



Dynamic succession and biodegradation potential of microplastic prokaryotic microbial communities in the Pearl River estuary

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ABSTRACT

Microplastics (MPs), as emerging pollutants, exhibit poorly understood dynamic characteristics and ecological effects of surface microbial communities in-situ, particularly regarding long-term succession patterns in estuarine environments. Through a 35-day in-situ experiment and multiple sampling in the Pearl River Estuary, combined with 16S rRNA gene sequencing and multidimensional ecological analyses, this study systematically revealed the temporal succession patterns and driving mechanisms of prokaryotic microbial communities on microplastics (polylactic acid, polypropylene, polystyrene) and natural particles (wood, stone, glass beads). Key findings include: (1) Microplastic surfaces exhibited significant substrate specificity: *Bacillota* and *Bacteroidota* rapidly colonized during short-term exposure (1 day), with alpha diversity significantly higher than natural particles. (2) Long-term exposure (35 days) reduced alpha diversity, while beta diversity analysis indicated enhanced heterogeneity, suggesting selective enrichment of functional taxa (e.g., *Campylobacterota*, *Desulfobacterota*). (3) Biomarker analysis confirmed the preferential enrichment of *Campylobacterota* on microplastics, whose metabolic traits may contribute to plastic degradation, providing potential targets for bioremediation. *Campylobacterota* enrichment suggests biodegradation potential, particularly for PLA. Inert PP/PS accumulate homogenized communities, heightening ecological risks. These findings advance the understanding of microplastic-microbe interactions and offer a theoretical foundation for ecological risk assessment and bioremediation strategies.

1. Introduction

Microplastics (MPs), as pervasive and persistent pollutants in marine and freshwater ecosystems, have become a global environmental and health concern (Ahmad et al., 2020; Ali et al., 2024). Estuaries, serving as critical interfaces between terrestrial and marine systems, are hotspots for microplastic accumulation (Wang et al., 2023b), where complex physicochemical conditions may profoundly influence microbial colonization and succession on microplastic surfaces. MPs can act as vectors for toxicological effects on key ecological taxa (e.g., zooplankton) via trophic transfer (Castro-Castellon et al., 2022). Previous studies suggest that microplastic surfaces, due to their hydrophobicity and large specific surface area (Jiang et al., 2023), selectively

enrich specific microbial taxa, altering local ecological functions or mediating pathogen dissemination (Junaid et al., 2022; Yan et al., 2024). Microplastics selectively enrich microbial taxa via polymer-specific properties, altering biofilm-mediated ecological processes (Xu et al., 2025). Recent evidence confirms that microbial colonization dynamics on MPs diverge from natural particles (Qiu et al., 2025) because of polymer leaching and UV-induced surface oxidation, driving substrate-specific succession patterns. However, knowledge gaps persist regarding the dynamic characteristics of prokaryotic communities on diverse microplastic types and their driving mechanisms (Oberbeckmann and Labrenz, 2020; Seeley et al., 2020; Zhang et al., 2024), particularly in estuarine environments with long-term in-situ data scarcity, hindering comprehensive ecological risk assessments.

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Due to urbanization and industrial activities, the Pearl River Estuary faces escalating microplastic pollution (Mai et al., 2021), with abundances reaching 10^3 – 10^4 particles/ m^3 and dominated by polyethylene terephthalate, polyethylene, and polypropylene, which have documented toxicological impacts on zooplankton (Yan et al., 2019; Wang et al., 2021). In recent years, studies utilizing remote sensing and other technological approaches have unveiled the spatial distribution patterns and seasonal migration dynamics of microplastics in the Pearl River Estuary and its adjacent coastal waters (Kaandorp et al., 2023; Pathira Arachchilage et al., 2025; Pathira Arachchilage et al., 2022; Yu et al., 2022), providing novel insights for deciphering their land-sea transport mechanisms. Existing research primarily focuses on static microbial communities on natural particles (e.g., sediments) (Wang et al., 2023a), while dynamic succession patterns on microplastics—artificial substrates—remain underexplored. Despite prior studies mapping microplastic distribution (Chau et al., 2023; Li et al., 2021), the temporal dynamics of microbial communities on microplastics and their divergence from natural particles (e.g., wood, stone) are unresolved.

This study conducted an in-situ experiment in the Pearl River Estuary to systematically investigate the composition, diversity, and temporal dynamics of prokaryotic communities on microplastics and natural particles. Key objectives included: (1) characterizing substrate-specific microbial communities on microplastics; (2) elucidating spatiotemporal succession patterns; (3) identifying microplastic-type-driven community heterogeneity. By integrating 16S rRNA gene sequencing and ecological analyses, this work reveals microbial succession dynamics and identifies potential degraders, offering novel insights into microplastic-ecosystem interactions and guiding bioremediation strategies. We hypothesized that microplastics drive divergent microbial succession patterns versus natural particles, with polymer-specific biodegradation potential linked to chemical properties.

2. Materials and methods

2.1. Sampling site and methods

The experiment was conducted at Xinzhou Wharf (23°09'58"N, 113°41'83"E) in the Pearl River Estuary, Guangzhou, China (Fig. 1). Six types of particles were selected: three microplastics—polylactic acid (PLA), polypropylene (PP), polystyrene (PS)—and three natural particles, wooden beads (WD), gravel (ST), glass beads (GL). Free-living (FL) and particle-attached (PA) microbial communities in the river water served as controls. Each material was placed in separate nylon bags with three biological replicates. The bags were secured in weighted iron cages and submerged approximately 2 m below the water surface. Nylon bags were pre-rinsed with ultrapure water to eliminate plastic leachates, and all particles (microplastics/natural substrates) were gently washed with sterile physiological saline (0.9 % NaCl) post-retrieval. Sampling occurred on days 1, 3, 7, 12, 21, and 35, yielding six batches of environmental samples. Collected samples were immediately stored in ice-boxes and transferred to the laboratory, where they were preserved at -80°C until analysis. PA and FL samples were obtained by sequentially filtering water samples through 3.0 μm and 0.22 μm pore-size filters (Pall Life Sciences, Ann Arbor, MI, USA). Field measurements of physicochemical parameters (temperature [T], salinity [S], conductivity [Cond], dissolved oxygen [DO], total dissolved solids [TDS], pH, turbidity [TU], and redox potential [ORP]) were conducted in-situ employing portable analytical instruments: a multi-parameter probe (HQD Field Kit, HACH 58258) and a portable turbidity meter (HACH 2100Q).

