplastics; T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust. The error bars mean the amount of standard deviation among three replications.

Fig. 2. SEM images and EDS spectra showing elemental contents of different microplastics. Figs. (a-d) show carbon and oxygen contents of polyhydroxyalkanoate (PHA) microplastics in the Control mixture during the composting process; Figs. (e-h) show the same in the T1 mixture. Control: mixture containing 0.5% PHA microplastics; T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust.

Fig. 3. FTIR spectra of polyhydroxyalkanoate (PHA) microplastics from the control (a) and T1 (b) compost mixtures. Control: 0.5% PHA microplastics; T1: 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust.

Fig. 4. Relative abundance of the top 10 bacterial (a) and fungal (b) taxa at genus level over the composting period. Control: mixture containing 0.5% PHA

microplastics; T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust.

Fig. 5. Linear discriminant effect size (LEFSe) analysis results showing bacterial (a) and fungal (b) biomarkers (at phylum level) sensitive to polyhydroxyalkanoate (PHA) microplastics during composting with biochar addition. Circles radiating from the inside out in the evolutionary branching diagram represent taxonomic levels from phylum to genus (or species). Each small circle at different taxonomic levels represents a taxon, and the diameter of the small circle is proportional to the relative abundance. Coloring principle: species without significant differences are uniformly colored yellow. Control: mixture containing 0.5% PHA microplastics; T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust.

Fig. 6. Network model showing the co-occurrence patterns between bacterial species (top 30 genera) and degradation characteristics of polyhydroxyalkanoate (PHA) microplastics in the control (a) and T1 (b) compost mixtures, and between fugal species (top 30 genera) and degradation characteristics of PHA microplastics in the control (c) and T1 (d) compost mixtures. Control: mixture containing 0.5% PHA microplastics, T1: mixture containing 0.5% PHA microplastics and 10% biochar. Both control and T1 contained equal amounts of cow manure and sawdust. A connection represents a significant correlation ($p < 0.05$) according to Spearmant's rank analysis. Pink color indicates positive correlation, while purple indicates negative correlation.

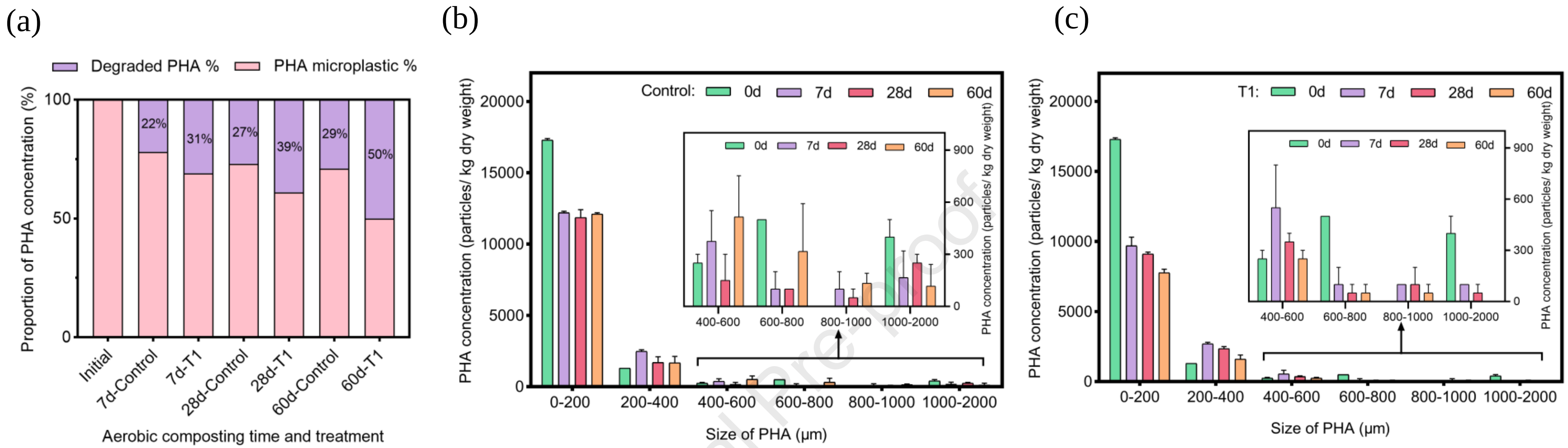

(a)
Degraded PHA %
PHA microplastic %
Proportion of PHA concentration (%)
100
50
0
22%
31%
27%
39%
29%
50%
Initial
7d-Control
7d-T1
28d-Control
28d-T1
60d-Control
60d-T1
Aerobic composting time and treatment
(b)
Control: 0d 7d 28d 60d
PHA concentration (particles/ kg dry weight)
20000
15000
10000
5000
0
0-200
200-400
400-600
600-800
800-1000
1000-2000
Size of PHA (μm)
0d 7d 28d 60d
900
600
300
0
PHA concentration (particles/ kg dry weight)
(c)
T1: 0d 7d 28d 60d
PHA concentration (particles/ kg dry weight)
Size of PHA (μm)

Fig. 1.

