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Distinct microbial community structures formed on the biofilms of PLA and PP, influenced by physicochemical factors of sediment and polymer types in a 60-day indoor study

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Microplastics (MPs) are colonized by biofilm-forming microbes. Biodegradable plastics, popular replacements for traditional plastics, still have unknown biofilm formation characteristics. We conducted a 60-day indoor experiment, where sediment was exposed to traditional MPs (polypropylene, PP), biodegradable MPs (polylactic acid, PLA), and glass beads (GLASS). The microbial communities in the MPs-biofilm were analyzed using high-throughput sequencing. Results indicated that Proteobacteria was the dominant phylum on all substrates, followed by Actinobacteria, and Firmicutes. At the genus level, the majority of microorganisms colonizing PP possessed nitrification and denitrification capabilities, while the dominant bacteria on PLA were capable of degrading lignin, cellulose and carbon metabolism. The genus *Sphingomonas*, a promising bacteria capable of degrading biodegradable microplastics, was particularly discovered on the PLA biofilm, meanwhile, bacterial colonization of PLA indirectly increased the potential for human transmission of pathogens. Redundancy analysis revealed that the pH and moisture significantly affected the bacterial communities. Pearson correlation heatmap indicated that the abundance of the majority of dominant bacterial genera of two MPs biofilms is negatively correlated with the physicochemical parameters of sediment (pH, moisture, TN, TP), except for salinity. The microbial communities associated with PP and PLA exhibited distinct differences caused by the combined effects of changes in physicochemical properties of sediment and different material substrates. This study provides further evidence of the significant selective features exhibited by

Abbreviations: MPs, microplastics; PP, polypropylene; PLA, polylactic acid; Glass beads, GLASS; PS, polystyrene; PET, polyethylene terephthalate; PA, polyamide; PE, polyethylene; PCL, polycaprolactone; PVC, polyvinyl chloride; PUF, polyurethane; PBAT, polyadipate/butylene terephthalate; LDPE, low density polyethylene; HDPE, high-density polyethylene; PHBV, 3-hydroxybutyrate-co-3-hydroxyvalerate; M. aeruginosa, Microcystis aeruginosa; TP, total phosphorus; TN, total nitrogen; EPS, extracellular polymeric substances; Mb, MaterBi.

microbial colonization on these two MPs when exposed to the same source community, offering insights into the exploration of promising bacteria for MPs degradation.

KEYWORDS

microplastic, PP, PLA, sediment, biofilm, bacterial community structure

1 Introduction

Plastic products are widely used in daily necessities packaging, transportation, cutting-edge industries, fishing gear applications and other fields (Ribic et al., 2010; Wagner et al., 2014; Karkanorachaki et al., 2021). Plastic fragments or particles with diameter < 5 mm are defined as microplastics (MPs), which are widely distributed in oceans and freshwater (Law and Thompson, 2014; Auta et al., 2017; Gigault et al., 2018; Yang et al., 2022; Kurniawan et al., 2023). Zhang et al. summarized relevant research of MPs on surface water in China in the past decade: the MPs abundances of rivers and lakes were 3.9–7900 and 340–8900 n/m^3, respectively (Zhang et al., 2020). Due to the influence of hydrological conditions, biological enrichment, and microplastic sedimentation, microplastics in the water column would settle into the sediment, resulting in the abundance of microplastics in the sediment often higher than that in the water column (Kukulka et al., 2012; Long et al., 2015; Scherer et al., 2020; Chauhan et al., 2021; Zhao et al., 2023).

Microplastics remain in sediment as an emerging pollutant, becoming a carrier for microbial colonization. As early as 1972, Carpenter et al. discovered that floating small pieces of plastic with attached microorganisms, such as diatoms, in the Sargasso Sea area of the North Atlantic Ocean (Carpenter et al., 1972). Microplastic biofilm enrichment special microorganisms, such as antibiotic-resistant bacteria, pathogenic microorganisms and plastic-degrading microorganisms that will have an impact on environmental microecological processes (Liu et al., 2022; Fajardo et al., 2023; Perveen et al., 2023; Zheng et al., 2023; Vercauteren et al., 2024); they may also increase the surface biology of microplastics, making them easier to be swallowed by organisms and enter the food chain (Rios et al., 2007). The community composition of plastic-associated biofilms was affected by microplastic properties, water types and environmental factors (Arias-Andres et al., 2018; Frère et al., 2018; Nguyen et al., 2022; Saygin et al., 2024). The polymer type of microplastics directly affects the carbon source of microorganisms colonizing its surface, and therefore is considered to be the most important factor affecting the microbial community on its surface (Qiang et al., 2021). It was reported that PS microplastic showed different bacterial communities than PE and PP in the Bay of Brest (Frère et al., 2018). Biofilms were more often detected on plastics (PS, PET, PA, PP) than on natural materials (stone, wood), furthermore, the biofilms detected on plastics were more complex, with higher microbial diversity (Raposo et al., 2022). Studying the interactions between MPs and biofilms is crucial for understanding the degradation of MPs in aquatic environments. As synthetic polymers are not water soluble, biofilm-forming bacteria degrade such materials more efficiently than planktonic strains.

