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# Diversity and succession of microbial communities on typical microplastics in Xincun Bay, a long-term mariculture tropical lagoon

by

Yunfeng Shi[1], Shuai Wang[1], Hui Wang[1], Zhaoyang Li[1], Jiali Cai[1], Qiuying Han[1], Muqiu Zhao[1,2,*]

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[1]*Yazhou Bay Innovation Institute/Modern Marine Ranching Engineering Research Center of Hainan/Key Laboratory of Utilization and Conservation for Tropical Marine Bioresources of Ministry of Education, Hainan Tropical Ocean University, Sanya, Hainan, China*

[2]*First Institute of Oceanography, Ministry of Natural Resources of China, Qingdao, Shandong, China*

* Corresponding author: zhaomuqiu@126.com

## Abstract

In this study, three polymer types of microplastics (MPs), polyethylene (PE), polystyrene (PS) and polypropylene (PP), were exposed for 60 days in Xincun Bay (Hainan, China), a long-term mariculture tropical lagoon. High-throughput sequencing and scanning electron microscopy (SEM) were used to investigate the succession of microbial community structure and function on MPs after 10, 30, and 60 days of exposure, respectively. The results showed that diversity indices for bacteria from MPs were higher than those for bacteria from seawater. Significant differences were observed in community structure and metabolic function between MPs and seawater. The microbial network structure on MPs was more complex and dispersed than that in seawater. No significant differences in bacterial community structure and metabolic function were observed among different types of MPs. The biofilm on PS was the thickest, and the network structure on PP was the most complex one. With increasing exposure time, the biofilm attached to the surface of MPs became thicker and microbial composition showed some differences. The analysis of potential degradation bacteria and pathogens with abundance above 0.01% showed that the abundance of several potential plastic biodegraders on MPs was higher than that in seawater, while no potential pathogen was found enriched on MPs.

**Key words:** microplastics, bacterial community, long-term mariculture tropical lagoon, succession

# 1. Introduction

Recently, mariculture has been growing rapidly to meet the global demand for seafood in many countries. Global mariculture and coastal aquaculture together produced 30.8 million tons of aquatic animals in 2018 (FAO, 2020), reaching a record high. Lagoon mariculture, in particular, is growing at an increasing rate. For example, Xincun Bay, a lagoon located on Hainan Island in China, began cage culture in 1984 and now has over 3.2 $km^2$ of mariculture area. The rapid development of mariculture has had significant environmental effects on the surrounding seawater, such as eutrophication (Meng & Feagin 2019; Li et al. 2021) and changes in microbial diversity and community structure (Wang et al. 2018; Shi et al. 2019). Various plastic products have been used in mariculture with a high annual growth rate, such as fishing nets, foams, ropes, floats, fish cages, crates, feeders, boxes, and tanks (Lusher et al. 2017). Abundant microplastics (MPs; defined as particles < 5 mm in diameter) can be generated from plastic products used and discarded in mariculture by mechanical abrasion, photooxidation or biological degradation. For example, mariculture-derived MPs accounted for approximately 55.7% and 36.8% of the MPs in seawater and sediment from Xiangshan Bay, where the abundance of MPs was 8.9 ± 4.7 items $m^{-3}$ in seawater and 1739 ± 2153 items $kg^{-1}$ in sediment, respectively (Chen et al. 2018). In the surface sediment from Sanggou Bay, mariculture-derived MPs accounted for approximately 57.72% of the MPs, where the abundance of MPs was 1674 ± 526 items $kg^{-1}$ (Sui et al. 2020).

As an emerging marine pollutant, MPs have recently attracted growing attention due to their potential threats to marine life and human health, particularly as carriers of microorganisms (Amaral-Zettler et al. 2020; Wright et al. 2020). Some studies have found that microbial communities attached to MPs are different from those in the surrounding seawater (Oberbeckmann et al. 2017; Sun et al. 2020). MPs may selectively enrich some microorganisms such as plastic biodegraders (Oberbeckmann & Labrenz, 2020), pathogens (Yang et al. 2020), invasive species, or antibiotic-resistant bacteria (Zhang et al. 2020). These microorganisms can be transported by MPs over long distances and represent a potential source of pollution for other ecosystems. Microbial communities forming colonies on MPs have been studied in the past decade, mainly in natural waters (Frere et al. 2018; Jiang et al. 2018; Dudek et al. 2020) and in mariculture in temperate and subtropical regions (Chen et al. 2018; Zhu et al. 2019; Chen et al. 2020; Sui et al. 2020). The related research from tropical areas with high temperature and high radiation is scarce. Some of the current studies suggested that microorganisms were selective toward the types of MPs due to varying properties of different materials (Rosato et al. 2020; Hou et al. 2021). However, other studies showed that there was no significant difference in microbial colonization on different polymer types of MPs (Li et al. 2019; Dudek et al. 2020). Nonetheless, another study revealed that microorganisms on MPs were closely related to environmental conditions and exposure time (Oberbeckmann et al. 2018). Therefore, it is necessary to study characteristics of microbial communities on MPs in the special environment of a tropical lagoon affected by mariculture, and to predict their ecological and environmental risks.

In this study, three typical types of MPs, polyethylene (PE), polystyrene (PS) and polypropylene (PP), originating from polymer products widely used in mariculture were incubated in situ in Xincun Bay. Using high-throughput sequencing (16S rRNA) and scanning electron microscopy (SEM), this study aimed to investigate: (1) differences in microbial structure and composition between MPs and surrounding seawater, and potential ecological and environmental risks in a tropical lagoon impacted by mariculture; (2) whether the polymer types of MPs and exposure time affect the structure and function of microorganisms on MPs.