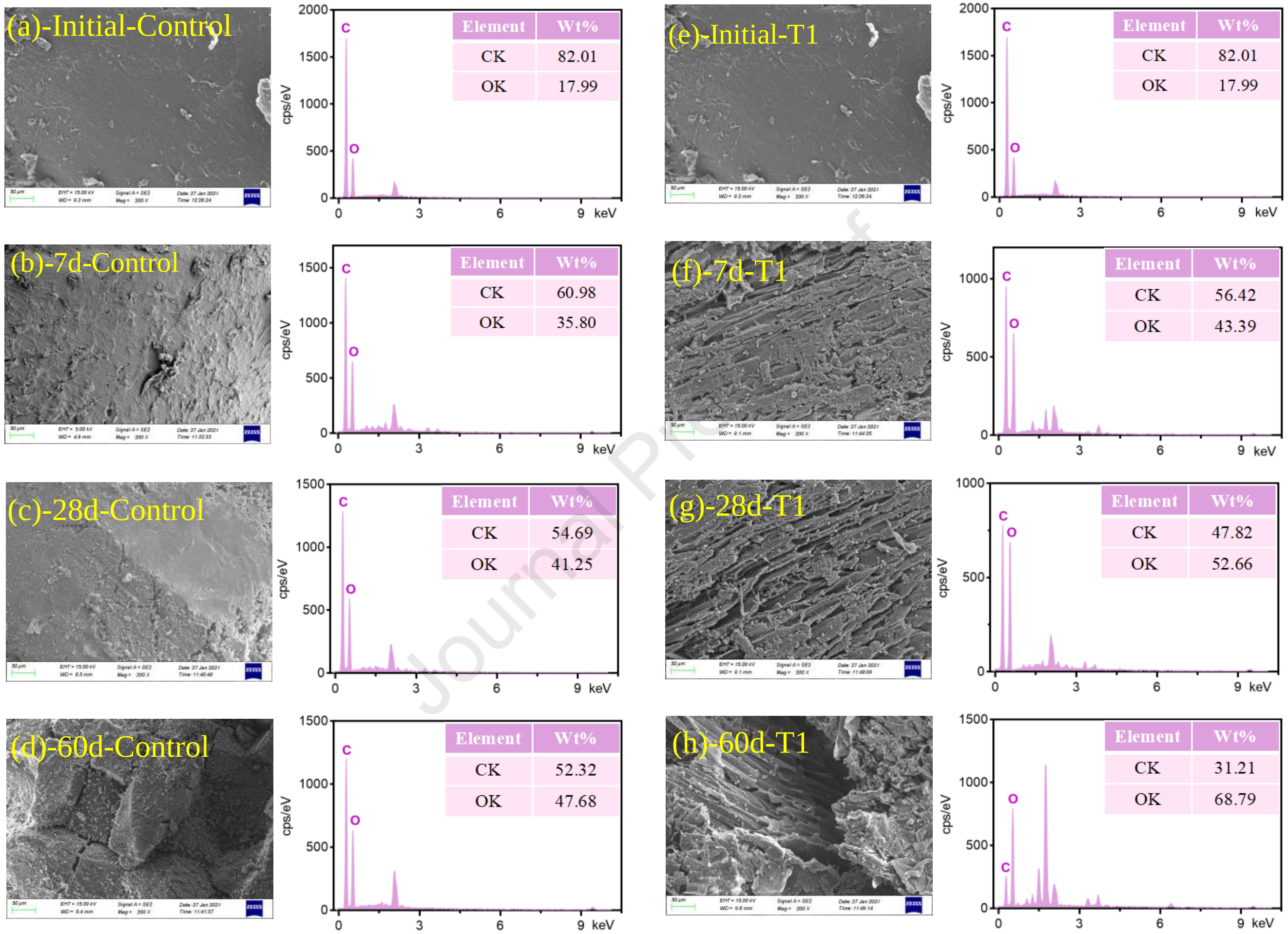

(a)-Initial-Control
Element Wt%
CK 82.01
OK 17.99
(b)-7d-Control
Element Wt%
CK 60.98
OK 35.80
(c)-28d-Control
Element Wt%
CK 54.69
OK 41.25
(d)-60d-Control
Element Wt%
CK 52.32
OK 47.68
(e)-Initial-T1
Element Wt%
CK 82.01
OK 17.99
(f)-7d-T1
Element Wt%
CK 56.42
OK 43.39
(g)-28d-T1
Element Wt%
CK 47.82
OK 52.66
(h)-60d-T1