2.2. Analysis of nutrient content in the water samples

The nutrient concentrations in the complete water samples were quantified using standardized methodologies (Lu et al., 2015). Dissolved

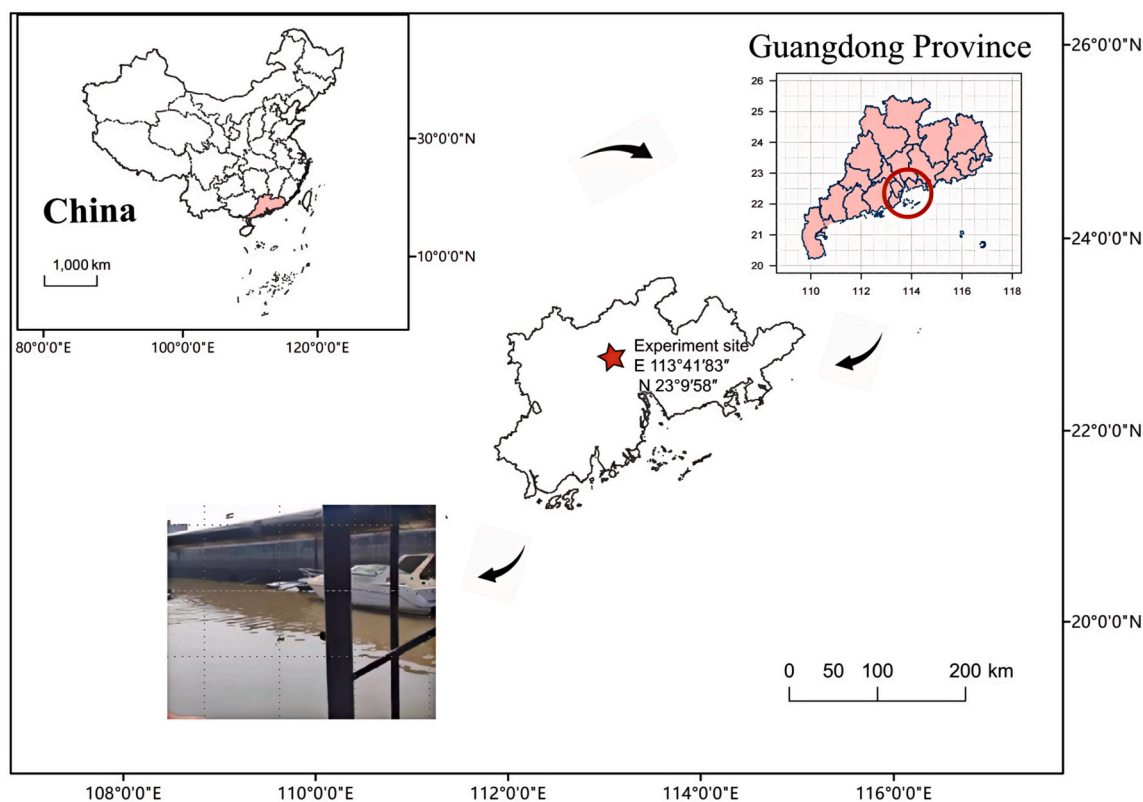


Fig. 1. Geographic location of the in-situ experimental site in the Pearl River Estuary. This experiment was conducted at Xinzhou Wharf (23°09'58"N, 113°41'83"E) in the Pearl River Estuary, Guangzhou, Guangdong province, China.

organic carbon (DOC) was analyzed via combustion-oxidation (TOC analyzer). Soluble reactive phosphorus (SRP) and nitrogen oxides (NO_x^-) were measured by molybdenum blue and hydrazine sulfate-NEDD spectrophotometry, respectively. Dissolved nitrogen (DN) and ammonium (NH_4^+) were determined using flow injection analysis (FIA). Particulate organic matter (POM) was quantified after HCl treatment to remove carbonates (Crump Byron et al., 2004). Chlorophyll-a (Chl a) was extracted with 90 % acetone and measured spectrophotometrically. Detailed descriptions of the determination of physicochemical factors are provided in Table S1.

2.3. DNA extraction, 16S rRNA gene amplification, and sequencing

DNA was extracted from particle surfaces using the Fast DNA SPIN Kit (MP Biochemicals, Solon, OH, USA) (Ahmad et al., 2021). To analyze prokaryotic community composition, the V4–V5 hypervariable regions of the 16S rRNA gene were amplified using primers 515F and 907R. Primers were synthesized by Novogene Bioinformatics Technology Co., Ltd. (Tianjin, China). PCR products were verified via 0.8 % agarose gel electrophoresis, purified using a gel recovery kit, and sequenced on the Illumina PE250 platform. DNA extraction included negative controls (sterile swabs). PCR amplification used triplicate reactions with non-template controls, and sequencing blanks confirmed no contamination.

2.4. Prokaryotic microbial community analysis

Raw sequencing data were processed using USEARCH v11. Paired-end reads were merged, and primer sequences were removed with CUTADAPT v2.4 (Mohideen et al., 2020). Sequences with a maximum expected error > 1.0 or length < 350 bp were filtered out. High-quality sequences were clustered into amplicon sequence variants (ASVs) via the unioise3 algorithm. Taxonomic annotation of ASVs was performed in QIIME2 using the SILVA 132 database and a Naïve Bayes classifier. Sequences not classified as bacteria or archaea were removed. An ASV table was generated by mapping trimmed reads to representative ASV sequences. To normalize sequencing depth, the ASV table was rarefied to the minimum number of reads across all samples. Further conduct alpha diversity analysis, beta diversity analysis, and biomarker analysis to explore differences between various particulate matters, thereby characterizing the community dynamics of prokaryotic microorganisms on the surfaces of microplastics and other particulates in the in-situ environment of the Pearl River water.

2.4.1. Alpha diversity analysis

After rarefaction normalization of the ASV table, alpha diversity analysis of prokaryotic microorganisms was performed based on the resampled data. This study selected the Hill diversity index as the reference metric. When q takes values of 0, 1, and 2 respectively, the Hill diversity index sequentially represents species richness (Hsieh et al., 2016), the exponential of the Shannon entropy (Haegeman et al., 2013), and the inverse Simpson index (Chao et al., 2014). Higher species richness values indicate greater diversity of prokaryotic microorganisms in the sample. Larger Shannon index values suggest both higher species numbers and more uniform distribution of prokaryotic microorganisms in the sample. The inverse Simpson index reflects both species richness and distribution uniformity of prokaryotic microorganisms in the sample to some extent.

2.4.2. Beta diversity analysis

Beta diversity characterizes and examines compositional differences in prokaryotic microbial communities across samples. The Bray-Curtis dissimilarity index, ranging between 0 and 1, measures intersample variation in species composition. Here, 0 denotes complete species overlap between two samples, whereas 1 represents complete dissimilarity with no shared species (Jami and Mizrahi, 2012).

2.4.3. Biomarker analysis

The samples were subjected to LEfSe (Linear Discriminant Analysis Effect Size) analysis using the Lianchuan Bio Cloud Platform (<https://www.omicstudio.cn/tool>) to identify statistically significant biomarkers (i.e., differentially abundant taxa) among different sample groups (Segata et al., 2011). In this study, LEfSe analysis was performed on the top 100 classes with the highest relative abundance in the microbial communities of each sample. The significance threshold for both the Kruskal-Wallis test and Wilcoxon test p -values was set at 0.05, and the linear discriminant analysis (LDA) score cutoff was defined as 3.

2.5. Statistical analysis

All statistical analyses were performed using R software (version 4.3.1). The significance of differences in alpha diversity between different particulate matter were determined using t -test. Beta diversity differences were assessed using permutational multivariate analysis of variance (PERMANOVA; Adonis 2 function) with Bray-Curtis dissimilarity and 999 permutations. Biomarker identification via LEfSe used a linear discriminant analysis (LDA) score > 3 and $P < 0.05$.

3. Results

3.1. Physical and chemical characteristics of in-situ water of the Pearl River estuary

We collected water samples from the experimental site and conducted physicochemical analyses (Fig. 1), the results of which are presented in Table S1. Salinity (0.12 ± 0.01 PSU) and conductivity ($269.33 \pm 5.51 \mu\text{S}\cdot\text{cm}^{-1}$) indicated a freshwater-dominated in studied area. The water temperature averaged 27.23 ± 0.90 °C, with near-neutral pH (7.26 ± 0.15). Dissolved oxygen (DO) levels were notably low ($2.36 \pm 0.34 \text{ mg}\cdot\text{L}^{-1}$), while oxidation-reduction potential (ORP) values (150.93 ± 14.07 mV) suggested weakly oxidative conditions. High concentrations of dissolved organic carbon ($\text{DOC}: 1.654 \pm 0.67 \text{ mg}\cdot\text{L}^{-1}$) and dissolved nitrogen ($\text{DN}: 3.16 \pm 0.27 \text{ mg}\cdot\text{L}^{-1}$) highlighted the eutrophic status of the water. The molar ratio of nitrate (NO_3^- : $1.37 \pm 0.06 \text{ mg}\cdot\text{L}^{-1}$) to soluble reactive phosphorus ($\text{SRP}: 0.24 \pm 0.01 \text{ mg}\cdot\text{L}^{-1}$) was approximately 5.7:1, significantly lower than the Redfield ratio (16:1), indicating potential phosphorus limitation. Additionally, elevated turbidity (26.54 ± 2.85 NTU) and particulate organic matter ($\text{POM}: 1.11 \pm 0.04 \text{ mg}\cdot\text{L}^{-1}$) suggested substantial suspended particulate loads. Chlorophyll a levels ($49.06 \pm 0.05 \mu\text{g}\cdot\text{L}^{-1}$) indicated active phytoplankton blooms, potentially contributing to biofilm formation through extracellular polymeric substance (EPS) secretion. These results collectively underscore the interplay between physicochemical gradients (e.g., hypoxia, eutrophication) and microbial colonization patterns on microplastics, providing a baseline for understanding biofilm-mediated ecological processes in estuarine ecosystems.

3.2. Prokaryotic microbial composition at phylum and class levels on particle surfaces

Based on 16S rRNA gene sequencing analysis, a total of 64 bacterial phyla and 12 archaeal phyla were identified across all samples. The top 10 phyla in relative abundance were selected to illustrate the temporal dynamics of prokaryotic microbial composition at the phylum level on different particle surfaces, as shown in Fig. S1. The dominant phyla on various particle surfaces included *Pseudomonadota* (34.50 %–95.42 %), *Bacteroidota* (0.98 %–32.53 %), *Actinomycetota* (0.21 %–23.78 %), *Bacillota* (0.12 %–41.25 %), *Chloroflexota* (0.08 %–7.53 %), *Planctomycetota* (0.05 %–3.85 %), *Verrucomicrobiota* (0.06 %–2.40 %), *Desulfobacterota* (0.09 %–9.09 %), *Acidobacteriota* (0.05 %–3.23 %), and others (0.18 %–14.21 %). Significant temporal fluctuations were observed on microplastic surfaces (PLA, PP, PS). For instance, the relative abundance of *Pseudomonadota* on PLA exhibited a “U-shaped” trend (initial decline

followed by recovery), while *Bacillota* on PP and *Bacteroidota*, *Actinomycetota*, and *Bacillota* on PS peaked at day 12 before declining. In contrast, natural particles (WD, ST, GL) showed relatively stable community compositions at the phylum level, with minor fluctuations such as a gradual increase in *Bacteroidota* on WD and a decline on ST. No significant temporal variations were observed in control group (FL, PA).

Further analysis of the class-level distribution in the samples revealed that the prokaryotic communities belonged to 179 bacterial classes and 19 archaeal classes. The top 20 most abundant classes were plotted to illustrate the temporal species composition of surface prokaryotic microorganisms across different samples (Fig. 2). The predominant classes included: *Gammaproteobacteria* (12.79 %–91.57 %), *Alphaproteobacteria* (2.51–67.06 %), *Bacteroidia* (0.90 %–32.40 %), *Actinobacteria* (0.15 %–22.86 %), *Bacilli* (0.03 %–36.42 %), *Clostridia* (0.08 %–3.51 %), *Anaerolineae* (0.04 %–5.44 %), *Planctomycetes* (0.05 %–3.73 %), *Verrucomicrobiae* (0.06 %–2.02 %), *Acidimicrobiia* (0.01 %–2.86 %), *Thermoleophilia* (0.02 %–1.82 %), *Fusobacteriia* (0.00 %–9.10 %), *Negativicutes* (0.01 %–5.33 %), *Desulfuromonadia* (0.02 %–6.81 %), *Deinococci* (0.00 %–13.42 %), *Vicinamibacteria* (0.02 %–1.81 %), *Campylobacteria* (0.00 %–1.50 %), *Cyanobacteria* (0.00 %–5.85 %), *Bdellovibrionia* (0.01 %–2.27 %), and others (0.30 %–12.25 %). The class-level community dynamics on microplastic surfaces (PLA, PP, PS) aligned with phylum-level trends: *Gammaproteobacteria* and *Alphaproteobacteria* exhibited inverse fluctuations in abundance, while *Bacteroidia* showed higher relative abundance during the initial experimental phase compared to mid- and late stages. For natural particulates (WD, ST, GL), the relative abundance of *Gammaproteobacteria* on WD surfaces decreased significantly over time, with marked increases in *Alphaproteobacteria* and *Bacteroidia*. In contrast, ST and GL surfaces demonstrated opposing trends between *Gammaproteobacteria* and *Alphaproteobacteria*. The community composition in control group samples (FL, PA) remained relatively stable.

3.3. Alpha diversity analysis of surface prokaryotic microbial communities

Alpha diversity metrics (ASV number, Shannon index, and inverse Simpson index) exhibited an initial decrease, followed by an increase and subsequent decline over time (Fig. 3), indicating reduced species richness, evenness, and diversity under prolonged exposure. The figure shown in Fig. 3 illustrates the alpha diversity of prokaryotic microbial community, from left to right, the relationships of ASV number, exponential of Shannon entropy, and inverse Simpson index over time are shown respectively, corresponding to Fig. 3a, Fig. 3b, and Fig. 3c, respectively. Alpha diversity analysis showed that the ASV number, Shannon index and the reciprocal Simpson index of microorganisms on the surface of particulate matter decreased first, then increased and then decreased with time, indicating that species richness, species number and species distribution uniformity decreased under long-term exposure.

The alpha diversity of different particulate matter in the in-situ environment of the Pearl River estuary was significantly different (Fig. 4a): the species richness was ranked as PA > GL > ST > PLA > FL > PS > PP > WD. In the control group FL and PA samples, the species richness of PA samples was higher, indicating that particulate matter in the environment would cause more prokaryotic microorganisms to attach to the surface. Among natural particulate matter, the species richness of prokaryotic microorganisms on the surface of ST and GL was at a relatively high level and there was no significant difference (t -test, $P > 0.05$), which was significantly higher than that of WD ($P < 0.01$). Among microplastics, the species richness of prokaryotic microorganisms on the surface of PLA was at a relatively high level, followed by PS, and the species richness of PP ($P < 0.01$) was the lowest.

Similarly, the Shannon index of prokaryotic microorganisms on the surface of different samples was consistent with the species richness (Fig. 4b). In the control group FL and PA samples, the Shannon index value of PA was higher, indicating that the species number and

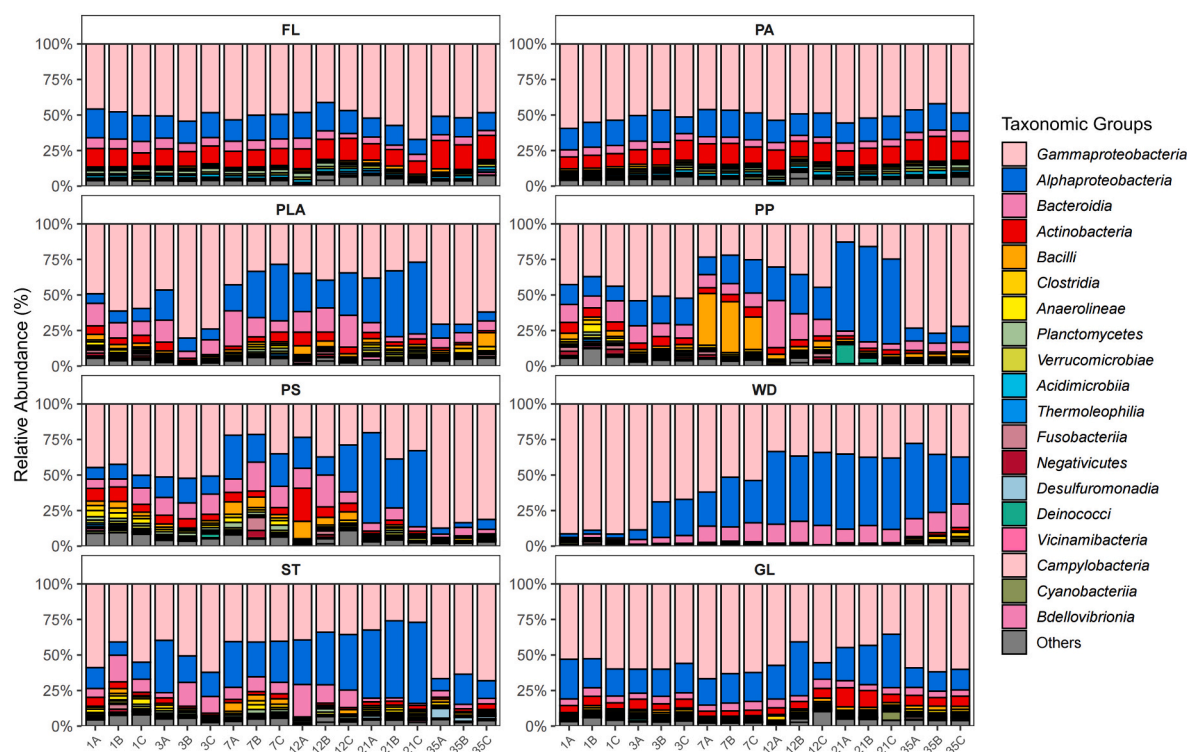


Fig. 2. The species composition of prokaryotes on the surface of different samples at the class level changes over time in the environment of Pearl River Estuary. FL: free living, PA: particle attached, PLA: polylactic acid, PP: polypropylene, PS: polystyrene, WD: wood, ST: stone, GL: glass; samples were taken on the 1st, 3rd, 7th, 12th, 21st, and 35th day, and 3 samples were recorded as A, B, and C respectively.

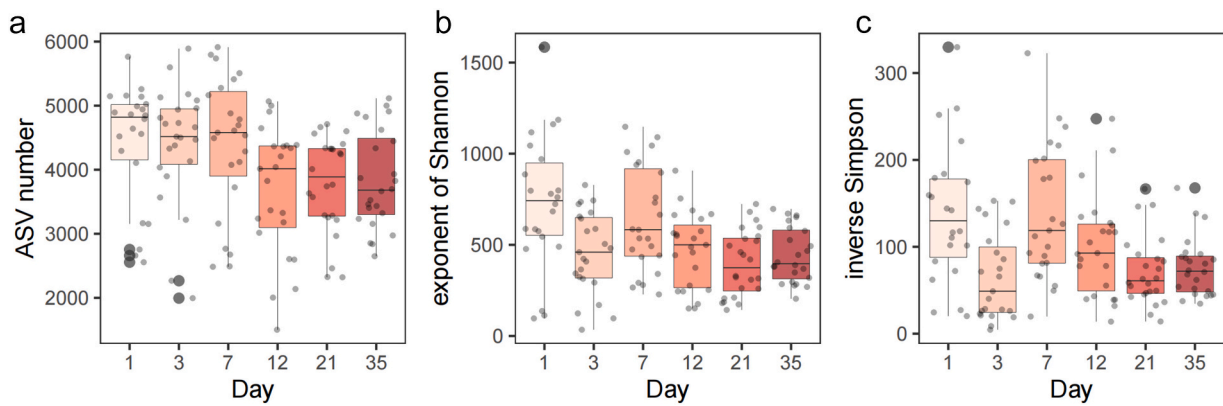


Fig. 3. The alpha diversity of prokaryotic microbial community at different time in the environment of Pearl River Estuary. The reciprocal of (a) ASV number ; (b) exponential of the Shannon entropy; and (c) inverse Simpson index of prokaryotes on the surface of samples at different time points from left to right.

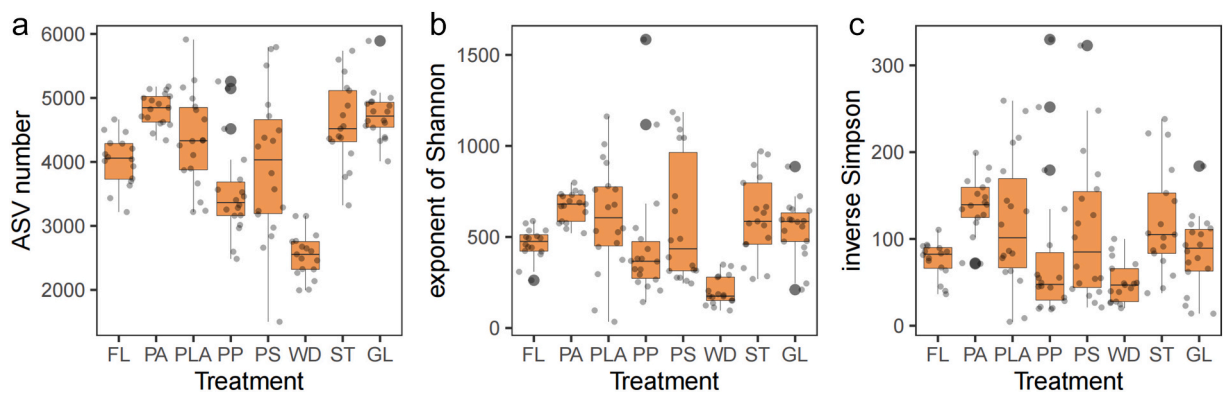


Fig. 4. The alpha diversity of prokaryotes on the surface of different samples in the environment of Pearl River Estuary. The reciprocal of (a) ASV number ; (b) exponential of the Shannon entropy; and (c) inverse Simpson index of prokaryotes on the surface of different particles from left to right.

distribution uniformity of prokaryotic microorganisms on the surface of particulate matter in the Pearl River water were higher than those of free-living prokaryotic microorganisms in the environment and single sample experimental groups. Moreover, the Shannon index values of prokaryotic microorganisms on the surface of FL and PA samples had significant differences (t -test, $P < 0.05$), indicating that particulate matter in the environment was more conducive to the attachment of prokaryotic microorganisms, and thus the diversity of prokaryotic microorganisms on the surface of particulate matter was higher. Among natural particulate matter, the Shannon index value of prokaryotic microorganisms on the surface of WD was significantly lower than other experimental groups (t -test, $P < 0.01$). In the experimental group, the Shannon index of prokaryotic microorganisms on the surface of PLA was the highest, and the Shannon index values in various particulate matters were also at a relatively high level, followed by PS, and the Shannon index value of PP was the lowest, but there was no significant difference among the three (t -test, $P > 0.05$).

The ranking of the inverse Simpson index of prokaryotic microorganisms on the surface of different samples was: PA > ST > PLA > GL > FL > PS > WD > PP, as shown in Fig. 4c. In the control group, the reciprocal of Simpson index of FL and PA had significant differences (t -test, $P < 0.05$), indicating that the species number and distribution uniformity of prokaryotic microorganisms on the surface of particulate matter in the Pearl River water were higher than those of free-living prokaryotic microorganisms in the environment and single sample experimental groups. Among natural particulate matter, the reciprocal of Simpson index of prokaryotic microorganisms on the surface of WD was significantly lower than that of ST; at the same time, there was no significant difference in the reciprocal of Simpson index between ST and

GL (t -test, $P > 0.05$). In the experimental group, the reciprocal of Simpson index of prokaryotic microorganisms on the surface of PLA was the highest, followed by PS, and the value of PP was the lowest; however, there was no significant difference among the three (t -test, $P > 0.05$).

The results of the t -test-based analysis of significant differences in species richness, Shannon index, and the inverse of the Simpson index of prokaryotic microorganisms on different particle surfaces in the in-situ environment of the Pearl River water are presented in Table S2–S4. In summary, the changes in the alpha diversity of prokaryotic microorganisms on the surface of different particulate matters in the in-situ environment of the Pearl River water can be summarized as follows: there were significant differences in the alpha diversity of prokaryotic microorganisms on the surface of different particulate matter; the alpha diversity of prokaryotic microorganisms on the surface of PLA, ST, and GL was significantly different from that of WD and PP, among which the alpha diversity of prokaryotic microorganisms on the surface of PLA, PS, ST, and GL was relatively high and there was no significant difference among them (t -test, $P > 0.05$); the alpha diversity of prokaryotic microorganisms on the surface of WD and PP was relatively low and there was no significant difference between them (t -test, $P > 0.05$).

3.4. Beta diversity analysis of surface prokaryotic microbial communities

The composition differences of prokaryotic microbial communities on different sample surfaces in the in-situ environment of the Pearl River Estuary significantly increased over time (Adonis 2 test, $P < 0.01$), as shown in Fig. 5a. Beta diversity analysis revealed that the prokaryotic microbial groups colonizing particle surfaces showed significant

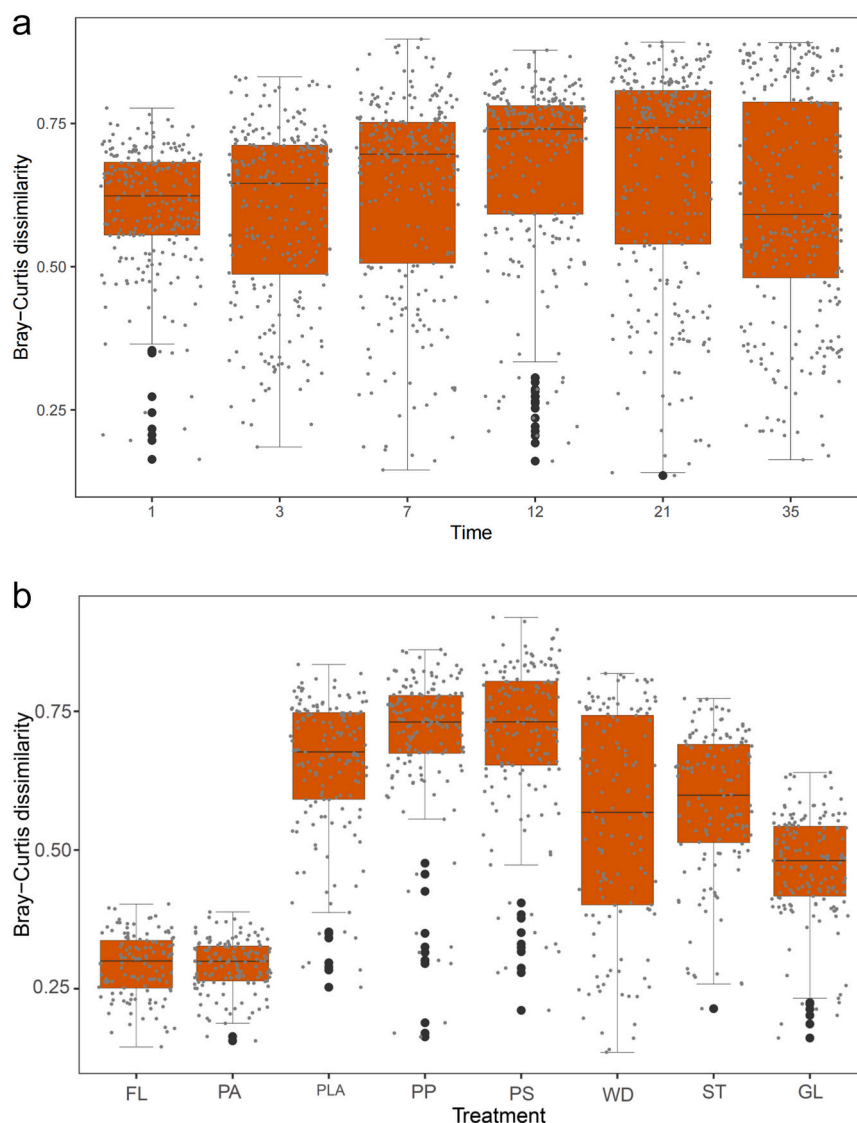


Fig. 5. The beta diversity of prokaryotes on the surface of samples in the environment of Pearl River Estuary, (a) at different time points; (b) in different samples.

differences over time (Adonis 2 test, $P < 0.01$; Fig. 5a, Table S5), suggesting that the type of particles may have a strong selective effect on the prokaryotic microbial communities colonizing their surfaces.

The beta diversity values of the prokaryotic microbial communities on different particle surfaces, ranked from high to low, are as follows: PP > PS > PLA > ST > WD > GL > FL > PA, as shown in Fig. 5b. The results of beta diversity analysis indicated that the beta diversity values on the surfaces of microplastics (PLA, PP, PS) (PP > PS > PLA) were significantly higher than those on natural particles (WD, ST, GL) and the control group (FL, PA) (Adonis 2 test, $P > 0.05$; Fig. 5b, Table S6), suggesting that the microbial communities on the surfaces of microplastics are more heterogeneous. The results of the significant difference analysis for the beta diversity of prokaryotic microorganisms in the in-situ environment of the Pearl River water across different times and sample surfaces, based on the Adonis 2 test, are provided in Table S5–S6.

3.5. Biomarker analysis of surface prokaryotic microbial communities

The phylum-level biomarker information of prokaryotic microbial communities on particle surfaces in the in-situ environment of the Pearl River water after 1 day of treatment is shown in Fig. 6a and Table S7. During short-term exposure (1 day), microplastics (PLA, PP, PS)

exhibited more biomarkers in their surface prokaryotic microbial taxa compared to natural particles (WD, ST, GL) (PLA = 3, PP = 3, PS = 3 vs. WD = 2, ST = 3, GL = 1). These taxa may utilize microplastics as a carbon source and preferentially adhere to them. Notably, *Bacillota* was a shared biomarker across all microplastics, *Bacteroidota* were enriched on PLA and PP surfaces, while natural particles (WD, GL) were dominated by *Pseudomonadota*.

The phylum-level biomarker information of prokaryotic microbial communities on particle surfaces in the in-situ environment of the Pearl River water after 21 days of treatment is shown in Fig. 6b and Table S8. With prolonged monitoring in the Pearl River's in-situ environment (21 days), the number of phylum-level biomarkers increased on PLA and GL surfaces (PLA = 5, GL = 6). For instance, *Crenarchaeota* and *Fusobacteriota* were significantly enriched on PLA surfaces, while *Cyanobacteriota* and *Bacillota* increased in abundance on GL surfaces, indicating these taxa are better adapted to long-term colonization. In contrast, the biomarker counts on PS and WD surfaces remained unchanged or decreased, suggesting enhanced selectivity in their surface communities.

The phylum-level biomarker information of prokaryotic microbial communities on particle surfaces in the in-situ environment of the Pearl River water after 35 days of treatment is shown in Fig. 6c and Table S9. Under long-term exposure (up to 35 days), the number of biomarkers on

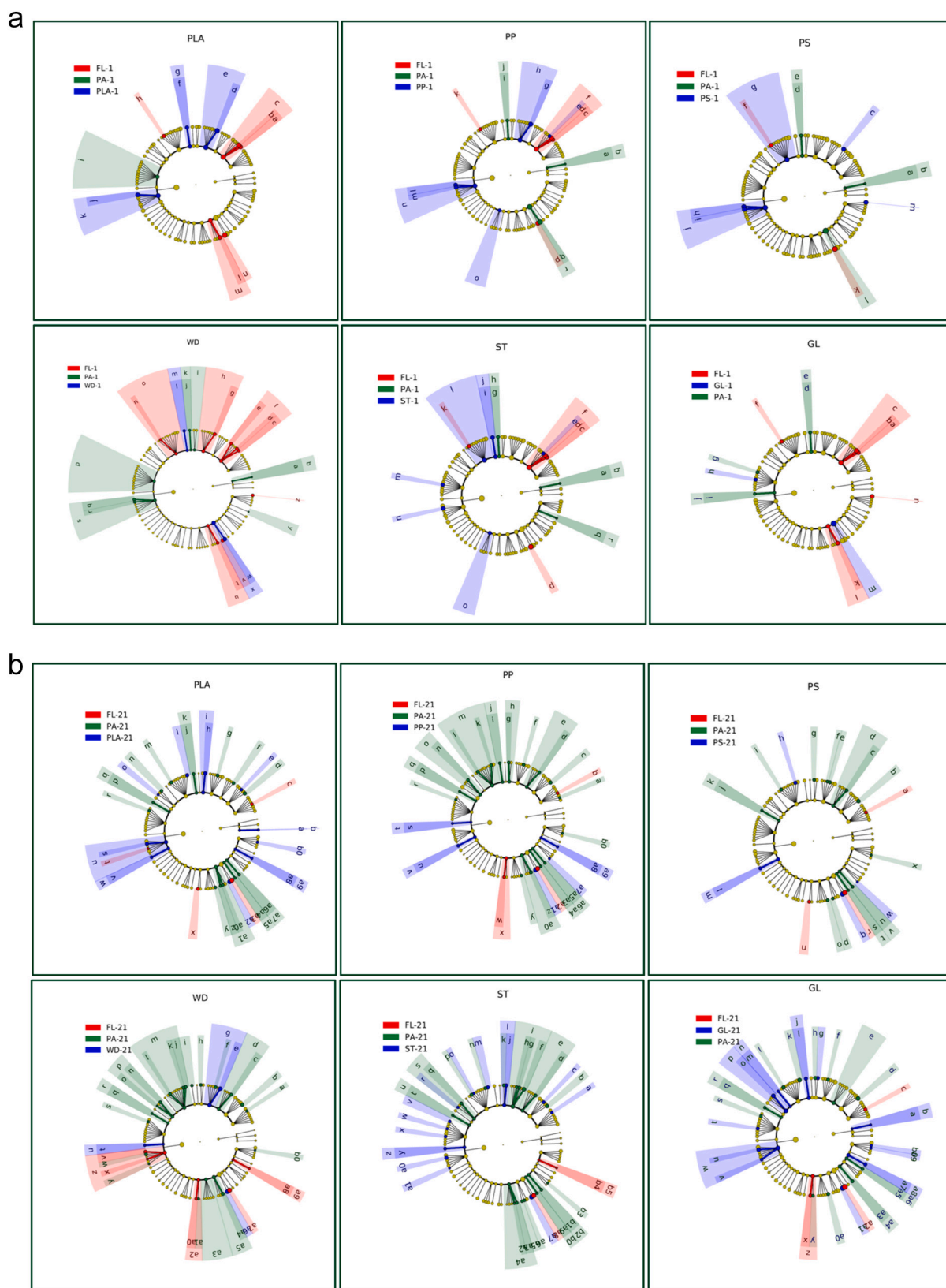


Fig. 6. Biomarkers of prokaryotic communities on different particle surfaces at different time in the environment of Pearl River Estuary. (a) After treated for 1 day, and taxonomic information of biomarkers is detailed in Supplementary Table S7, including *Bacteroidota*, *Campylobacterota* and *Bacillota* etc.; (b) After treated for 21 days, and taxonomic information of biomarkers is detailed in Supplementary Table S8, including *Thermoproteota*, *Bdellovibrionota* etc.; (c) After treated for 35 day, and taxonomic information of biomarkers is detailed in Supplementary Table S9, including *Campylobacterota* and *Desulfobacterota* etc. PLA: polylactic acid, PP: polypropylene, PS: polystyrene, WD: wood, ST: stone, GL: glass; each figure represents a prokaryotic microorganism with significant differences.

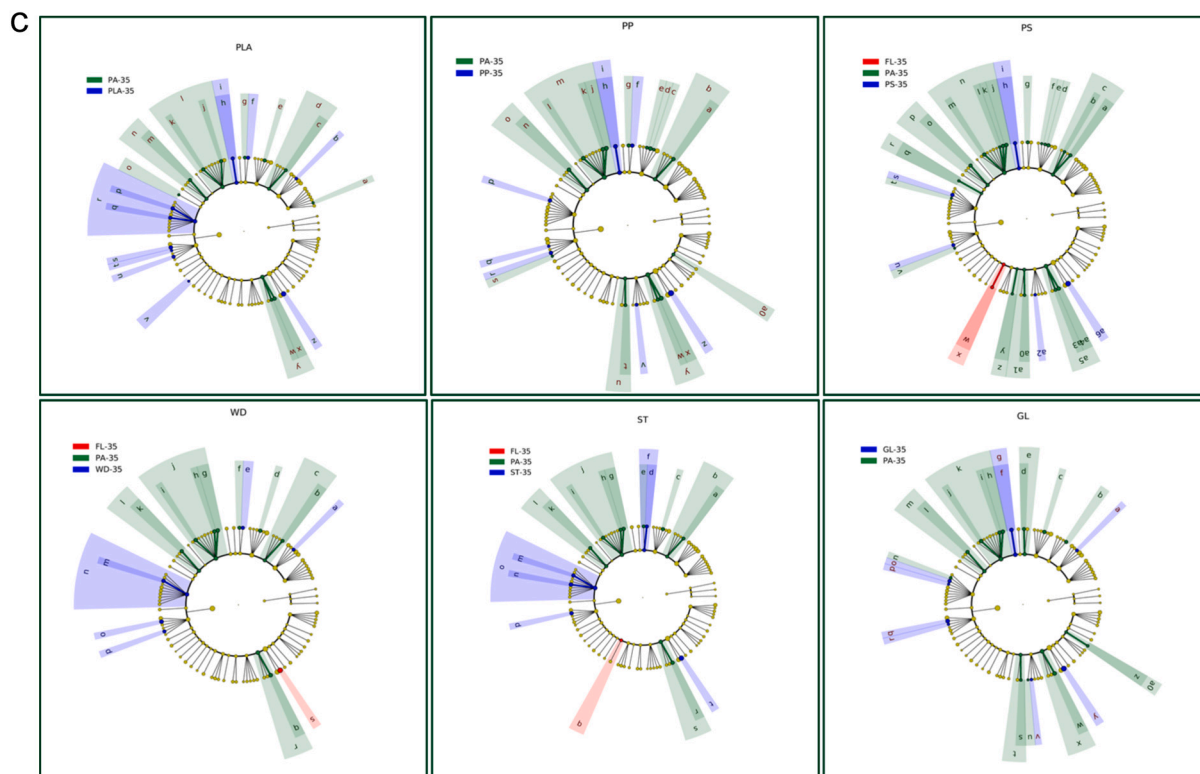


Fig. 6. (continued).

all particle surfaces decreased and converged (e.g., PLA = 2, PP = 1, PS = 1). This indicates that prolonged exposure to the Pearl River's in-situ environment led to stabilization of dominant prokaryotic microbial taxa on particle surfaces. Late-stage biomarkers at the phylum level likely represent taxa better suited for adhesion and growth on these particles.

4. Discussion

This study resolves long-term prokaryotic succession on diverse microplastics in the Pearl River Estuary through in-situ experiments—addressing a critical gap identified by Oberbeckmann and Labrenz (2020). By revealing how polymer properties drive functional taxon enrichment, we provide actionable targets for microbiota-driven bioremediation.

4.1. Microplastic surfaces exhibit nonlinear diversity dynamics during biofilm maturation

This study elucidates the temporal succession patterns of prokaryotic microbial communities on microplastic surfaces in the Pearl River Estuary. During short-term exposure (1 day), the alpha diversity (e.g., ASV number and Shannon index) of microplastic-associated communities was significantly higher than natural particles (Fig. 3, Fig. 4), consistent with the rapid colonization phase during initial biofilm formation (Lemonnier et al., 2022). Over time (up to 35 days), alpha diversity exhibited a nonlinear “decline-rise-decline” trend (Fig. 3), while beta diversity remained significantly higher on microplastics than on natural particles (Fig. 5), showing stronger heterogeneity and dynamism in microplastic-associated communities. This phenomenon may relate to the staged progression of biofilm development: stochastic microbial attachment initially elevates diversity; competitive dominance of specialized functional taxa (e.g., *Bacillota* and *Bacteroidota*) emerges during biofilm maturation; and environmental stressors (e.g., chemical additive release or physical abrasion) may subsequently drive community simplification (Moyal et al., 2023; Oberbeckmann and Labrenz,

2020). Notably, PLA, a biodegradable polymer, maintained relatively high alpha diversity in later stages (Fig. 4), likely because of its gradual degradation releasing organic carbon substrates (Sheridan et al., 2022), which sustains metabolic activity for diverse functional taxa (e.g., *Campylobacterota*).

Furthermore, the decline in beta diversity over prolonged exposure (Fig. 5a) suggests community convergence, reflecting enhanced adaptation of dominant taxa (e.g., *Bacillota*, *Bacteroidota*) to microplastic surfaces. This aligns with studies demonstrating that microplastic-associated niches enhance microbial network stability in Taihu Lake sediments, implying selective pressures drive long-term homogenization (Li et al., 2022). In contrast, natural particles (WD, ST, GL) exhibited stable communities (Bray-Curtis dissimilarity <0.6) (Fig. 5b), dominated by *Pseudomonadota*, which are likely generalists adapted to the Pearl River Estuary's physicochemical conditions.

4.2. Microplastics select substrate-specific prokaryotic communities

Microplastic surfaces harbored distinct microbial compositions compared to natural particles (WD, ST, GL) (Fig. S1, Fig. 2). The enrichment of *Bacillota* and *Bacteroidota* on microplastics (Table S7) may relate to their hydrophobic surfaces and leached carbon sources (e.g., plasticizers) (Wang et al., 2025; Zhu et al., 2020), which favor taxa with competitive organic matter degradation capabilities. In contrast, natural particles were dominated by *Pseudomonadota* (Table S7), whose stability may reflect conserved surface properties (e.g., charge distribution and roughness) (Nguyen et al., 2021). Furthermore, combined with the significant temporal differences in beta diversity of prokaryotic microbial composition on particle surfaces (Adonis 2 test, $P < 0.01$) and the aforementioned analyses, these findings suggest that microplastics may exert strong selective pressures on prokaryotic microorganisms colonizing their surfaces. Beta diversity analysis further revealed greater temporal fluctuations in microplastic-associated communities (Fig. 5a) and stronger inter-substrate divergence among microplastics than natural particles (Fig. 5b), supporting the role of microplastics as “artificial

niches" exerting strong selective pressures (Yang et al., 2020). For instance, PLA surfaces selectively enriched *Campylobacterota* and *Desulfobacterota* during long-term exposure (Table S9), suggesting their potential involvement in PLA degradation via sulfur reduction or anaerobic metabolic pathways, consistent with biodegradation mechanisms reported for polyester plastics (Jin et al., 2022; Niu et al., 2023).

4.3. Enriched taxa imply polymer-degrading functions

Biomarker analysis identified *Bacillota*, *Bacteroidota*, and *Campylobacterota* as key taxa enriched on microplastics (Table S7–S9), which are widely implicated in plastic degradation (Qin et al., 2023). *Bacillota* secrete esterases and lipases capable of hydrolyzing polyesters (e.g., PLA), while *Bacteroidota* may facilitate biofilm formation through polysaccharide degradation (Sooriyakumar et al., 2022). The persistent enrichment of *Campylobacterota* (Table S9) highlights its ecological role in colonizing microplastics, potentially via hydrophobic carbon utilization.

PLA surfaces showed increasing Biomarker numbers over time (Table S8), with *Campylobacterota* and *Desulfobacterota* dominating at day 35 (Table S9). *Campylobacterota* showed persistent enrichment on microplastics (PLA, PP, PS), suggesting potential involvement in plastic degradation (Fig. 6c). The relatively higher biomarker count on PLA surfaces (25 % higher than PP/PS) may relate to its degradability and structural/chemical properties that promote microbial adhesion. In contrast, the limited dominant biomarkers on PP and PS surfaces may correlate with their recalcitrance to degradation. This may reflect redox state shifts in localized microenvironments during PLA degradation (Li et al., 2024). Conversely, PP and PS surfaces hosted fewer Biomarkers (Table S9), likely due to their chemical inertness (e.g., C–C backbone in PP) limiting bio-accessibility, consistent with low degradation efficiency reported for polyolefins (Lin et al., 2022).

4.4. MP-driven community shifts impact estuary ecological risks

The dynamic microbial patterns on microplastics, modulated by polymer type and exposure duration, hold critical implications for ecological risk assessment. For instance, the sharp decline in alpha diversity on PP and PS (Fig. 4) may reduce community resilience, increasing functional vulnerability (Menicagli et al., 2023). Additionally, microplastic biofilms could serve as vectors for pathogens (e.g., pathogenic *Campylobacter* strains) or antibiotic resistance genes (Oliver et al., 2024; Wright et al., 2020), underscoring health risks in estuarine ecosystems.

On the other hand, the finding of late-stage *Campylobacterota* dominance (Fig. 6c), and the sustained enrichment of *Campylobacterota* on PLA (Table S9) offers bioremediation opportunities: these taxa could serve as biomarkers for microplastic pollution or be genetically engineered to enhance degradation efficiency (Barone et al., 2024; Zeng et al., 2023). For recalcitrant polymers like PP, pretreatment strategies (e.g., photo-oxidation or surface modification) may improve bio-accessibility (Wu et al., 2025), informing the development of microbiota-driven mitigation technologies.

5. Conclusion

This in-situ study delineates the temporal dynamics and ecological drivers of prokaryotic communities on microplastics in the Pearl River Estuary. MPs exhibited substrate-specific communities with higher diversity than natural particles. Biodegradable PLA supported richer diversity due to carbon release, while inert PP and PS showed homogenized communities. *Bacillota* and *Bacteroidota* dominated during short-term exposure (1 day), whereas *Campylobacterota*—potential degraders—were enriched over 35 days, with increased beta diversity, reflecting functional adaptation. PLA-associated *Campylobacterota* implied degradation potential, while PP/PS inertness may elevate

ecological risks. This study advances understanding of microplastic-microbe interactions in aquatic environments. Future work integrating multi-omics approaches could unravel molecular mechanisms underlying these interactions, guiding the development of targeted bio-accessibility strategies for microplastic pollution.

CRedit authorship contribution statement

Weicong Yan: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Danling Tang:** Writing – review & editing, Supervision, Project administration. **Manzoor Ahmad:** Methodology, Investigation, Conceptualization. **Pandeng Wang:** Methodology, Funding acquisition, Data curation. **Jialing Li:** Writing – review & editing, Investigation, Conceptualization. **Ziqi Peng:** Investigation, Conceptualization. **Wenjun Li:** Validation, Supervision, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2025.118992>.

Data availability

Complementary experimental datasets supporting the conclusions of this work are maintained by the corresponding investigator team and can be accessed through formal data request protocols.

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