Biodegradable plastics as an alternative of conventional plastics have been considered as a sustainable solution to plastic pollution, therefore, there will be an increasing presence of biodegradable MPs in water. It is worth noting that the accumulation of biodegradable MPs may cause more potential risk in aquatic environment than conventional MPs (Bandopadhyay et al., 2018). Among currently available bioplastics (biodegradable plastics), PLA is one of the bio-based and biodegradable plastics of great commercial value (Karan et al., 2019). It was reported that the interaction between PLA-MPs and *M. aeruginosa* could promote the degrading of PLA-MPs meanwhile favoring *M. aeruginosa* growth after 63 days, causing increasing harmful cyanobacteria biomass (Tang et al., 2024). To date, most studies have focused on the effects of nondegradable plastic types commonly found in aquatic environments on biofilm formation (Akdogan and Guven, 2019; Chen et al., 2020; Shabbir et al., 2020; Sun et al., 2023). Furthermore, metabolic pathway analysis showed that microorganisms embedded in microplastic biofilms have greater potential for biodegradation and metabolism (Jiang et al., 2018). Biofilms formed on wood and the biodegradable polymer (polycaprolactone, PCL) exhibited a higher abundance of *Alphaproteobacteria*, whereas biofilms on conventional synthetic polymers like polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) were predominantly comprised of *Gammaproteobacteria* (Bhagwat et al., 2021). Therefore, studying the characteristics of biodegradable MPs biofilms is more beneficial for a comprehensive understanding of the interactions between such MPs and microbial communities in water, as well as understanding the behavior and potential toxicity of biodegradable plastic polymers in aquatic environment.

Up to now, our understanding of biofilm formation on microplastics mainly relies on *in situ* observations. Field studies have shown that various factors such as seasonality, geography, and hydrology can affect the community composition of plastic-associated biofilms (Oberbeckmann et al., 2014). Furthermore, field sampling *in situ* is subject to inherent variability and uncertainty, while replicable studies with controlled exposure conditions are lacking. In this study, we aimed to 1) examine the effects of substrate type on microbial community assembly, using traditional MPs (polypropylene, PP), biodegradable plastics (polylactic acid, PLA), and a non-plastic substrate (glass beads). The glass beads acted as an inert substrate, providing a surface for colonization but not serving as an energy source for microorganisms; 2) Notably, sediment samples collected from Nan Lake in Wuhan city were subjected to a 60-day exposure to these substrates under precisely controlled conditions. The correlation analysis between changes in soil physicochemical properties caused by two different types of MPs and microbial communities on the biofilms also needs to be explored. This study contributes to a comprehensive understanding of the relationship between MPs and microbial communities, providing

a reference method for screening promising microorganisms capable of degrading MPs. It will be beneficial for the prevention and control of MPs pollution in water. Additionally, it allows for a more comprehensive understanding of the behavior and potential toxicity of MPs in aquatic environments.

2 Materials and methods

2.1 Sediment and water sampling

The test sediment and water were collected from Nan Lake, a typical urban lake (30°28′59.2″N, 114°23′3.6″E) in Wuhan, China. Water samples were pre-filtered with a 6-μm sieve to remove potential grazers that may feed on bacteria during the incubation. Sediment samples were sealed in aluminum foil bags and transported back to the lab. After removing impurities (such as plant residues and shells), the sediment was thoroughly mixed and then naturally dried before biofilm experiment.

Microplastics were extracted from sediment samples using a density separation method (Rodrigues et al., 2018; He et al., 2020), three replicates were carried out for each sample. Under a stereo microscope (XTD-7045A, China Coissm) with magnifications ranging from 40 to 100 x, observations and documentation of the color and shapes of plastic particles on the filter paper are documented. Subsequently, particles within the size range of 0.63–5.00 mm are analyzed through a Fourier Transform Infrared Spectrometer (FTIR, Vertex 70, Germany Bruker) within a scan frequency spectrum of 4000 to 400 cm^{-1}. This analysis aims to identify the types of polymers present in the sediment of Nan Lake.

2.2 Biofilm development experiment

According to the results of MPs investigation, traditional microplastic beads (PP), biodegradable microplastics (PLA), as well as non-plastic beads (GLASS) within a diameter of 2 mm were used to evaluate biofilm formation. To prepare for the indoor experiment, the above-mentioned substrates were first soaked in a 2% SDS solution. Afterward, they were rinsed several times with sterile water to remove any leftover reagents from the surface. Following this, the substrates were soaked in 75% ethanol for 24 h and rinsed three more times with sterile water. They were then dried in a drying oven. To minimize the presence of microorganisms, the substrates were exposed to ultraviolet light for 1 hour for sterilization. Lastly, they were stored in sterile bags at a temperature of 4°C until ready for use. The ratio of substrate (M) to sediment (M) was 0.70 g:1 kg. Each substrate weighing 0.7 g was added separately to 1 kg of sediment. This process was repeated three times, ensuring thorough mixing for a duration of 60 days. The incubation was performed under controlled light (12:12 h of light: dark) and temperature (25°C). The whole process of microplastic dosing was carried out on the aseptic operation table. At the beginning of the experiment, a sterile sealing film was used to seal the beaker mouth to avoid contamination by external microorganisms. Pure water was added daily to keep the water level at 1 cm.

2.3 DNA extraction and sequencing

On the day 60, the substrate material was vacuum filtered on a hydrophilic nylon membrane (0.2 μm Millipore), rinsed with sterile water and separated from the filter using sterile stainless-steel pincers or scraper into Eppendorf tubes, after which half of each sample was used for DNA extraction. The TGuide S96 Magnetic soil DNA kit (Tiangen Biotech Beijing Co., Ltd.) was used to extract genomic DNA from substrate samples according to the manufacturer's protocol. Primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the 16S rRNA hypervariable region V3-V4. PCR merchandise was examined with agarose gel and purified by omega DNA ablution kit (Omega Inc., Norcross, GA, United States). Illumina Novaseq 6000 platform was used for the collection of pure PCR products.