Element Wt%
CK 31.21
OK 68.79
cps/eV
keV

Fig. 2.

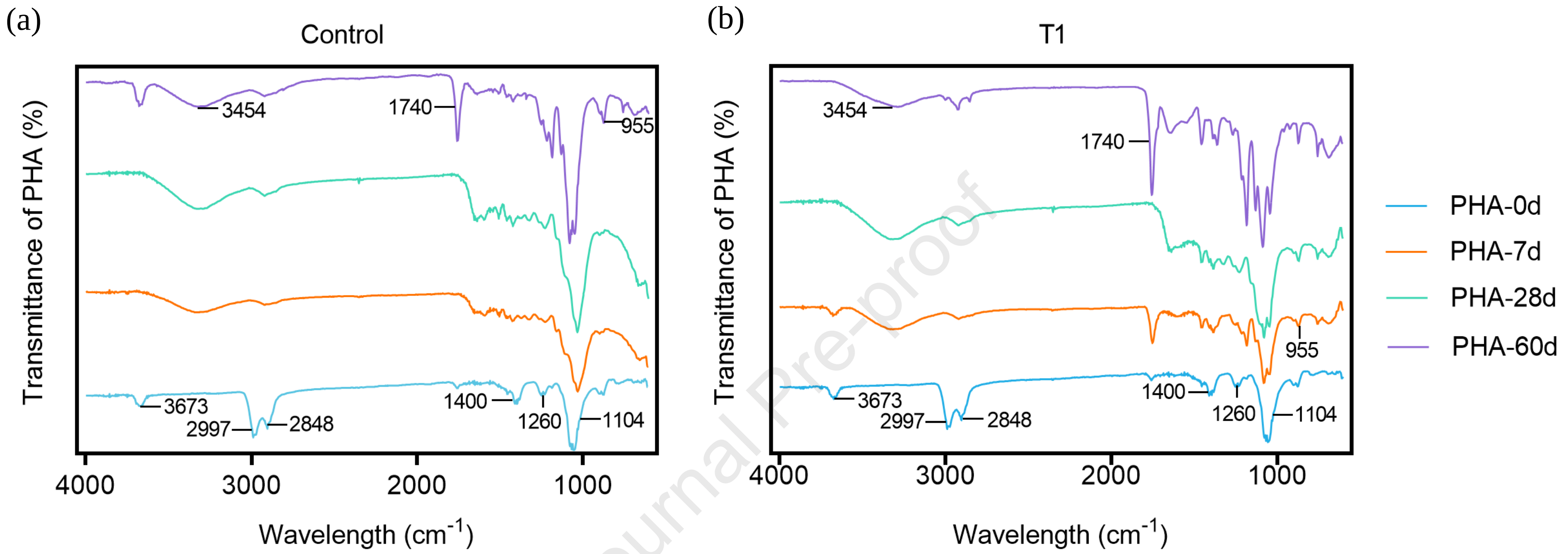

(a)
Control
Transmittance of PHA (%)
3454
1740
955
3673
2997
2848
1400
1260
1104
4000
3000
2000
1000
Wavelength (cm-1)
(b)
T1
Transmittance of PHA (%)
3454
1740
955
3673
2997
2848
1400
1260
1104
4000
3000
2000
1000
Wavelength (cm-1)
PHA-0d
PHA-7d
PHA-28d
PHA-60d

Fig. 3.