# 2. Materials and methods

## 2.1. Study location

An in situ experiment was conducted in Xincun Bay, which is located in the southeast of Hainan Island (18°24′ – 18°27′N; 109°58′ – 110°02′E), covering an area of 22.5 $km^2$ with an average water depth of 4.2 m and a tidal range of 0.7 m, and connecting to the South China Sea through only one narrow channel of 156 m width (Fig. 1A). Marine aquaculture and tourism have been developed in the lagoon, with an aquaculture area of more than 3.2 $km^2$ and more than one million tourists per year. Seagrass beds and mangrove forests are typical and important ecosystems in the lagoon, covering 3.28 $km^2$ and 1.48 $km^2$, respectively (Fang et al. 2020; Huang et al. 2020).

## 2.2. Experimental design

Three types of MPs (1–5 mm diameter) were obtained by shredding commercial mariculture plastics (fishing nets, foams and woven bulk bags) purchased from markets. Shredded MPs were pre-cleaned with

3% $HNO_3$ to remove organic materials and microbial organisms, washed three times with sterile seawater and then dried in a germ-free room at 20°C (Sun et al. 2020). Each type of pretreated MPs was put in nine nylon net bags (20 × 20 cm, 0.3 mm mesh size). To ensure a comparable surface area for colonization, each bag was loaded with 100 MP particles. These bags, tied with ceramic sinkers to submerge them below the seawater surface, were fastened to floating collars of a cage (Fig. 1), which was mainly used for *Epinephelus*. Three bags of each MP were retrieved after different incubation periods of 10, 30 and 60 days (from 26 September 2019 to 26 November 2019). Half of the MPs in the retrieved bags were immediately frozen with liquid nitrogen and stored at –80°C for DNA extraction. The remainder was fixed with glutaraldehyde (2.5%) and stored at 4°C for type identification and biofilm observation.

Simultaneously with the retrieval of MPs bags, seawater samples (1 l × 3) were also collected from 30 cm below the surface, right next to the incubated bags and stored at –20°C until DNA extraction. Water parameters were also determined in situ using a Combo Water Quality Meter (AZ86031; $T$ = 26.4 – 27.7°C; pH = 7.72 – 7.81; salinity = 20.2 – 33.2; dissolved oxygen = 7 – 8.2 mg $l^{-1}$). The concentrations of phosphate and nitrogen compounds were measured using an autoanalyzer (SEAL, Germany; phosphate = 0.008 – 0.037 mg $l^{-1}$, nitrate = 0.108 – 0.228 mg $l^{-1}$).

### 2.3. MPs characteristics

MPs components were identified by Fourier transform infrared spectroscopy (FTIR, Bruker ALPHA II). The FTIR spectrum range was 400–4000 $cm^{-1}$ with a spectral resolution of 4 $cm^{-1}$; 24 scans were performed.

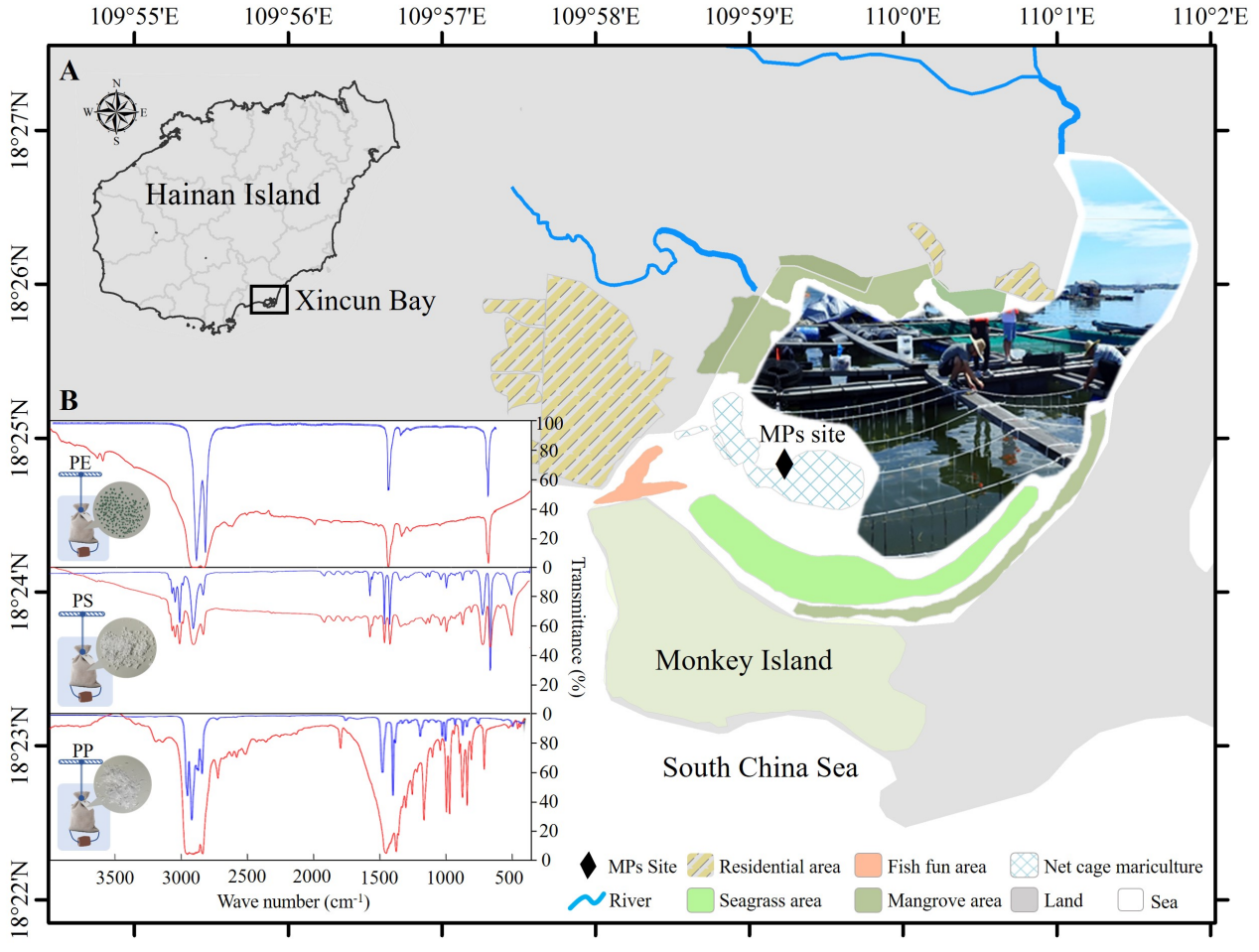



**Figure 1**

(A) Location of the experiment and spatial distribution of ecosystem types within the bay. (B) FTIR spectra of MPs originating from fishing nets, foams and woven bulk bags in red; reference spectra of polyethylene, polystyrene and polypropylene in blue.

All spectra were processed offline using the automatic baseline correction mode in the OMNIC software. The Bruker Optics ATR-Polymer Library was used to determine the composition of polymers. The results of FTIR showed that polyethylene (PE), polystyrene (PS) and polypropylene (PP) were the main components of experimental MPs originating from fishing nets, foams and woven bulk bags (Fig. 1B). Therefore, PE, PS, PP and WR in this paper indicate samples of fishing nets, foams, woven bulk bags and seawater, respectively. Preserved MPs samples for SEM were fixed with 2.5% glutaraldehyde, and subsequently dehydrated by a graded series of 20 min exposures to 50%, 90% and absolute ethanol on ice, and then stored in a vacuum drying oven (Sun et al. 2020). Dehydrated MPs were coated with 10–15 nm of platinum using a JEOL JEC-3000FC sputter coater. Images were obtained by SEM (JEOL, JSM-7610F Plus) at an accelerating voltage of 5.00 Kv.

### 2.4. DNA extraction

The collected MPs were rinsed three times using 0.1 M sterile phosphate buffer saline (PBS, pH = 8) and further ultrasonicated for 30 s in sterile PBS. After centrifugation at 14 000 *g* for 10 min, MPs-associated microorganisms were collected and frozen at –80°C for further analysis. Seawater samples were filtrated through sterile 0.22 µm polysulfone filter membranes (Millipore, USA). Membranes were stored at –80°C before DNA extraction.

The following steps of DNA extraction were conducted by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Microbial DNA was extracted from samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis.

### 2.5. 16S rRNA gene sequencing and data processing

The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCR reactions for each sample were performed in triplicate in 20 µl mixtures containing 4 µl of 5 × FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.4 µl of FastPfu Polymerase and 10 ng of template DNA. PCR reactions were conducted using the following program: 3 min of denaturation at 95°C, 27 cycles of 30 s at 95°C, 30 s for annealing at 55°C, 45 s for elongation at 72°C, and a final extension at 72°C for 10 min. The resulting PCR products were extracted from 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the standard manual. Purified amplicons were pooled in equimolar ratios and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, USA).

Raw fastq files were demultiplexed, quality filtered by Trimmomatic and merged by FLASH based on the following criteria: (i) the reads were truncated at any site that received an average quality score < 20 over a 50 bp sliding window; (ii) the primers were exactly matched, allowing a 2-nucleotide mismatch, and reads containing ambiguous bases were removed; (iii) sequences with overlaps longer than 10 bp were merged according to their overlapping sequence. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of the 16S gene sequence was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the 16S rRNA database (SILVA 132/16S bacteria) using a confidence threshold of 70%.

### 2.6. Statistical analysis

Alpha diversity of bacterial assemblages, including Chao1, Shannoneven and Shannon indices were calculated using QIIME (version 1.9.1). The community structure of bacterial communities, including visualization of community turnover over time and between treatments, was assessed using Principal Coordinates Analysis (PCoA) with Bray–Curtis dissimilarity. These analyses were conducted in R with the vegan package (version 3.3.1). Functional inferences were made from the Kyoto Encyclopedia of Gene and Genomes (KEGG) catalogue using the Tax4Fun program, which is connected to the SILVA database. Further, a linear discriminant analysis effect size (LEfSe) algorithm was implemented in the Galaxy module (http://huttenhower.sph.harvard.edu/galaxy/) to evaluate biomarkers and functions that are primarily responsible for dissimilarities between community structures. STAMP (version 2.1.3) was used to statistically analyze KEGG level 3 abundance to identify significant pathways by a two-sided Welch's t-test. Co-occurrence network analyses were carried out through the Molecular Ecological Network

Analyses Pipeline (MENAP, http://ieg4. rccc.ou.edu/MENA/main.cgi) at the OTU level (Deng et al. 2012). Poorly represented OTUs that were present in fewer than 80% of the samples and had < 0.1% average relative abundance in each group were removed from the network analyses. Using the Spearman correlation coefficient and similarity thresholds of 0.92, networks were constructed and visualized in Gephi 0.9.2. Intra-module connectivity (Zi) and inter-module connectivity (Pi) were used to sort nodes as peripheral nodes ($Zi \leq 2.5$, $Pi \leq 0.62$), module hubs ($Zi > 2.5$, $Pi \leq 0.62$), connectors ($Zi \leq 2.5$, $Pi > 0.62$) and network hubs ($Zi > 2.5$, $Pi > 0.62$) (Olesen et al. 2007; Deng et al. 2012).

## 3. Results and discussion

### 3.1. Dynamics of biofilm morphology

Microbial colonization and biofilm formation were observed by SEM at all MPs samples exposed at different exposure times (Fig. 2). According to the SEM images, a large variety of biofilm morphological types were observed on the surface of MPs, including cocci-, rod- and disc-shaped cells, as well as a dense layer of extracellular polymeric substances. No microbial community was found on the MPs surface on day 0, which was due to the precleaned process with $HNO_3$. After 10 days of exposure, there were sparse microbial communities on the MPs surface, and some microalgae were observed based on morphology. Compared with 10 days, a very intense microbial community was observed on the surface of MPs after 30 days of exposure. A biofilm layer was formed on the original surface, and some mycelia were observed after a month of incubation. A very dense biofilm layer was observed after 60 days of exposure, resulting in obscured surface of MPs. Many cocci-, rod- and disc-shaped bacterial cells and algae were covered and entangled by mycelia. In general, the biofilm density on the surface of MPs increased with exposure time (Tu et al. 2020). Compared with PE and PP, PS had a thicker biofilm due to more folds that can provide more space for microbial colonization.

### 3.2. Dynamics of diversity

A total of 36 samples were collected, including 27 substrate samples and nine seawater control samples. After assembling and quality filtering, high quality sequences, ranging from 30 027 to 67 403 per sample, were clustered into 9767 OTUs at a similarity level of 97%. The confidence level used by the RDP classifier database was 0.7. The rare faction curves for

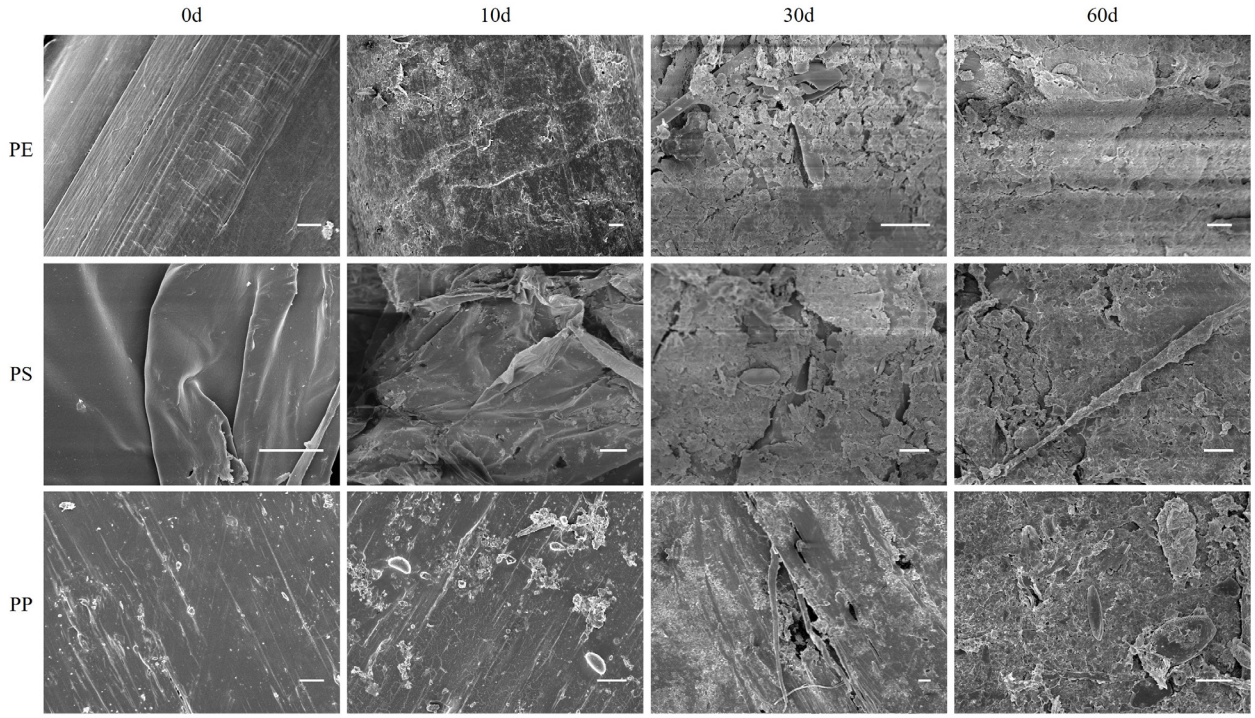



**Figure 2**

Scanning electron microscopy images of the surface of different MPs showing biofilm after 0, 10, 30, and 60 days (scale bar = 10 μm).

all samples tended to approach the saturation plateau, indicating sufficient sequencing depth and accuracy. The sequencing coverage of all samples was > 97%.

Based on the OTUs, bacterial alpha diversities were calculated by Chao1, Shannoneven and Shannon indices (Fig. 3A). After 10, 30 and 60 days of exposure, the Chao1, Shannoneven and Shannon indices of all MPs were higher than those of seawater (Wilcoxon rank-sum test). This suggested that the bacterial community richness, evenness and diversity on MPs were higher than in seawater. For example, the Chao1 and Shannon indices for MPs were in the range of 2461–4732 and 5.13–6.27, thus higher than those for seawater (1525–3307 and 3.79–5.27). This is consistent with some previous studies (Wu et al. 2019; Zhu et al. 2019; Feng et al. 2020), but contradictory to other studies where higher species richness and diversity were found in seawater than on MPs (Zettler et al. 2013; De Tender et al. 2017; Sun et al. 2020; Wang et al. 2020). This inconsistency may be attributed to variable environmental conditions. The studies that found higher diversity in water than on MPs were conducted in natural water without human activities. In contrast, our research was conducted in a lagoon with many mariculture facilities, which may reduce the current flow and ultimately affect the diversity of bacteria. The biofilm formed on MPs may provide physical support and protection from mechanical damage and predators, consequently enhancing the diversity of bacteria (Oberbeckmann et al. 2015; Shen et al. 2019). Our research also showed that richness, evenness and diversity of bacteria was higher on PS than on PE and PP, especially at the exposure of 60 days. This phenomenon proved again the importance of properties of polymer types for the biofilm formed on MPs (Yang et al. 2020). Combined with SEM images (Fig. 2), this result can be partly explained by differences in initial surface morphologies of different MPs. For example, PS exhibited a rougher surface than PE and PP, which may provide more surface area for microbial colonization.

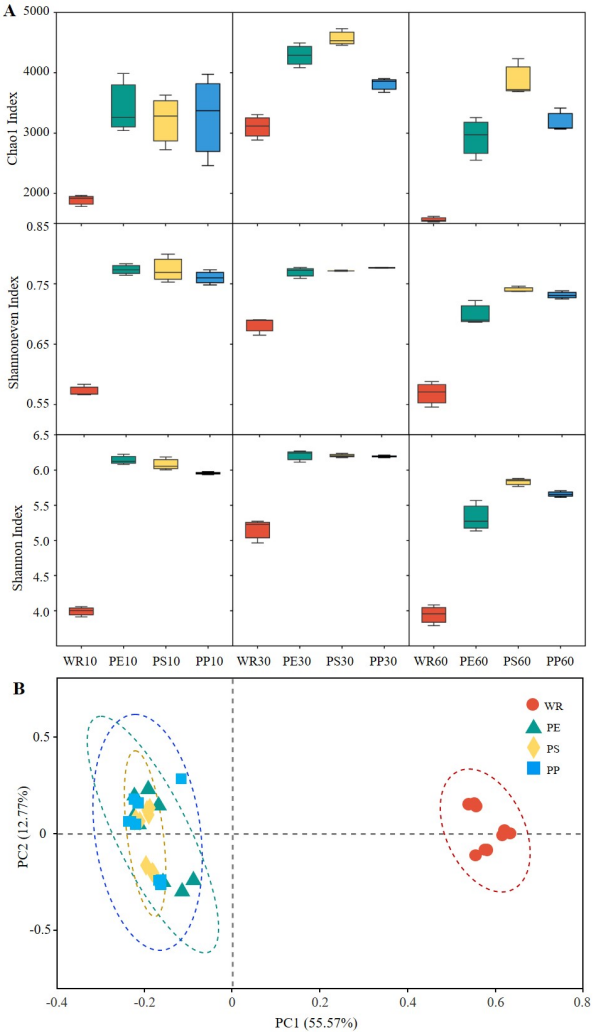



**Figure 3**

(A) Alpha diversity (Chao1, Shannoneven and Shannon indices) of bacterial communities on MPs and seawater after 10, 30 and 60 days of exposure. (B) Principal Coordinates Analysis (PCoA) based on Bray–Curtis distance, calculated from OTUs of bacterial communities.

Type- and time-dependent variation in bacterial communities was revealed by PCoA (Fig. 3B). The clear separation between the confidence ellipse of bacterial communities on various types of MPs and seawater suggested that the composition of bacterial communities on MPs differed significantly from that in seawater. The composition of bacterial communities on different types of MPs overlapped to a large extent. However, the composition of bacterial communities at different exposure times was relatively scattered, particularly in the case of exposure lasting 60 days (figure not shown). The effect of MPs exposure time on bacterial communities was much greater than the types of MPs. Changes in microbial communities on MPs with exposure time were likely related to changes in the surrounding environment, as the microbial community in water varied at the corresponding time. Even in a stable laboratory simulation environment such as a microcosm experiment, microorganisms on MPs also showed obvious succession with exposure time (Tu et al. 2021). The above results demonstrated that the composition of bacterial communities on MPs differed from that in seawater, and the exposure time had a stronger effect on changes in the composition of bacterial communities on MPs than the polymer types.

### 3.3. Composition and structure of bacterial communities on MPs and in seawater

Significant differences in the composition of bacterial communities at the phylum level were found between MPs and seawater (Fig. 4A), but no significant differences were observed between the types of MPs. In addition, the bacterial composition on MPs showed some differences at different exposure times. Bacteria in seawater mainly belonged to Proteobacteria, Bacteroidetes and Actinobacteria, with the average abundance of Proteobacteria up to 59.9%. In contrast, the composition of bacteria on MPs was relatively decentralized, with Proteobacteria, Firmicutes and Actinobacteria being the dominant phyla. The abundance of Proteobacteria and Bacteroidetes in seawater was much higher than that on MPs, while the abundance of Actinobacteria, Firmicutes and Chloroflexi on MPs was significantly higher than in seawater. It is worth noting that Chlamydiae, Acidobacteria and Dadabacteria were only found on MPs although their abundance was very low. This indicates that bacteria on MPs were not exclusively derived from seawater. The structure of bacterial communities on MPs consistently differs from those in the surrounding water (Harvey et al. 2020; Oberbeckmann & Labrenz 2020; Xue et al. 2020). Dadabacteria have the potential to degrade dissolved organic matter, specifically peptidoglycan and phospholipids (Graham & Tully, 2021). The prolongation of exposure time leads to a significant increase in the abundance of Proteobacteria and Bacteroidetes on MPs and a decrease in the abundance of Firmicutes and Chlamydiae, showing time-dependent succession

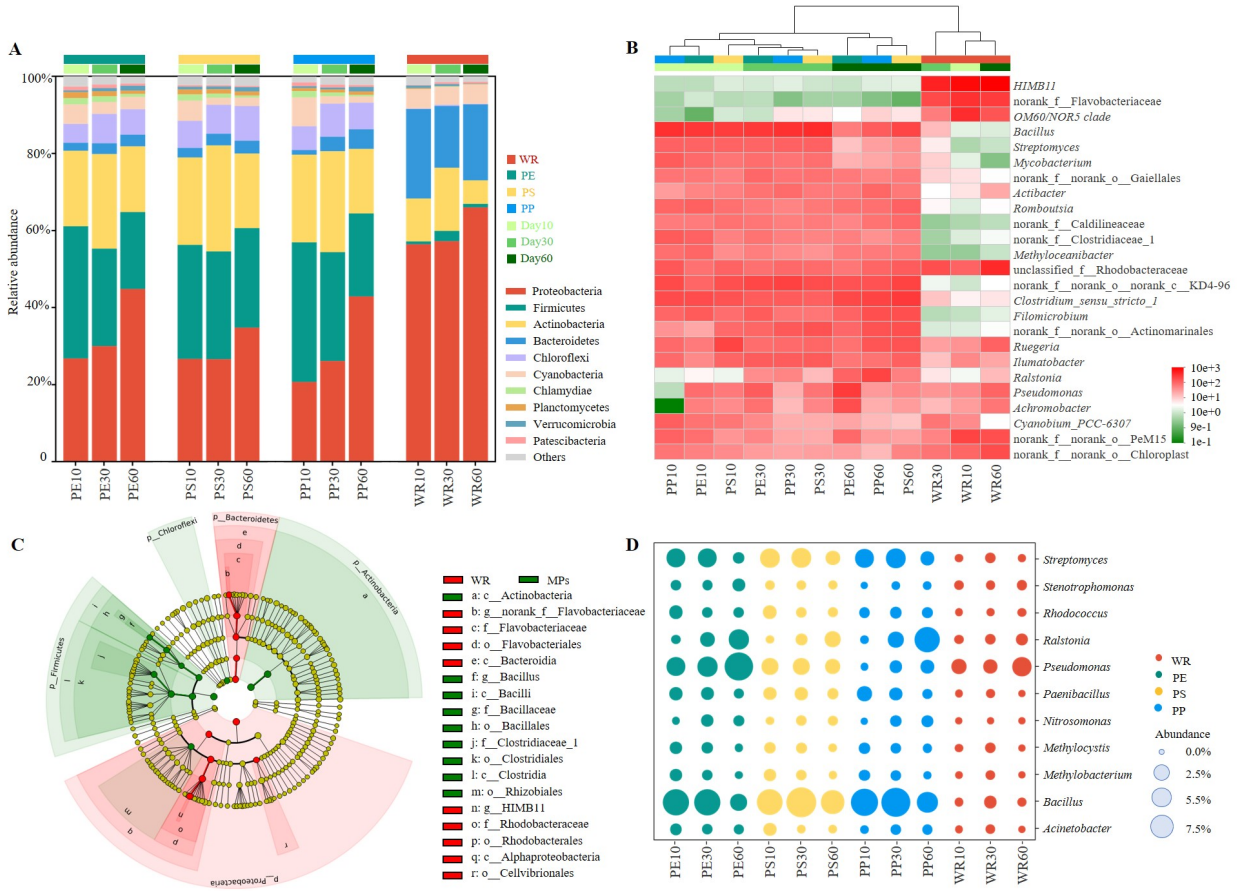



**Figure 4**

(A) Histogram of abundance distribution at the phylum level. (B) Heatmap of abundant bacterial genera (top 25) present in the microbial community of different groups. (C) Cladograms of LEfSe analysis using abundance of the full taxonomy for MPs and seawater. Bacterial groups from phylum to species level are listed from the center out. Biomarkers were selected based on the Kruskal–Wallis test ($p < 0.05$) and the linear discriminant analysis score greater than 4.5. (D) Abundance bubble chart of potential degradation bacteria and pathogens at the genus level.

characteristics. Consistent with previous reports, Bacteroidetes were always significantly more abundant at later time points (Wright et al. 2021).

The detailed composition of bacterial communities at the genus level (top 25) was further revealed using heat maps (Fig. 4B), which was similar to that at the phylum level. The results of cluster analysis showed that the relative abundance and composition of bacteria on MPs were significantly different from those in seawater after 60 days of exposure. This suggests that MPs may provide a unique habitat for bacterial communities (Amaral-Zettler et al. 2020; Yang et al. 2020). Furthermore, Wilcoxon rank-sum test showed that the abundance of HIMB11 and OM60/NOR5 clades in seawater was significantly higher than that on MPs ($p < 0.001$). HIMB11 has the potential to utilize various energy sources (such as organic matter, reduced inorganic sulfur, light and carbon monoxide; Durham et al. 2014). The OM60/NOR5 clade can play an important role in the marine carbon cycle (Spring & Riedel 2013). The abundance of *Bacillus*, *Streptomyces*, *Romboutsia*, *Clostridium sensu stricto 1* and *Filomicrobium* on MPs was significantly higher than that in seawater ($p < 0.001$). Among them, *Clostridium sensu stricto 1* with higher abundance was considered well adapted to higher DOC and lower DO conditions (Zhao et al. 2017), which are typical environmental characteristics in the mariculture lagoon. There was no significant difference in the composition of bacterial communities among the three polymer types of MPs, which could be due to the environmental characteristics of the long-term mariculture water covering the difference of MPs types. It is not the polymer types, but environmental factors that have the largest impact on microbial composition (Wright et al. 2021). There were some differences in the composition of bacterial communities at different exposure times. The non-parametric Kruskal–Wallis sum-rank test showed that the abundance of *Mycobacterium* decreased significantly, while the abundance of *Actibacter* and *Ralstonia* increased significantly with the prolongation of exposure time. The LEfSe method identified the key bacteria taxa in different samples, where a threshold of 4.5 was used for logarithmic LDA scores (Fig. 4C). Specifically, we found that the phylum Firmicutes, the genus *Bacillus* and the family Clostridiaceae_1 were significant biomarkers on MPs; the phyla Bacteroidetes and Proteobacteria, the genus *H1MB11* and the family Flavobacterium were significant biomarkers in seawater.

According to other studies (Amaral-Zettler et al. 2020; Rogers et al. 2020; Yang et al. 2020; Bowley et al. 2021), 12 genera of bacteria (abundance above 0.01%) were selected as potential plastic biodegraders and pathogens (Fig. 4D). Even though pathogenic *Vibrio* was frequently found on MPs in different water environments (Zettler et al. 2013; Li et al. 2019; Oberbeckmann & Labrenz 2020; Sun et al. 2020), the average abundance of *Vibrio* in this study was below 0.01%. Moreover, we found no potential pathogens enriched on MPs, and only observed some potential plastic biodegraders on MPs whose abundance was higher than in seawater. Bacteria from several taxa associated with plastic degradation, including *Streptomyces*, *Ralstonia* and *Bacillus*, were more abundant on MPs than in seawater, with *Ralstonia* showing a significantly increasing trend in abundance with exposure time.

### 3.4. Prediction analysis of bacterial community function

Changes in bacterial community composition may be accompanied by changes in bacterial community function. To analyze the effect of MPs on microbial functional diversity, the sequences obtained from 16S rRNA data were annotated in the KEGG database. Hierarchical cluster analysis (Fig. 5A) showed that metabolic pathways in seawater at level 3 were significantly different from those on MPs. No significant differences were observed between different polymer types of MPs or different exposure times. Therefore, Figure 5B and Figure 5C focused on the differences between seawater and MPs.

Based on the prediction of functional potential of bacteria, six metabolic pathways were obtained at level 1: metabolism (77.34%), genetic information processing (6.38%), environmental information processing (5.76%), cellular processes (4.46%), human diseases (4.17%) and organic systems (1.89%). The observed abundance of metabolism was higher than in other studies (Wang et al. 2020; Wen et al. 2020).

The results of metabolic pathway prediction (Fig. 5B) showed that metabolic differences between the bacterial community in seawater and on MPs were mainly related to human diseases and metabolism at level 1. In the pathways related to human diseases at level 1, the relative abundance of three metabolic pathways at level 2, such as infectious disease: bacterial, was significantly lower on MPs than in seawater ($p < 0.05$). In the pathways related to metabolism at level 1, the relative abundance of five metabolic pathways at level 2, including Xenobiotics biodegradation and metabolism, was significantly lower on MPs than in seawater, while the relative abundance of Carbohydrate metabolism on MPs was higher than that in seawater. In the pathways related

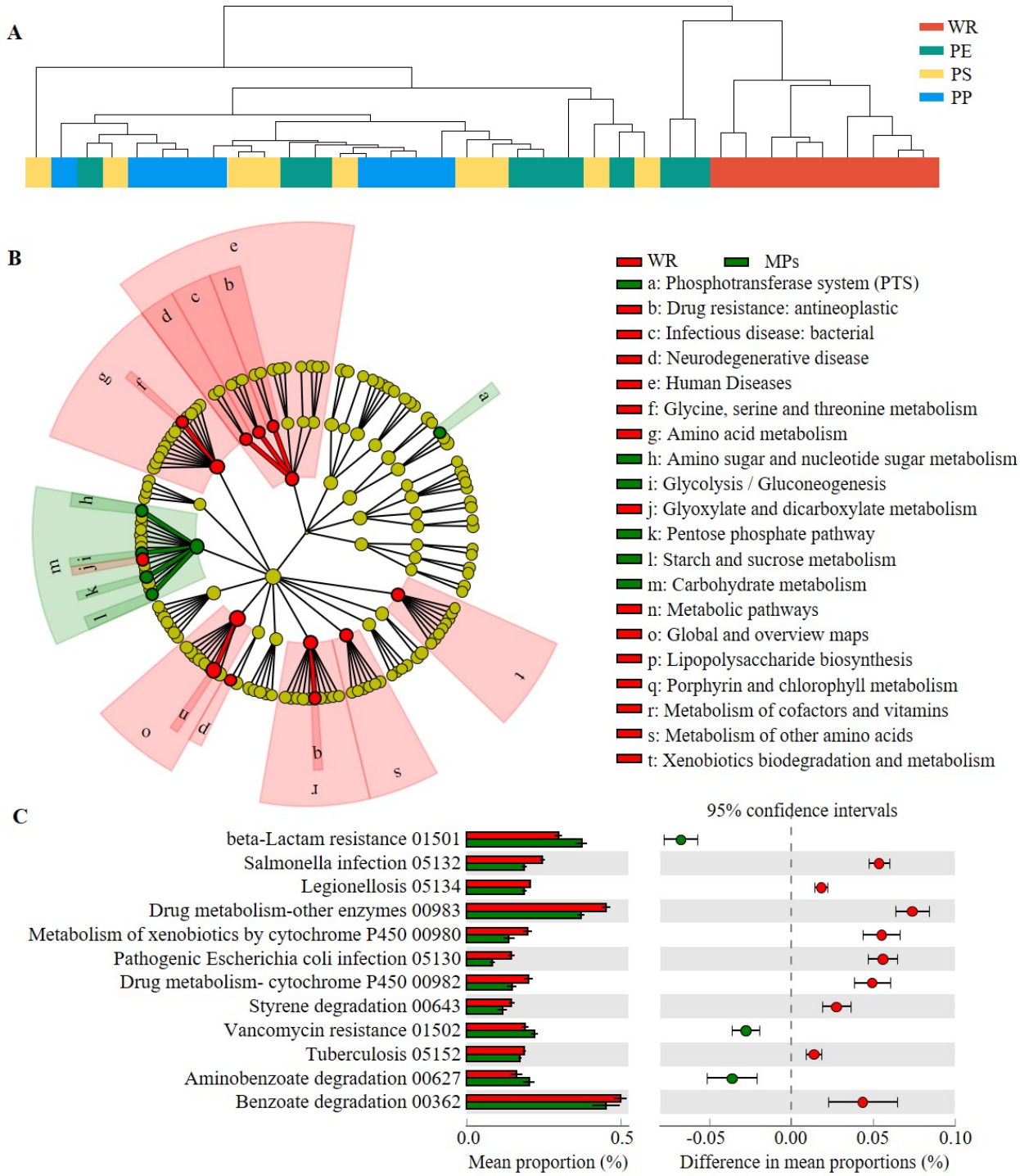



**Figure 5**

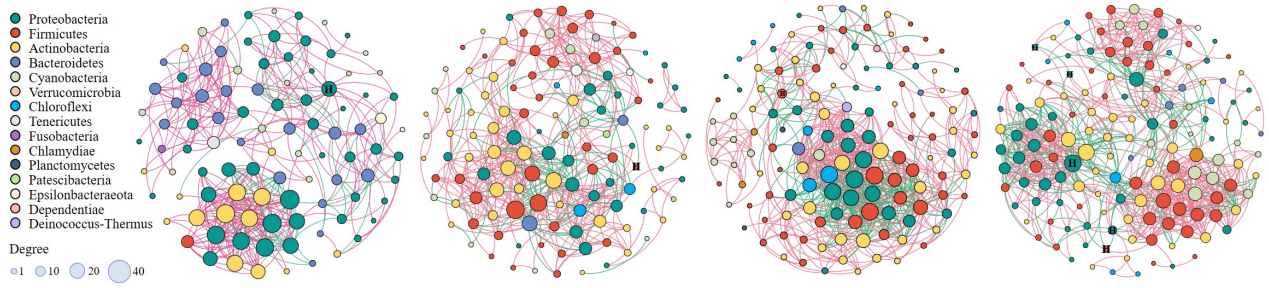
(A) Hierarchical cluster analysis of KEGG categories at level 3. (B) LEfSe analysis using abundance of the predicted metabolic pathway. Biomarkers were selected based on the Kruskal–Wallis test ($p < 0.05$) and the linear discriminant analysis score greater than 2.8. (C) Comparison of the abundance of predicted metabolic pathways of Drug resistance: antimicrobial, Infectious disease: bacterial and Xenobiotics biodegradation at level 3. A positive value indicates a significantly ($p < 0.01$) higher abundance of metabolic pathways in bacteria associated with seawater compared with this those associated with MPs.

to Carbohydrate metabolism at level 2, the relative abundance of Amino sugar and nucleotide sugar metabolism, Glyoxylate and dicarboxylate metabolism, Starch and sucrose metabolism at level 3 was significantly higher on MPs than in seawater, which may affect the carbon and nitrogen cycle.

We focused more on the differences ($p < 0.01$) in seawater and on MPs in three metabolic pathways at level 2, including Drug resistance: antimicrobial, Infectious disease: bacterial and Xenobiotics biodegradation and metabolism. As shown in Figure 5C, the relative abundance of metabolic pathways in Drug resistance showed significant differences: antimicrobial were significantly higher on MPs, and those in Infectious disease: bacterial were significantly lower on MPs than in seawater. As for Xenobiotics biodegradation and metabolism, other metabolic pathways were lower on MPs than in seawater, except for Aminobenzoate degradation (PATH: ko00627). As a distinct microbial habitat, the polymer types of MPs may affect the metabolic performance and nutrient metabolism of microbial communities (Arias-Andres et al. 2018; Miao et al. 2019; Wang et al. 2021). Furthermore, we observed some differences in the metabolic pathways between PE and other MPs, but no significant differences between PS and PP.

### 3.5. Network analysis

The co-occurrence network can describe potential interactions or niche overlap relationships among microorganisms (Zhou et al. 2010). As shown in Figure 6, nodes and links of MPs were higher than those of seawater, and the microbial network structures of MPs were more complex. Among MPs, the structure of PP was the most complex one, followed by PS. The connections of four networks were mainly positive, suggesting that most microbial species cooperated to resist the environmental conditions of long-term mariculture. The lower positive connection ratio of MPs compared to seawater indicated that the cooperative relationship of the bacterial community on MPs was weaker. Moreover, a smaller average path length of MPs was observed compared to seawater. This suggests that the interaction between microorganisms on MPs may be stronger than that in seawater, and the response to changes in the external environment may be faster and more sensitive to interference. The centralization of stress centrality on MPs was much lower than in seawater, indicating that microbial connections on MPs were more dispersed. There were no network hubs in each network, but there were five general lists in PP and only one in the others. One connector of PP was Pseudomonas, which may be a pathogen or plastic biodegrader. The keystone phylum with high connectivity were all Firmicutes on MPs and Proteobacteria in seawater. As pioneer species, Firmicutes can form endospores to reduce environmental pressure and to improve the colonization environment. Proteobacteria contain many photosynthetic bacteria that can carry out photosynthesis. In general, the microbial network




| Topological indices | WR | PE | PS | PP |
|---|---|---|---|---|
| Nodes | 98 | 126 | 139 | 152 |
| Links | 413 | 511 | 771 | 793 |
| Positive links | 82.57% | 79.45% | 71.21% | 74.27% |
| Average path length | 4.78 | 3.30 | 4.12 | 3.39 |
| Centralization of stress centrality | 17.46 | 1.83 | 6.81 | 1.40 |
| Module hubs | 1 | 0 | 0 | 1 |
| Connectors | 0 | 1 | 1 | 4 |
| Key Phylum | Proteobacteria | Firmicutes | Firmicutes | Firmicutes |

**Figure 6**

Overview of bacterial networks in seawater, PE, PS and PP. Node color represents different phylogenetic phyla. Pink lines indicate positive interactions and green lines indicate negative interactions.

structure of MPs was more complex and more sensitive to the external environment compared with seawater. PP had the most general lists, and the keystone phyla of MPs and seawater were significantly different.

## 4. Conclusions

The biofilm adhering to the MPs surface became thicker with increasing exposure time, with the PS biofilm being the thickest. There were significant differences in the bacterial community between MPs and seawater, but no significant differences among MPs. The composition of the bacterial community on MPs changed with exposure time. Further analysis shows that the abundance of several potential plastic biodegraders on MPs was higher than that in seawater, while no potential pathogen was found. In addition, we found slight differences in metabolic function between different types of MPs and different exposure times, but significant differences between MPs and seawater. Compared with seawater, the microbial network structure of MPs was more complex and dispersed, of which PP with the most general lists was the most complex one.

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