2.4 Data analysis

The quality of the original DNA sequence was optimized through the QIIME program, and the optimized sequences were clustered into operational taxonomic units (OTUs) for species classification based on 97% similarity. The diversity index (Shannon, Simpson), richness index (Chao1) and coverage index of bacterial communities were calculated using the QIIME2 software (Bolyen et al., 2019). Weighted principal coordinate analysis (PCoA) based on Bray-Curtis distance was used to determine the changes in microbial community structure. Differentially abundant taxa were identified by linear discriminated analysis (LDA) with effect size (LEfSe) (Segata et al., 2011). Redundancy analysis (RDA) and correlation heatmap analyses of physicochemical factors and microbial community were carried out with Majorbio Cloud Platform (www.majorbio.com).

3 Results

3.1 Link between bacterial community on biofilms and physicochemical factors of sediments

The physicochemical properties of test sediment, as well as the characteristics of MPs were determined. The results showed that the abundance of MPs in sediment was relatively low (68 ± 1.98 n/kg), thus, the influence of background value on subsequent experiment can be ignored. Besides, as shown in Table 1, MPs identified in the sediment of Nan Lake are commonly found and include PP, with all particles having a diameter less than 4 mm. Although PLA was not detected in this sampling, it is not excluded that other sampling sites or freshwater lakes may contain such biodegradable microplastics. Therefore, we selected PP and PLA for comparative analysis of the dissimilarity in microbial communities growing on biofilms. The effects of adding three matrix materials on the physicochemical of sediment were also analyzed as shown in Table 2, the addition of both PP and PLA decreased the physicochemical indicators, including total phosphorus (TP), total nitrogen (TN), and

TABLE 1 Physicochemical properties of test sediment used in the study.

Test items	pH	Moisture content (%)	Salinity (%)	TP content (mg•g^{-1})	TN content (mg•g^{-1})
Day 1 (CK)	7.60	52.62	1.98	1.61	2.17
Day 60 (CK)	7.52	46.13	2.28	1.52	2.08
Day 60 (PP group)	7.25	43.45	2.03	1.36	1.78
Day 60 (PLA group)	6.95	42.28	1.95	1.02	1.36
Day 60 (Glass group)	7.45	45.93	2.19	1.59	2.01

TABLE 2 Characteristics of MPs in sediment of Nan Lake.

Polymer	Main size	Main color	Shape
Polyethylene terephthalate (PET) Polystyrene (PS) Polypropylene (PP) Polyethylene (PE) Nylon	<4 mm (over 95%)	Transparent (40%–45%) Blue (25%–30%) Purple (5%) Red (10%) Others (10%)	Fiber (>40%) Granule (>45%) Pellet, Film (<15%)

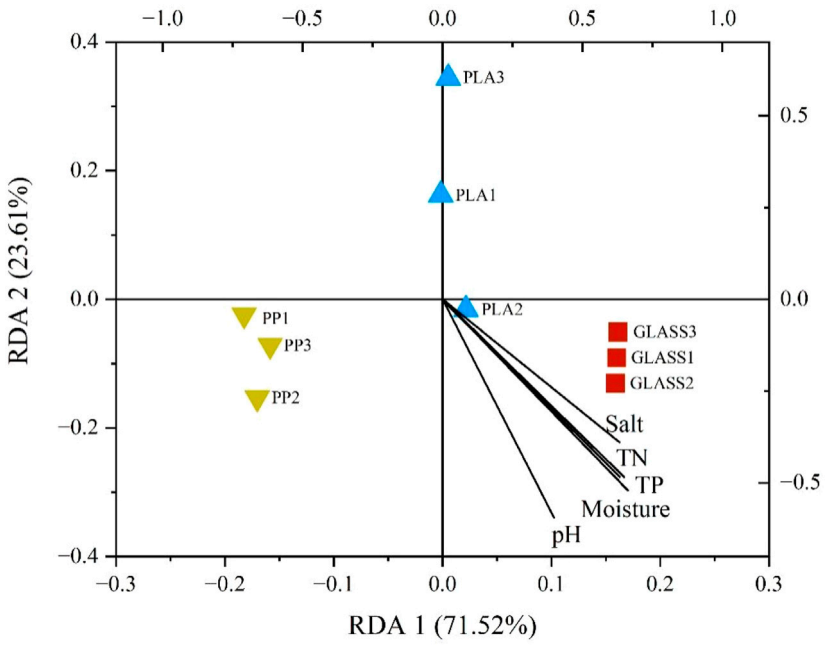

FIGURE 1
Redundancy analysis (RDA) graph for the microorganism community and sediment parameters.

pH value ($p < 0.05$). However, the addition of GLASS had no effect on the physicochemical properties of sediment ($p > 0.05$).

Based on redundancy analysis (RDA), as shown in Figure 1, results revealed that TN, TP and moisture have a strong correlation with each other. Furthermore, the effects of pH and moisture on the overall diversity distribution of the sample communities are the most significant. Compared to GLASS, the bacterial communities of MPs are most influenced by the physicochemical factors. The spearman correlation heatmap analysis was used to investigate the relationship between the sediment physicochemical parameters and relatively microbial abundance of dominated bacterial genera (top 20) during 60-days biofilms formation (Figure 2). Results showed that four physicochemical parameters (pH, moisture, TP and TN) showed a significant negative correlation with the dominant bacterial genera of PLA, such as *Sphingomonas* and *Arthrobacter*, while these four parameters showed no correlation with genus *Acinetobacter* and *Staphylococcus,* some species of these bacteria being common pathogens. Salinity did not correlate with the above bacteria on the PLA biofilm; conversely, it exhibited a positive correlation with *Rhizobacter*, *Staphylococcus*, and *Arthrobacter* on the PP biofilm. The three parameters (moisture, TP and TN) indicated a negative correlation with the predominant