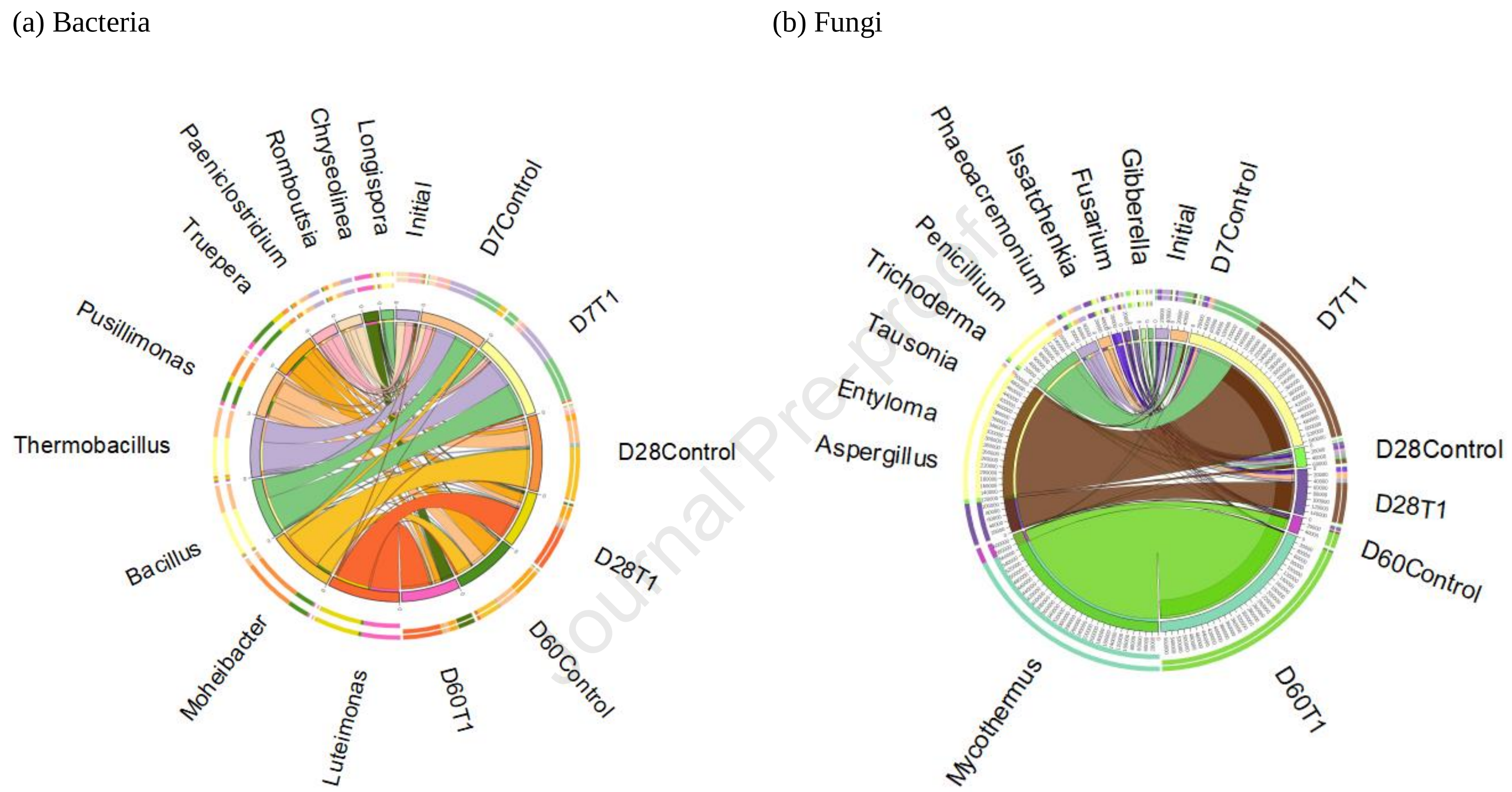

(a) Bacteria
Paeniclostridium
Romboutsia
Chryseolinea
Longispora
Initial
D7Control
D7T1
Truepera
Pusillimonas
Thermobacillus
D28Control
Bacillus
D28T1
Moheibacter
Luteimonas
D60T1
D60Control
(b) Fungi
Phaeoacremonium
Issatchenkia
Fusarium
Gibberella
Initial
D7Control
Penicillium
Trichoderma
Tausonia
Entyloma
Aspergillus
D7T1
D28Control
D28T1
D60Control
Mycothermus
D60T1

Fig. 4.

(a) Bacteria

(b) Fungi

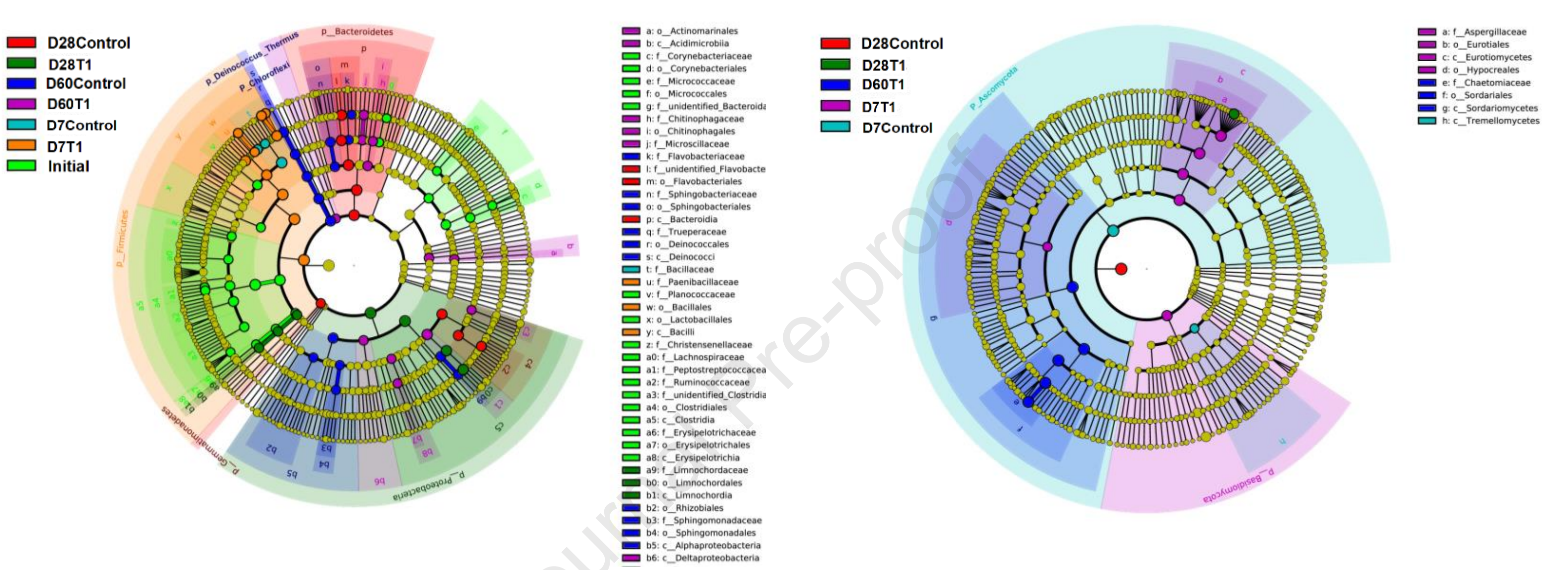
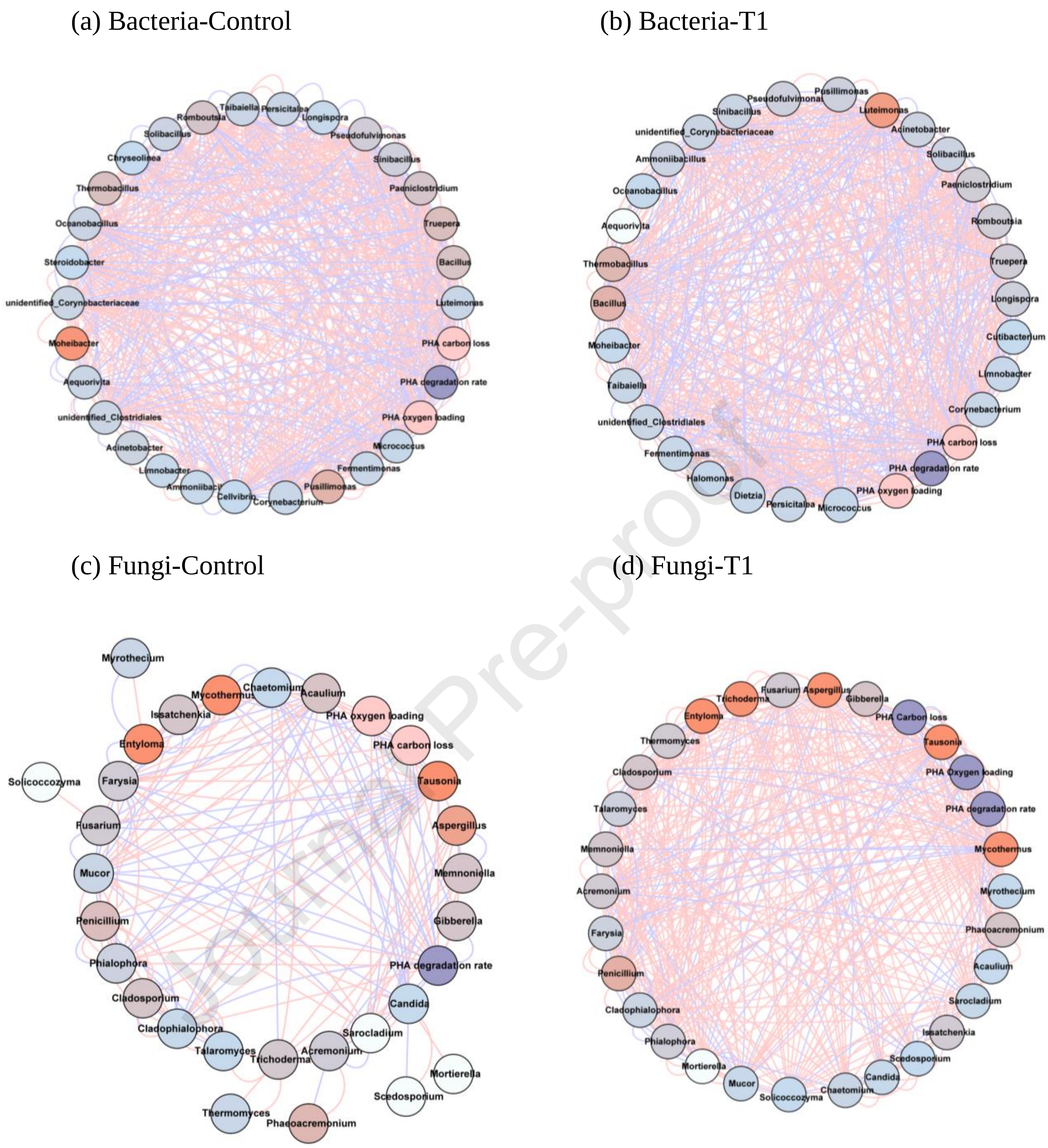