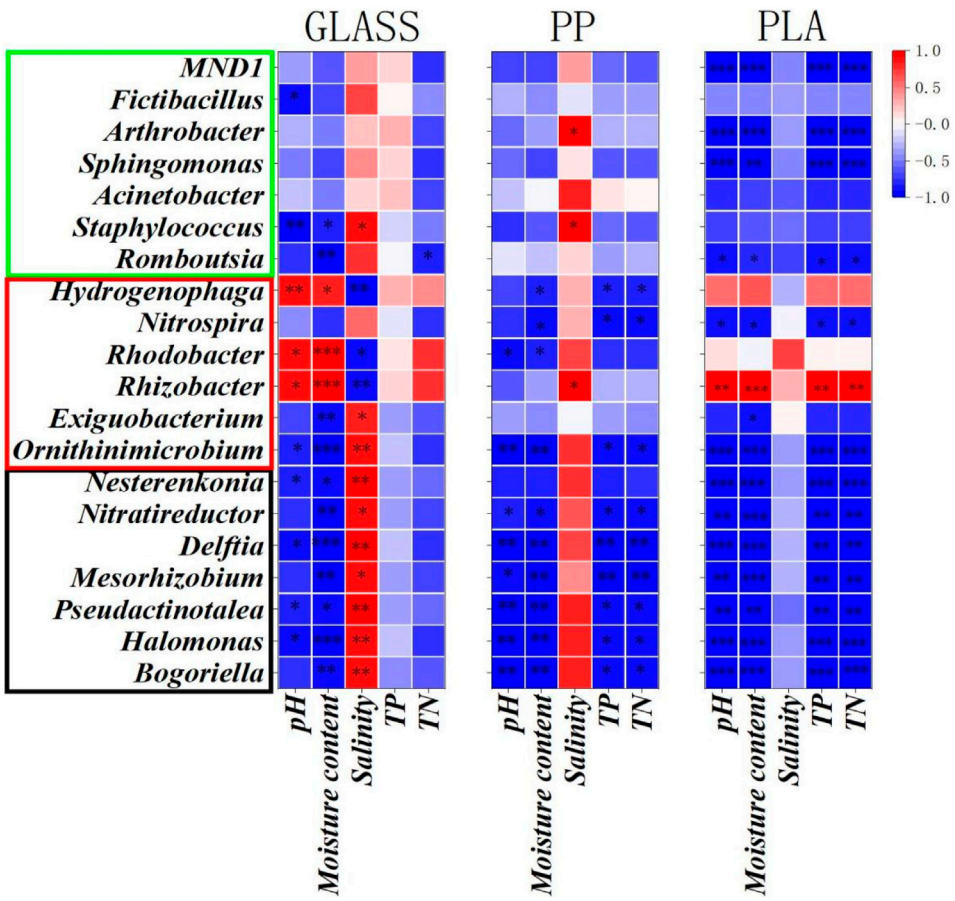

FIGURE 2
Correlation heatmap of the top 20 genera and physicochemical factors during the 60-day biofilms formation in GLASS, PP, and PLA. The bacteria within the red box, green box, and black box represent the dominant genera in PLA, PP, and GLASS biofilms, respectively. (P < 0.05, P<0.01 and P < 0.001 are demoted as *, **, and ***, respectively.).

bacterial genera in PP, specifically *Nitrospira and Hydrogenophaga,* both of which were involved in nitrification and denitrification processes. TP and TN are not correlated with the dominant genera on GLASS, but salinity showed a significant positive correlation with them.

3.2 Alpha diversity analysis of bacterial community composition on different substrate surfaces

After 60 days of incubation, a total of 12,149 OTUs were acquired. The numbers of bacterial OTUs on the surfaces of GLASS, PP and PLA were 3125, 3095, and 2944 respectively. The results of Shannon, Simpson, Chao1 and Goods_coverage indexes of each substrate surface as shown in Figure 3. In each treatment group, there was no significant difference in Goods_coverage index, and all were greater than 0.98, indicating that the 16S sequences in the samples were completely detected. The Shannon and Simpson index of the bacterial community on the PP surface were both higher than those of other substrates, especially the Simpson index of the bacterial community on the PP surface was significantly higher than that of other substrates ($p < 0.05$), indicating that the bacterial community diversity on the PP biofilm was higher.

3.3 Beta diversity analysis of bacterial community composition on different substrate surfaces

In Figure 4, the PCoA analysis showed alterations in microbial communities (OTUs based) across three treatments. A significant disparity (ANOISM test, $p < 0.01$) was observed among the different treatments, with PC1 and PC2 explaining 74% and 10% of the total variation, respectively. The PCoA analysis reveals that the microbial community structures on the PLA and GLASS surfaces are remarkably similar, whereas those on the PLA and PP surfaces show much less similarity. As a non-plastic material, GLASS itself does not contain carbon sources and cannot sustain the growth and proliferation of microorganisms; it only provides a colonization surface for microorganisms. Therefore, the microbial community on the glass surface can indicate the native microorganisms in the sediment.

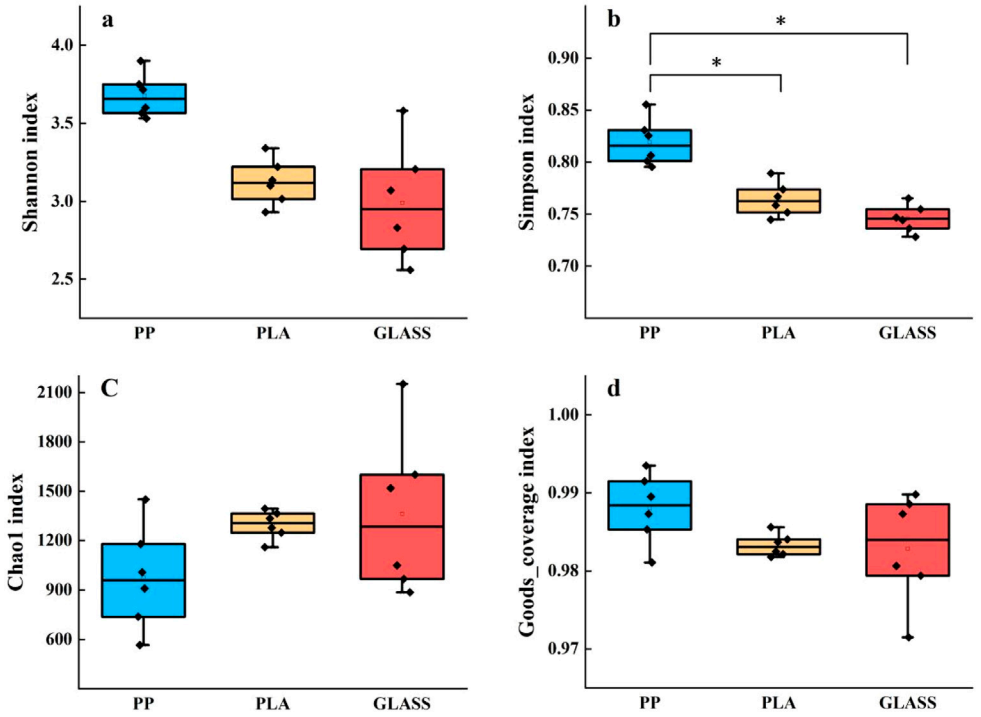

FIGURE 3
α diversity of bacterial communities on different substrates. **(A)** Shannon index; **(B)** Simpson index; **(C)** Chao1 index; **(D)** Goods_coverage index (* represented a significant difference between groups ($p < 0.05$)).

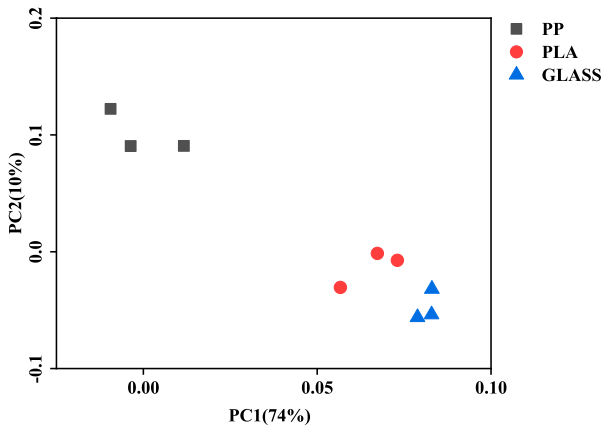

FIGURE 4
Principal coordinate analysis (PCoA) analysis of bacterial communities based on Bray-Curtis distance across three treatments at the OTUs level.

3.4 Distinct bacterial community structures in different substrates

The distribution of relative abundance of different bacterial community structures at phylum levels of different substrates was shown in Figure 5. The top 10 bacterial categories on each substrate surface were listed. It can be seen that Proteobacteria was the dominant phylum in each substrate (66.43%–71.83%), followed by Actinobacteria (20.52%–23.23%) and Firmicutes (1.59%–6.13%). The relative abundance of Firmicutes on the surface of PLA (6.13%) was higher than that of PP (1.59%) and GLASS (3.82%) ($p < 0.05$). It was worth noting that Cyanobacteria phylum occupied a higher abundance on the PP surface compared to PLA and GLASS ($p < 0.001$).

As shown in Figure 6, the heatmap indicated that the dominant genera (top 20) on the surface of different substrates were different from each other. In terms of the microbial communities formed on the PP, the prominent genera were *Hydrogenophaga, Nitrospira, Rhodobacter* and *Rhizobacter*. While the bacterial communities of PLA are dominated by *MND1, Fictibacillus, Arthrobacter, Sphingomonas, Acinetobacter* and *Staphylococcus*. The bacteria in GLASS were mainly *Nesterenkonia, Nitratireductor, Delftia* and *Halomonas*, which were significantly higher than those two MPs substrates ($p < 0.01$).

In order to determine the differences in the abundance of bacterial species on the surface of three substrates, LEfSe was used to search for metagenomic biomarkers. Differential information for all bacteria at the phylum, class, order, family, and genus level was represented in Figure 7. At the phylum level, there were significant differences in bacteria belonging to the Verrucomicrobiota in the PP samples. At the genus level, bacteria of the genus *Halomonas* was significantly different in GLASS, bacteria of the genus *Aeromicrobium, Ruminococcus_gnavus_group and Acidibacter* were significantly different in GLASS, respectively. In the PP sample, there were significant differences in bacteria of the genus *Nitrospira* from the phylum Nitrospirota, as well as bacteria of the genus *Rhodobacter*,

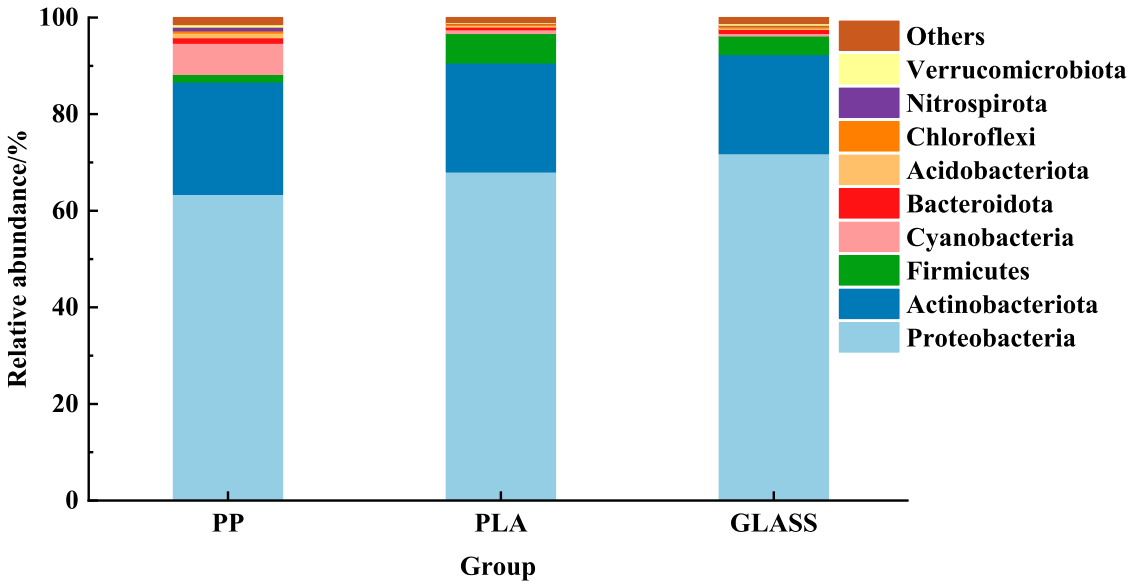

FIGURE 5
Distribution of relative abundance of different bacterial community structures at phylum levels of different substrates.

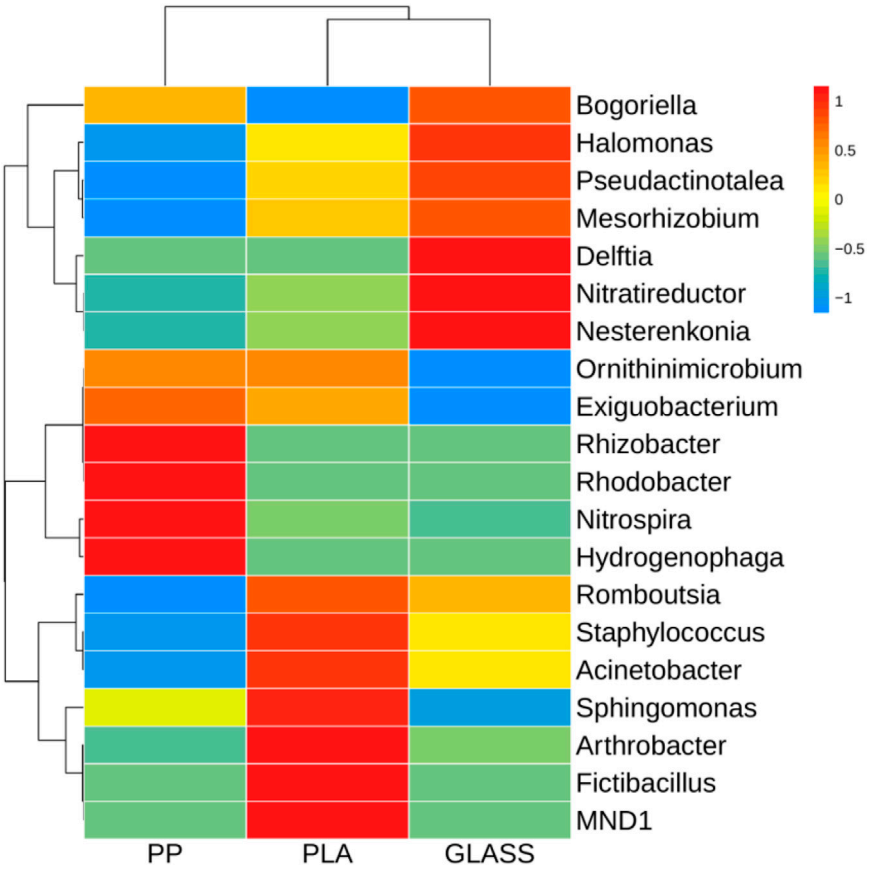

FIGURE 6
Microbial community structure (genus-level heatmap) of different sample groups.

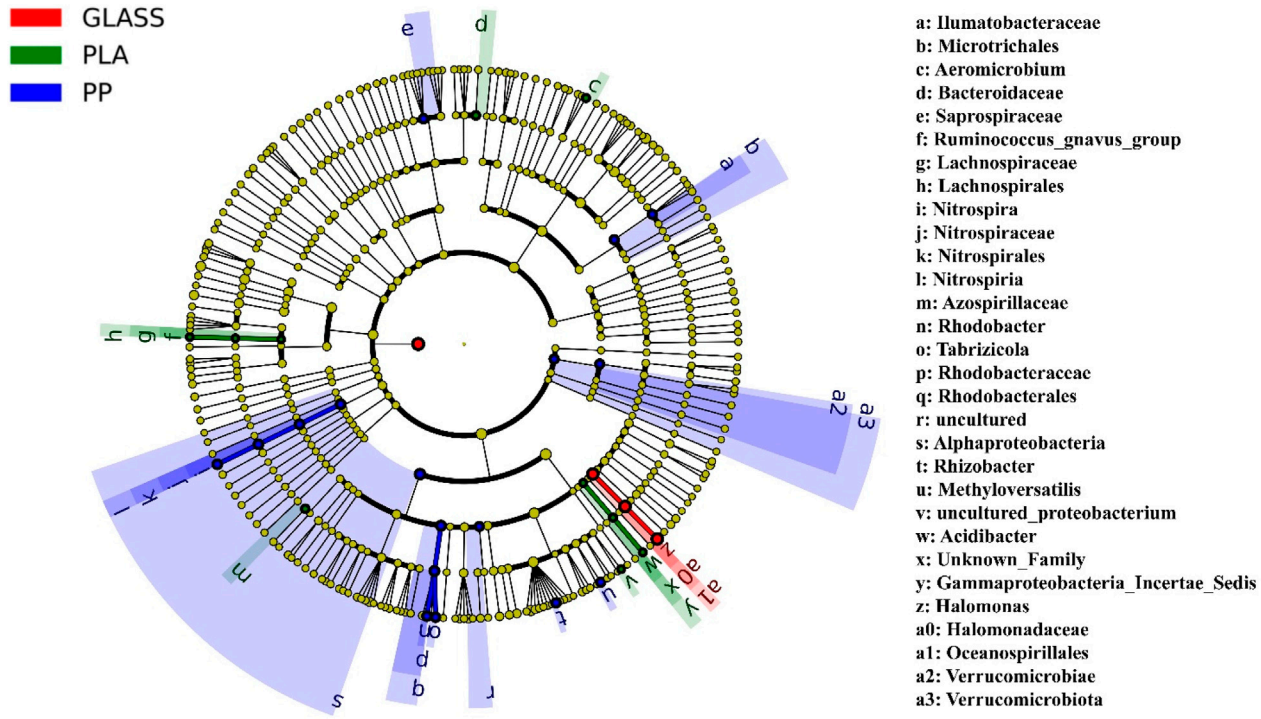

FIGURE 7
Cladogram indicating the phylogenetic distribution of microbial lineages associated with three substrates; lineages with LDA values of 3.0 or higher determined by LEfSe are displayed. Differences are represented in the color of the most abundant genus (red indicating GLASS, green PLA, blue PP). Each circle's diameter is proportional to the taxon's abundance. Circles represent phylogenetic levels from domain to genus inside out. Labels from the inside to the outside, the order is phylum, class, order, family and genus.

Tabrizicola, *Rhizobacter* and *Methyloversatilis* from the phylum Proteobacteria.

4 Discussion

In this indoor study, Proteobacteria was the dominant phylum in each substrate, which is in agreement with previous findings (Roager and Sonnenschein, 2019; Bae and Yoo, 2022), followed by Actinobacteria and Firmicutes. Most of studies have shown that Proteobacteria can adhere to large amounts of the substrate at the early stage of biofilm formation, showing non-specific selection for plastics (Roager and Sonnenschein, 2019). It was also reported that Actinobacteria are able to degrade bioplastics in either laboratory conditions or field trials (Butbunchu and Pathom-Aree, 2019). Notably, we found that the relative abundance of Firmicutes on the surface of PLA was higher than other two matrix. It has also been reported that biodegradable MPs can increase the abundance of Firmicutes (Fan et al., 2023). Microorganisms from the Firmicutes secrete enzymes such as cellulase, lipase, and protease, which may be one of the reasons why these bacteria can stably adsorb on microplastics (Blasco et al., 2011; Ma et al., 2015; Yadav et al., 2016; Dong et al., 2021). Most research studies currently report that the phylum Firmicutes can be isolated from animal bodies and possess the ability to degrade MPs (Lwanga et al., 2018; Janakiev et al., 2023). Besides, in our study, it was worth noting that Cyanobacteria occupied a higher abundance on the PP surface, which was significantly different from the other matrix. Studies have demonstrated that under the stimulation of microplastics (PS, PP), cyanobacteria will secrete more extracellular polymeric substances (EPS) to form hetero-aggregates with microplastics (Jiao et al., 2022; Parsai et al., 2022; Wu et al., 2023). However, the interaction between biodegradable microplastics (such as PLA) and cyanobacteria is still unclear. This phenomenon deserves further study to provide more data for exploration of the interactions between different polymer types and Cyanobacteria.

In particular, the genus *Sphingomonas*, recognized as an eco-friendly bacterium, was dominated on the PLA biofilm. A recent study indicated that the genera *Sphingomonas* with higher abundance in the biofilm of Mater-Bi (innovative biodegradable materials) during *in situ* incubation (Sabatino et al., 2024), this finding is somewhat in-line with our current results. However, Nguyen et al. conducted *in situ* experiments on HDPE, PLA and PHBV biofilms in freshwater reservoirs, and found that the genus *Gemmatimonas* and *Crenothrix* were increased on PLA (Nguyen et al., 2023). Di Pippo et al. investigated LDPE, PET, PLA, and starch-based MaterBi (Mb) biofilms exposed to lakes and found that LDPE, PET, and PLA surface biofilms were predominantly Actinobacteria during early development. The main Actinobacteria genera are *Cutibacterium*, *Propionicella* and *Olsenella* (Di Pippo et al., 2023). Given the variation in habitats, whether analyzing MPs biofilms through laboratory experiments or *in situ* studies, there exist significant background differences in the formation of microbial communities on these materials. *Sphingomonas*, has a very rich set of degradation genes, which have application value in degrading aromatic compounds such as

pesticides, dioxins, polycyclic aromatic hydrocarbons, and heterocyclic aromatic hydrocarbons, and can coordinate multiple degradation pathways (Zhang et al., 2021). Other studies indicated that PLA-degraded bacteria included *Bacillus*, *Sphingomonas*, *Pseudomonas* (Satti et al., 2018; Zhang et al., 2019). It has been reported that *Pseudomonas* and *Sphingomonas* were found to positively related to the degradation of PLA/PBAT film (Zhang et al., 2022). Therefore, we believe that *Sphingomonas* is a promising friendly bacteria capable of degrading biodegradable microplastics such as polylactic acid (PLA).

In this study, *Acinetobacter* and *Staphylococcus* were also particularly identified in the PLA-attached biofilm, with some species of these bacteria being common pathogens associated with high resistance rates and causing nosocomial infections (Antunes et al., 2014; Oliveira et al., 2018; Iruegas et al., 2023; Yao et al., 2023). Microplastics, as an attachment matrix for microorganisms, may also serve as carriers of pathogens, resulting in ecological risks that may harm human health (Bhatt et al., 2021 Lai et al., 2022; Bydalek et al., 2023; Kim and Yoo, 2024). Kesy et al. (2019) reported that salinity, as well as the "life history" of the particles, can greatly influence the composition of biofilm assemblages on microplastics. Our results also showed that the PLA-added group had the smallest salinity at the end of the experiment, and the biofilm was in a mature and stable phase after 60 days cultivation. It was found that microplastic biofilm (PVC) selectively enriched opportunistic human pathogens, but no enrichment was observed in two natural biofilms (rock and leaf) (Wu et al., 2019). Moreover, our findings suggested that there was no correlation between the abundances of the two pathogenic bacteria detected on the PLA biofilm and the variations in the physicochemical properties of the sediment. However, there is currently a lack of relevant research on whether the biodegradable microplastic-biofilms can carry more pathogens than traditional microplastic-biofilms, which requires further validation by indoor and outdoor experimental studies.

In this experiment, we found that the predominant bacterial genera on the surface of PP and GLASS biofilms exhibit the capability for both nitrification and denitrification (Xie et al., 2018; Yang et al., 2020; Ye et al., 2020; Wang et al., 2023). In contrast, the dominant bacterial genera on the PLA surface possess the ability to degrade lignin, cellulose, and engage in carbon metabolism processes (Zhang et al., 2021). This highlights the distinct micro-biological activities and functionalities that are supported by different materials under study. It is noteworthy that there were distinct differences in the relative abundance of genera between PLA and PP. The relative abundance of *Fictibacillus* on PLA was 37.6 times that of GLASS, but this genus was not found on PP. It has been reported that *Fictibacillus phosphorivorans* as a common bacterial specie isolated from the gut of *Chironomus riparius*, one of the sediment-dwelling invertebrate in freshwater ecosystems, exhibited the most effective proteolytic activity to degrade PVC and PA, but not PE, after 1 week of cultivation (Janakiev et al., 2023). In particular, the relative abundance of the genus *Arthrobacter* on the surface of PLA was 136 times and 15.3 times that of PP and GLASS respectively. A study has shown that *Arthrobacter* and *Sphingomonas* were enriched on biodegradable plastic mulch films compared to non-biodegradable PE, meanwhile, these microbial consortia were able to degrade the plastics in laboratory enrichment cultures (Bandopadhyay et al., 2018).

Most studies have demonstrated that the addition of MPs alters the physicochemical properties of soil as well as its microbial community structure (Seeley et al., 2020; Yin et al., 2023; Li et al., 2024). Li et al. reported that PBAT, one of biodegradable microplastic, is beneficial to soil nitrogen fixation, but it will significantly reduce the soil P content (Li et al., 2023). It has been reported that age-PE MPs can significantly reduce soil TN, TP (Zhang et al., 2023). Our results also revealed that compared to the GLASS group, MPs reduced P content in sediments after 60 days, particularly observed in the PLA group. In addition, some studies have reported that during the process of microbial degradation of MPs, extracellular enzymes (hydrolases) are secreted, causing the pH of the culture medium decreased (Wallace et al., 2017; Joo et al., 2018). Therefore, we hypothesize that bacteria with potential degradation capabilities also exist in the biofilms on the surface of MPs in this experiment, leading to a decrease in the pH value of the sediment, especially noticeable in the PLA group.

Furthermore, this study reveals a correlation between changes in the physicochemical properties of sediments and the abundance of specific bacterial taxa on the MPs. In our study, salinity did not correlate with most bacteria in the biofilms of MPs; conversely, it exhibited a significant positive correlation with the dominant bacterial genera in the GLASS biofilm. It has been reported that salinity was the primary factor affecting bacterial diversity of the colonies on plastic debris in Haihe Estuary (Li et al., 2019). In this study, on one hand, we focus on the environmentally friendly bacterium *Sphingomonas* that appears on PLA, which has a significant negative correlation with pH, moisture, TN, and TP; on the other hand, the dominant bacterial genera (*Nitrospira* and *Hydrogenophaga*) appearing on PP also show a negative correlation with moisture, TN, and TP. A study revealed that Pearson correlation analysis between soil properties and bacterial communities showed that both *Sphingomonas* and *Nitrospira* were negatively correlated with pH, TN, and TP (Sun et al., 2020).

Whether obtained from field *in situ* experiments or indoor experiments, the bacterial communities on MP biofilms show distinct differences at the genus and species levels due to geographical factors, physicochemical factors of water bodies, and different seasons, even with the same microplastic polymers. It has been reported that the structure and composition of plastisphere communities showed notable differences among the locations, and the taxonomic composition on MP samples was associated with their origins in sedimentary and aquatic environments (Jiang et al., 2018). According to the report: the organic content in the water emerged as the primary determinant of biofilm communities on MPs (Nguyen et al., 2022). MP biofilm colonization will also be affected by factors such as polymer type, particle size, surface charge, aging, and hydrophobicity, etc. (Amaral-Zettler et al., 2015; Didier et al., 2017; Mercier et al., 2017; Liu et al., 2020). In our study, the addition of MPs to sediment leads to alterations in the micro-environment, which in turn influences the abundance of specific bacterial genera on various materials. The disparity in bacterial communities between MPs and GLASS is predominantly attributed to the intrinsic properties of the materials themselves along with changes in the physical chemical attributes of the sediments. The impact on biofilm formation by combining the properties of MPs

themselves and sedimental environmental factors, which deserves further study in the future.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YJ: Funding acquisition, Writing–original draft. AZ: Conceptualization, Formal Analysis, Writing–review and editing. DZ: Project administration, Writing–review and editing. MC: Supervision, Visualization, Writing–review and editing. LW: Funding acquisition, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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