Fig. 5



(a) Bacteria-Control
Romboutsia
Taibaiella
Persicitalea
Longispora
Pseudofulvimonas
Solibacillus
Sinibacillus
Chryseolinea
Paeniclostridium
Thermobacillus
Truepera
Oceanobacillus
Bacillus
Steroidobacter
unidentified_Corynebacteriaceae
Luteimonas
Moheibacter
PHA carbon loss
Aequorivita
PHA degradation rate
unidentified_Clostridiales
PHA oxygen loading
Acinetobacter
Micrococcus
Limnobacter
Fermentimonas
Ammoniibacillus
Pusillimonas
Cellvibrio
Corynebacterium
(b) Bacteria-T1
Pseudofulvimonas
Pusillimonas
Sinibacillus
Luteimonas
unidentified_Corynebacteriaceae
Acinetobacter
Ammoniibacillus
Solibacillus
Oceanobacillus
Paeniclostridium
Aequorivita
Romboutsia
Thermobacillus
Truepera
Bacillus
Longispora
Cutibacterium
Moheibacter
Limnobacter
Taibaiella
Corynebacterium
unidentified_Clostridiales
PHA carbon loss
Fermentimonas
PHA degradation rate
Halomonas
PHA oxygen loading
Dietzia
Persicitalea
Micrococcus
(c) Fungi-Control
Myrothecium
Chaetomium
Mycothermus
Acaulium
Issatchenkia
PHA oxygen loading
Entyloma
PHA carbon loss
Solicoccozyma
Farysia
Tausonia
Fusarium
Aspergillus
Mucor
Memnoniella
Penicillium
Gibberella
Phialophora
PHA degradation rate
Cladosporium
Candida
Cladophialophora
Sarocladium
Talaromyces
Trichoderma
Acremonium
Mortierella
Scedosporium
Thermomyces
Phaeoacremonium
(d) Fungi-T1
Fusarium
Aspergillus
Trichoderma
Gibberella
Entyloma
PHA Carbon loss
Thermomyces
Tausonia
Cladosporium
PHA Oxygen loading
Talaromyces
PHA degradation rate
Memnoniella
Mycothermus
Acremonium
Myrothecium
Farysia
Phaeoacremonium
Penicillium
Acaulium
Cladophialophora
Sarocladium
Phialophora
Issatchenkia
Scedosporium
Mortierella
Candida
Mucor
Chaetomium
Solicoccozyma

Fig. 6

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: