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Distinct community structure and microbial functions of biofilms colonizing microplastics

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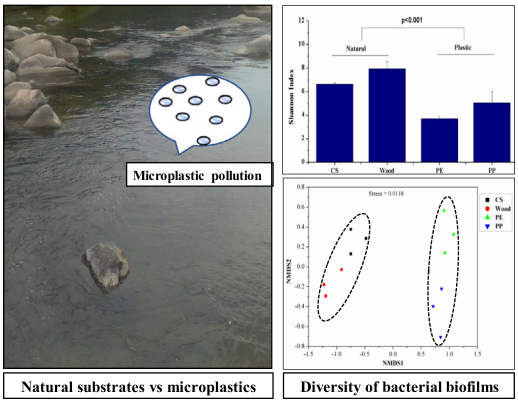
HIGHLIGHTS

- Alpha diversity of biofilms was lower on microplastic than on natural substrates.
- Community structure and composition varied between biofilms on different substrates.
- Metabolic pathways were altered in biofilms colonizing microplastic.
- Microplastic is a new microbial niche affecting microbial structure and function.
- This alteration in biofilms may have an ecological impact on aquatic ecosystems.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics are frequently detected in freshwater environments, serving as a new factitious substrate for colonization of biofilm-forming microorganisms. Distinct microbial assemblages between microplastics and surrounding waters have been well documented; however, there is insufficient knowledge regarding biofilm colonization of plastic and non-plastic substrates, despite the fact that microbial communities generally aggregate on natural solid surfaces. In this study, the effects of substrate type on microbial communities were evaluated by incubation of biofilms on microplastic substrates (polyethylene and polypropylene) and natural substrates (cobblestone and wood) for 21 days under controlled conditions. Results from high-throughput sequencing of 16S rRNA revealed that the alpha diversity (richness, evenness, and diversity) was lower in the microplastic-associated communities than in those on the natural substrates, indicating substrate-type-coupled species sorting. Distinct community structure and biofilm composition were observed between these two substrate types. Significantly higher abundances of *Pirellulaceae*, *Phycisphaerales*, *Cyclobacteriaceae*, and *Roseococcus* were observed on the microplastic substrates compared with the natural substrates. Simultaneously, the functional profiles (KEGG) predicted by Tax4Fun showed that the pathways of amino acid metabolism and metabolism of cofactors and vitamins were increased in biofilms on the microplastic substrates. The findings illustrate that microplastic acts as a distinct microbial habitat (compared with natural substrates) that could not only change the community structure but also affect microbial functions, potentially impacting the ecological functions of microbial communities in aquatic ecosystems.

Abbreviations: ANOVA, analysis of variance; CS, cobblestone; KEGG, Kyoto Encyclopedia of Gene and Genomes; MP, microplastics; NMDS, non-metric multidimensional scaling; OTUs, operational taxonomic units; PE, linear low-density polyethylene; PP, polypropylene; STAMP, Statistical Analysis of Metagenomic Profiles.

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1. Introduction

Global plastic production has increased rapidly in the past 60 years, reaching 322 million tons in 2015 with an upward trend (PlasticsEurope, 2016). Large amounts of plastic debris are continuously released into the environment directly or indirectly (Dris et al., 2015; Ivleva et al., 2017) and then fragment into smaller particles collectively termed microplastics (MP; particles with diameter < 5 mm) by biological, photo-, and/or mechanical degradation (Shim et al., 2017). MP have been identified as an emerging environmental threat to aquatic ecosystems because of their negative effects on a range of aquatic organisms from phytoplankton to zooplankton, fish, and cetaceans (Cole et al., 2011; Connors et al., 2017; Eerkes-Medrano et al., 2015; Ogonowski et al., 2018; Sharma and Chatterjee, 2017). MP are widespread in marine habitats, and numerous studies have been performed to investigate their temporal and spatial distribution, fate, and ecological impacts in marine environments (Harrison et al., 2011; Ivleva et al., 2017; Oberbeckmann et al., 2015). Recent investigations revealed that MP were also discovered in various freshwater environments, such as rivers, lakes, and reservoirs (Di and Wang, 2018; Klein et al., 2015; McCormick et al., 2014; Su et al., 2016). Thus, the fate and potential impact of MP in freshwater systems should be further investigated.

When released in aquatic habitats, buoyant MP may float in the water for months and finally accumulate in benthic environments (Besseling et al., 2017; Eerkes-Medrano et al., 2015). During their long-distance transport by water flow and winds, MP can serve as factitious surfaces for planktonic microorganism colonization and assemblage formation (De Tender et al., 2015; Harrison et al., 2014; Rummel et al., 2017). Studies have reported that rapid formation of microbial biofilms was observed on MP surfaces within 1–2 weeks in aquatic environments, and the taxonomic composition of biofilms on plastic particles was distinct from the microbial assemblages of the surrounding water (De Tender et al., 2017; McCormick et al., 2014; McCormick et al., 2016). These MP are therefore suggested as a specific niche for microbial life, known as the "plastisphere" (Zettler et al., 2013). Moreover, MP were hypothesized as vectors for transport of pathogens and harmful algae species in natural ecosystems (Arias-Andres et al., 2018a; Koelmans et al., 2016; Viršek et al., 2017). For example, Eckert et al. (2018) demonstrated that increasing quantities of MP promote the survival of wastewater treatment plant (WWTP)-derived bacteria in fresh water. Therefore, the introduction of MP colonized by specific assemblages is likely to alter the microbial communities and genetic exchange in natural water and consequently affect the ecological function of the microbial communities.

Generally, in natural water, microbial communities exist in the form of biofilm attached to natural solid surfaces (such as rock and wood) with an assortment of colonies and cellular and extracellular polymers (Flemming et al., 2016). Biofilms have been demonstrated to be of great significance to microbial function and ecological processes in fresh water (Battin et al., 2016). The formation and growth of biofilms are significantly affected by environmental conditions, among which the types and properties of solid surfaces are directly associated with early biofilm formation (Cardinale et al., 2002). As MP serve as a new surface for biofilm colonization, it is essential to compare the microbial communities developing on plastic and natural substrates inoculated with the same source communities. Nonetheless, most current research is focused on the comparison of MP-associated and aquatic communities (Chae and An, 2017; De Tender et al., 2015; De Tender et al., 2017; Jiang et al., 2018), and investigations of biofilm formation on plastic and non-plastic surfaces are scarce (Ogonowski et al., 2018). More importantly, the specific assemblages colonizing MP might reveal distinct microbial functions compared with those of assemblages on natural substrates, resulting in notable ecological consequences.

Thus, in this study, we hypothesized that compared with natural substrates, the introduction of MP as a new substrate may result in a shift of the community structure of biofilms formed on these substrates and then change the functional diversity of biofilm communities, which consequently might result in unpredictable influences on the freshwater ecosystems. To test this hypothesis, an indoor biofilm culture experiment was performed using two types of substrates—natural (cobblestone and wood) and MP (polyethylene and polypropylene)—with a bacterioplankton community from Xuanwu Lake (Nanjing, China) as the inoculum. The microbial richness, composition, and structure of biofilm communities were compared between the natural and MP substrates. Moreover, comparative analysis of the predicted functional diversity was performed to investigate the ecological impacts of MP on biofilm in aquatic ecosystems.

2. Materials and methods

2.1. Microplastics and natural substrates

To evaluate the potential impacts of substrate type (natural and MP) on aquatic biofilm communities, four different substrates (two of each type) were selected for microbial colonization. Linear low-density polyethylene particles (PE; diameter 3–4 mm, density 0.92 g cm^{-3}) and polypropylene particles (PP; diameter 3–4 mm, density 0.91 g cm^{-3}) were purchased from Aladdin Biochemical Technology Co. LTD (Shanghai, China) and served as MP substrates. PP and PE were used because they represent the most abundant plastics detected in the aquatic environments (Cózar et al., 2014), and to compare with previous studies (Zettler et al., 2013), in which bacterial communities colonizing PP and PE were studied. Cobblestone (CS; diameter 3–4 cm) and shortcut wood (length 5 cm; width 2 cm; depth 1 cm), which are ubiquitous in freshwater environments, served as the natural reference substrates.

The source community was retrieved from fresh water collected from Xuanwu Lake, Nanjing, East China. Fifty liters of water was collected in sterile jars, transported to the lab on ice, and then filtered with a 10-μm sieve to remove the large particles and small organisms. The water quality parameters were also determined (pH = 7.7; total nitrogen = 2.3 mg L^{-1}; total phosphorous = 0.13 mg L^{-1}; ammonia = 0.62 mg L^{-1}; and nitrate = 0.85 mg L^{-1}).

2.2. Biofilm incubation

Bacterial biofilms were incubated in 12 experimental tanks (length 50 cm; width 50 cm; depth 30 cm), with three tanks per substrate type. To provide comparable surface areas for colonization, tanks were loaded with 200 particles of microplastics (PE and PP), and 10 pieces of natural substances (CS and Wood) (see Table S1). The tanks were situated in a greenhouse that was exposed to natural light, and the roofs were covered with black cloth to block approximately 50% of the incoming solar radiation. Evaporation loss was replenished daily by adding dechlorinated tap water. In order to facilitate the development of biofilms, 100 mL Woods Hole culture medium (Table S2) was preadded into experimental tanks (described below) to provide normal levels of nutrition (Sun et al., 2018). The tanks were stirred manually four times per day for 5–6 min. After 21 days of incubation, the substrate material was washed three times with sterile water, and the biofilms were collected and used in further experiments.

2.3. DNA extraction, amplification, and sequencing

Approximately 0.5 g biofilm was collected, from which genomic DNA was extracted using the E.Z.N.A.® Tissue DNA kit (Omega Biotek, Norcross, GA, USA). DNA integrity and purity were monitored on 1% agarose gels. DNA concentration and purity were measured simultaneously using the NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA). 16S rRNA genes of the V4 region were amplified using the 515F and 806R primers for bacterial communities. Then, the PCR products were detected by 1% agarose gel electrophoresis and purified. Sequencing libraries were generated using NEBNext® Ultra™ DNA

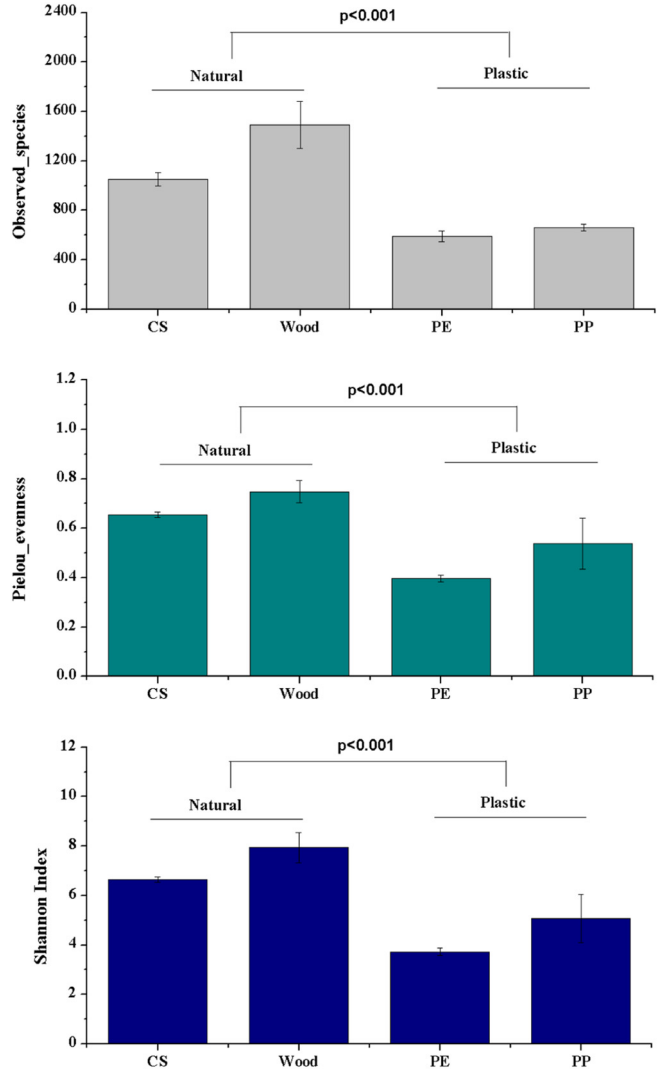

Fig. 1. Alpha diversity of biofilms, including observed species, Pielou's evenness, and Shannon index, for natural (CS: cobblestone; wood) and plastic substrates (PE: polyethylene; PP: polypropylene). P-values were determined by one-way analysis of variance followed by Tukey's posthoc tests.

Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following manufacturer's recommendations, and index codes were added. Finally, the library was sequenced, and the microbial communities were analyzed by the Illumina HiSeq 2500 platform (Guangdong Magigene Biotechnology Co., Ltd. China). Detailed information is provided in Supplementary Material.

2.4. Sequence processing and data analysis

The amplicons with sequences shorter than 200 bp and of low quality (quality score < 25) were removed from the raw sequence data. Then, the normalized samples were individually classified and analyzed by the Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu/). High-quality reads related to 16S rDNA were clustered into operational taxonomic units (OTUs), and then all sequences were subsampled to 27,658 sequences to adjust to the lowest number of sequences observed in a sample. Alpha diversity including observed species, Pielou, and Shannon indexes in the samples was calculated with QIIME (V1.9.1) and displayed with R software (V2.15.3). Bacterial community structure and composition were compared using non-metric multidimensional scaling (NMDS) analysis by means of Bray–Curtis similarities or weighed UniFrac distances in R using the metaMDS function, and a combination of these metrics allows for a more comprehensive community description (Caporaso et al., 2012; Legendre and Gallagher, 2001). Permutational multivariate analyses of variance (PERMANOVA; "adonis and anosim" in vegan R package) with 999 random permutations were performed to assess the influence of substrate on the community variances.

Bacterial metagenome content was predicted from the OTU (taxonomic) data, and functional inferences were made from the Kyoto Encyclopedia of Gene and Genomes (KEGG) catalog using the Tax4Fun program, which is connected to the SILVA database (Asshauer et al., 2015). Statistical Analysis of Metagenomic Profiles (STAMP) (Parks et al., 2014) was used to perform taxonomic (OTUs) and functional (KEGG level 3) comparison of biofilm samples on the natural and MP substrates. Significant differences were determined by Welch's unequal variances *t*-test and then corrected for multiple test according to the Benjamini–Hochberg false discovery rate (FDR) procedure, with q-values lower than 0.01.

2.5. Statistical analysis

All biochemical analyses were performed in triplicate, and the experimental values are expressed as the mean ± standard deviation (SD). The alpha diversity index, including observed species, Pielou's evenness, and Shannon indexes, and the relative abundances of dominant lineages (phyla and class) from natural and MP substrates were compared by one-way ANOVA followed by Tukey's posthoc tests.

3. Results

3.1. Taxonomic annotation and alpha diversity

After quality filtering, 416,626 sequences were detected and assigned to 12,328 OTUs from all the biofilm samples, and then 27,658 sequences were subsampled to compare community structure and composition. The rarefaction plots (Fig. S1) plateaued with the current sampling effort, and the coverage was high (>97%) for all biofilm samples, indicating that the OTUs of each bacterial library were adequately sampled.

Bacterial community complexity was evaluated using the alpha components including the total species richness (observed species), evenness (Pielou's evenness), and diversity (Shannon index) (Engelbrektson et al., 2010). Diverse microbial assemblages were observed on PE, PP, CS, and wood, with averaged observed OTUs of 669, 687, 1158, and 1595, respectively, suggesting different amounts of unique species. Significant differences in the observed species (range from 1490 to 592) (ANOVA, $P < 0.0001$), Pielou's evenness (range from 0.75 to 0.40) (ANOVA, $P < 0.0001$), and Shannon index (range from 7.94 to 3.72) (ANOVA, $P < 0.0001$) suggested community complexity differences between the four substrates. The highest species richness, evenness, and diversity were observed on the wood substrate, and the biofilm samples from PE exhibited the lowest values (Fig. 1).

Then, the four sample types were divided into two categories—natural substrates (CS and wood) and MP substrates (PE and PP)—to determine the influence of substrate type on the biofilm communities. As shown in Fig. 1, substrate type had a significant effect on the alpha diversity in biofilm communities, with significantly higher observed species on natural substrates than on MP (ANOVA, $P < 0.0001$) (Fig. 1). In addition, the distributions of the bacterial communities on natural substrates were more even, as indicated by Pielou's evenness, compared with those on MP (ANOVA, $P < 0.0001$) (Fig. 1). The higher Shannon index on natural substrates suggested that the biofilms formed on CS and wood were more diverse than those formed on MP (PE and PP) (ANOVA, $P < 0.0001$) (Fig. 1).

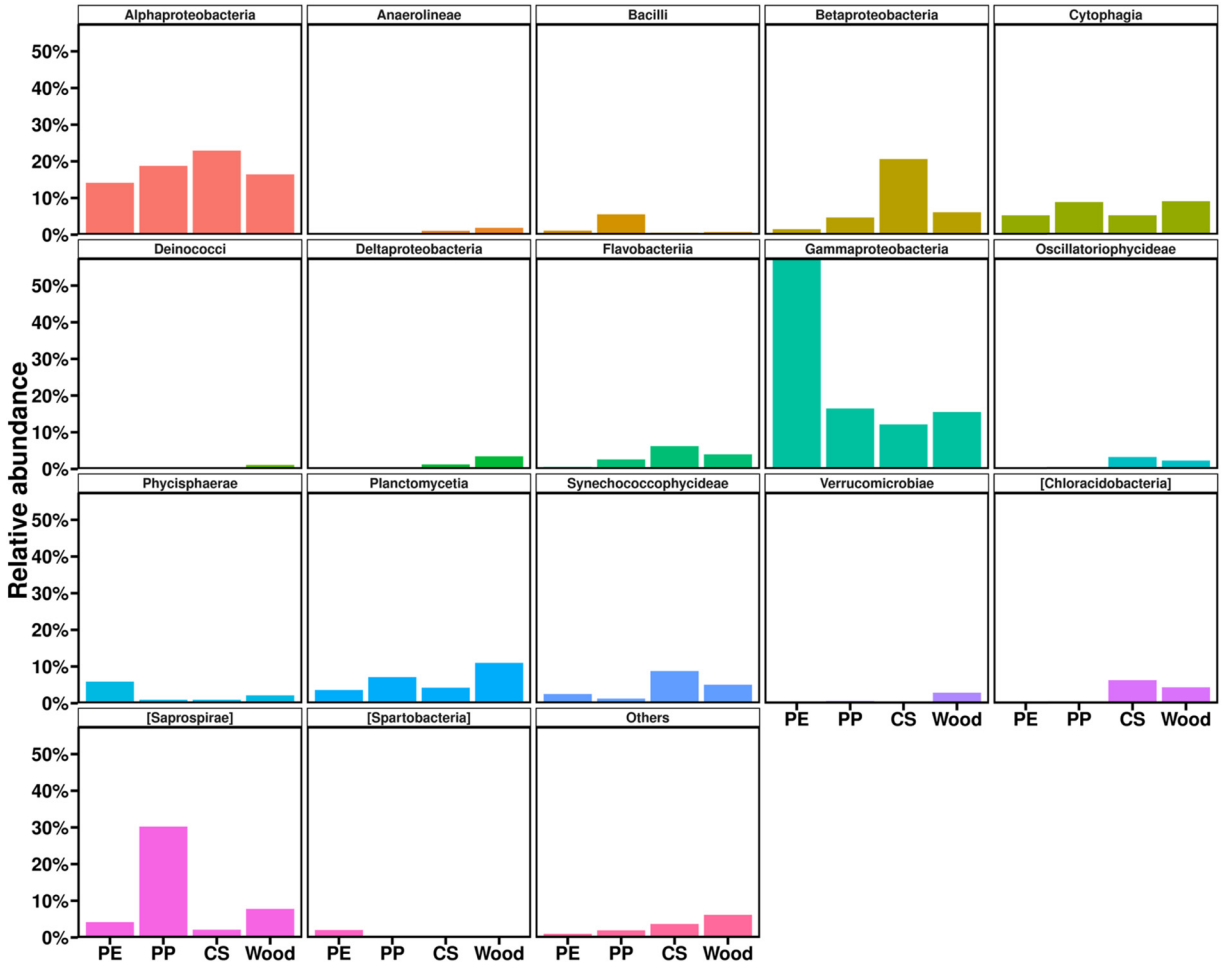

Fig. 2. Relative abundance of the top 18 most abundant classes in bacterial biofilms from natural (CS: cobblestone; wood) and plastic substrates (PE: polyethylene; PP: polypropylene). Statistical analysis between the substrate types was performed using one-way analysis of variance followed by Tukey's posthoc tests, and results are provided in Table S4 in SI.

3.2. Biofilm community composition and structure

Substrate type significantly affected the composition and structure of biofilm communities in this study. Proteobacteria (40.34–73.32%) was the dominant phylum in all collected biofilm samples, followed by Bacteroidetes (10.05–42.03%) (Fig. S2 and Table S3). The phyla Cyanobacteria, Acidobacteria, Chloroflexi, and Actinobacteria were significantly overrepresented on the natural substrates compared with

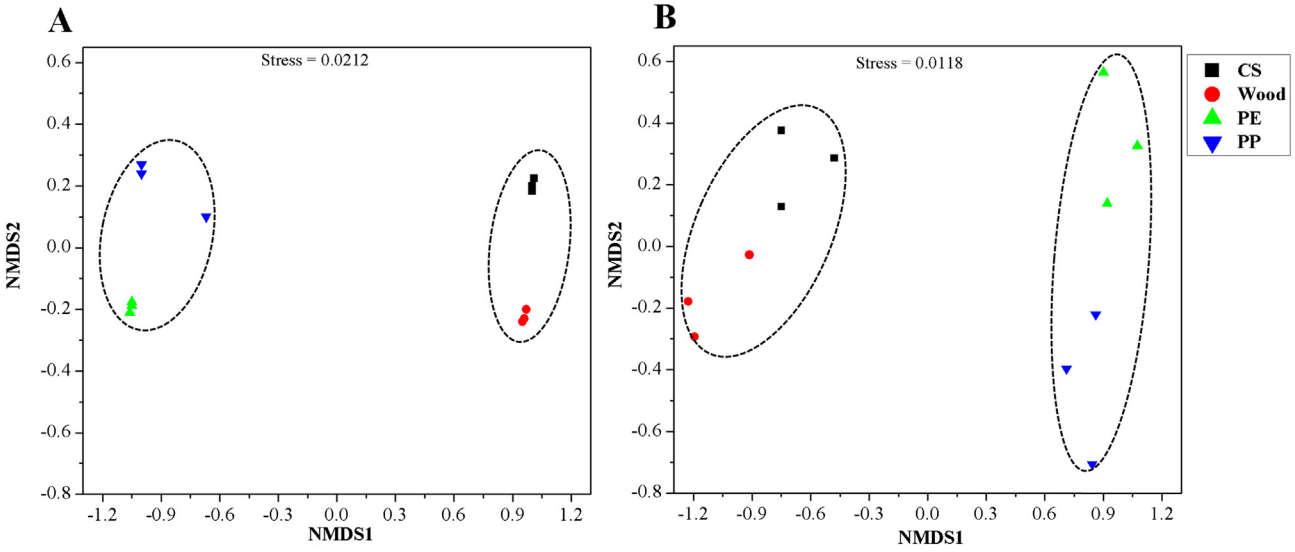

Fig. 3. Non-metric multidimensional scaling (NMDS) plots of operational taxonomic unit tables from all substrates (CS: cobblestone; wood; PE: polyethylene; PP: polypropylene) using the Bray–Curtis distance matrix (A) and phylogenetically weighted UniFrac distance matrix (B).

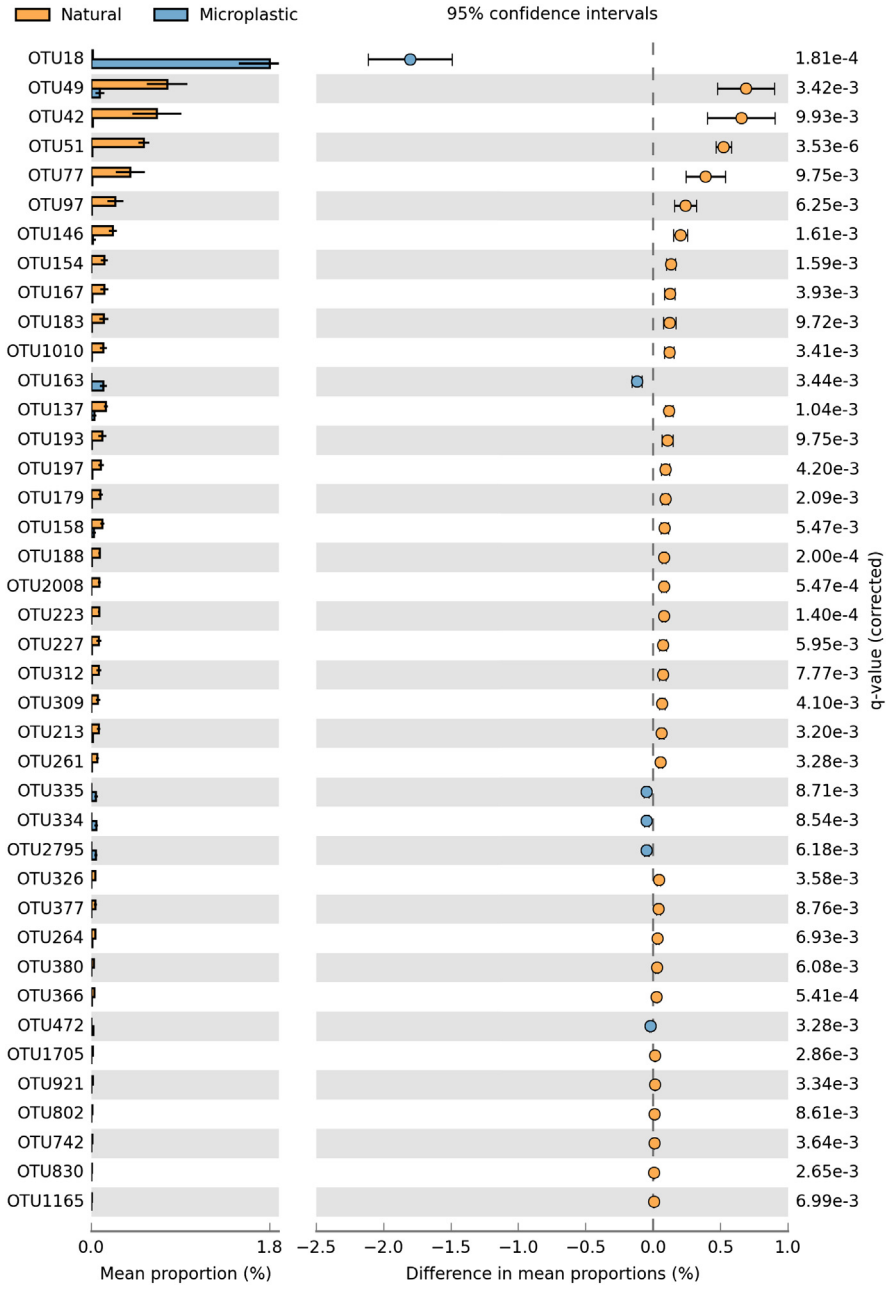

Fig. 4. Comparison of the bacterial operational taxonomic unit (OTU) abundance between the natural (CS: cobblestone; wood) and microplastic substrates (PE: polyethylene; PP: polypropylene). Positive values indicate a significantly ($P < 0.01$) higher abundance of OTUs in the communities associated with natural substrates compared with those associated with MP substrates. See also SI Table S6.

the MP substrates ($P < 0.01$, ANOVA). At the class level, the relative abundances of *Betaproteobacteria* and *Deltaproteobacteria* were higher on the natural substrates than on the MP ($P < 0.05$, ANOVA), while *Gammaproteobacteria* was more enriched on the MP ($P < 0.05$, ANOVA), especially on PE, where *Gammaproteobacteria* was the most dominant class (Fig. 2 and Table S4). Levels of *Flavobacteriia*, *Synechococcophycideae*, and *Oscillatoriophycideae* (the latter two belonging to Cyanobacteria) were significantly higher on the natural substrates ($P < 0.01$, ANOVA). Similar trends were also observed for the classes *Chloracidobacteria* and *Anaerolineae* ($P < 0.001$, ANOVA). Meanwhile, *Bacilli*, classified as Firmicutes, was more enriched on the MP substrates ($P < 0.05$, ANOVA) (Fig. 2 and Table S4).

Two-dimensional NMDS was performed based on a Bray–Curtis distance matrix and a phylogenetically weighted UniFrac distance matrix. In these plots, microbial communities that are similar are in closer proximity than dissimilar communities (Ramette, 2007). As shown in Fig. 3, NMDS demonstrated that all biofilm samples were obviously clustered into two groups (natural and MP substrates) and were separated primarily along the first coordinate axis. These two groups were significantly different as conformed by the Adonis ($F = 9.7$, $R^2 = 0.49$, $P = 0.003$) and Anosim analyses ($P = 0.003$, $R = 1$) (Table S5), consistent with the heatmap analysis (Fig. S3). Collectively, the results from analysis of community structures indicated that the bacterial communities are significantly different between the two types of substrates both at phylogenetic and taxonomic resolution. There was a lack of significant discrepancies in community structure among the single substrates (Table S5), which might be due to the low replication ($n = 3$) and high intra-treatment variability.

To better understand the influence of substrate type on the bacterial communities, the abundance of bacterial OTUs was compared between

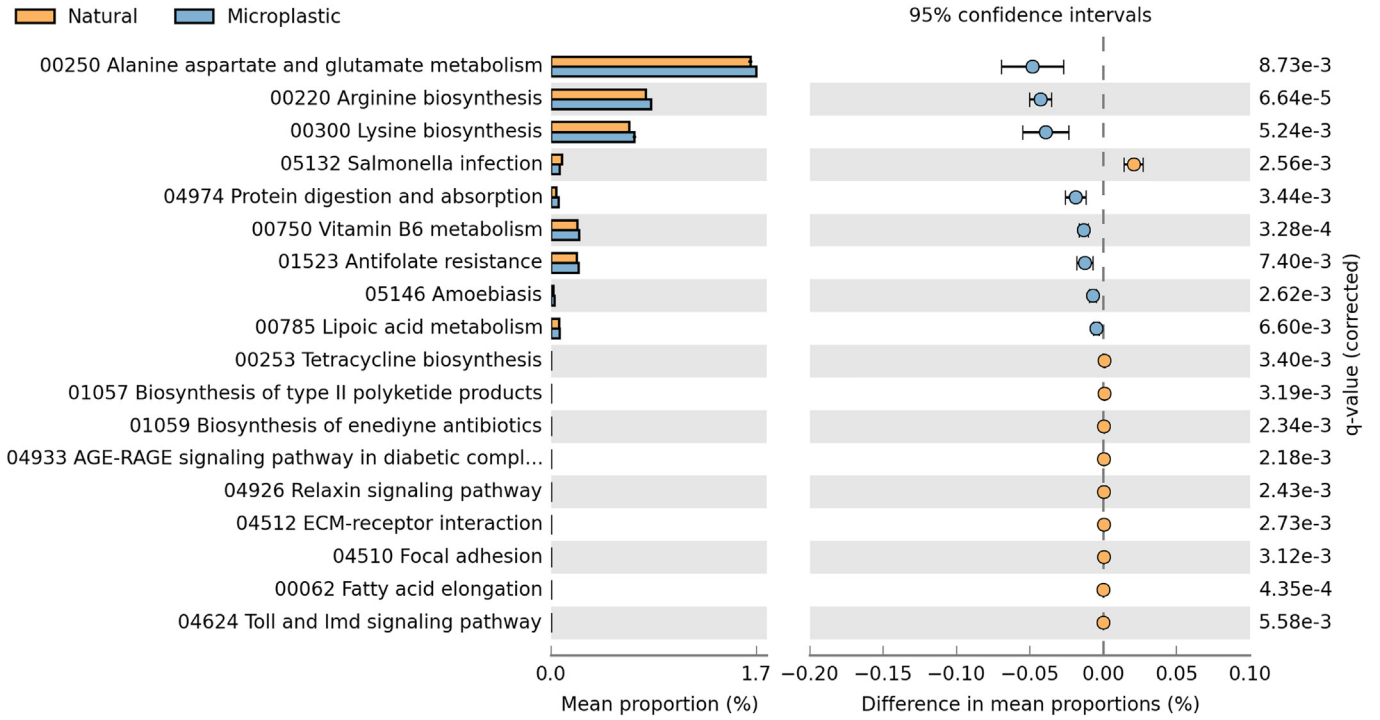

Fig. 5. Comparison of the abundance of the predicted metabolic pathways between the natural (CS: cobblestone; wood) and microplastic substrates (PE: polyethylene; PP: polypropylene). Positive values indicate a significantly ($P < 0.01$) higher abundance of metabolic pathways in the biofilms associated with the natural substrates compared with those associated with the MP substrates.

the natural and MP substrates using STAMP. Collectively, a total of 40 differentially represented OTUs were observed between natural and MP substrates ($P < 0.01$) (Fig. 4 and Table S6). Only six of the 40 OTUs, mainly belonging to the phyla Planctomycetes, Bacteroidetes, Verrucomicrobia, and Proteobacteria, were more abundant on the MP substrates compared with the natural substrates. In particular, OTU18 assigned to *Pirellulaceae* (Planctomycetes) contributed most to this variation, as its abundance was 120 times higher on the MP compared with the natural substrates. OTU163, OTU2795, and OTU334 were annotated to *Phycisphaerales* (Planctomycetes), *Cyclobacteriaceae* (Bacteroidetes), and *Luteolibacter* (Verrucomicrobia), respectively, with ~20- to 78-times higher abundance on MP compared with the natural substrates. OTU335 (*Phycisphaerales*) and OTU472 (*Roseococcus*) were only detected on the MP substrates. The OTUs that were overrepresented on the natural substrates were more diverse and mainly belonged to Proteobacteria (13 of 34), Bacteroidetes (8 of 34), and Cyanobacteria (6 of 34).

Interestingly, no OTU was significantly different in relative abundance within the natural group (CS vs. wood) and the MP (PE vs. PP) group in this study. These results were consistent with those of NMDS and demonstrated the potential impact of substrate type on the microbial communities in biofilms.

3.3. Microbial functional potential of biofilm communities

To investigate the effects of substrate type on microbial functional diversity in biofilm communities, the sequences obtained from 16S data were annotated to the KEGG database (Asshauer et al., 2015). Forty functional categories were detected with metabolism (40.68% of the total predicted genes) of the highest abundance, which mainly consisted of carbohydrate (12.35%), amino acid (12.01%), energy (7.91%), and cofactor and vitamin (7.18%) metabolism. In the comparison between the natural and MP substrates, interesting trends were observed for the predicted microbial functions of the biofilms. Unlike in the community structure, there was no significant difference in functional diversity between the two types of substrates (Adonis, $F = 12.3$, $R^2 = 0.73$, $P = 0.1$; Anosim, $P = 0.1$, $R = 1$). PE was separated from the other three substrates (PP, CS, and wood), as confirmed by the cluster analysis (Fig. S4).

There were still significant differences in the functional composition between the two types of substrates, although the magnitude of difference was relatively low. Some metabolic pathways were overrepresented on the MP substrates compared with the natural substrates (Fig. 5). Specifically, the pathways of amino acid metabolism (alanine, aspartate, and glutamate metabolism, PATH:ko00250; arginine biosynthesis, PATH:ko00220; lysine biosynthesis, PATH:ko00300) and metabolism of cofactors and vitamins (vitamin B6 metabolism, PATH:ko00750; lipoic acid metabolism, PATH:ko00785) were enriched on the MP ($P < 0.01$). The pathway of amoebiasis (PATH:ko05146) was more abundant on MP, while Salmonella infection (PATH:ko05132) was overrepresented on the natural substrates ($P < 0.01$); both these pathways belong to the pathways of infectious diseases.

4. Discussion

MP were documented to be widespread in freshwater environments, and their ecological impact has attracted the attention of many researchers (Besseling et al., 2017; Canniff and Hoang, 2018; Eerkes-Medrano et al., 2015; Kalcikova et al., 2017). However, investigations of the microbial assemblages colonizing MP in fresh water are relatively lacking (Blettler et al., 2018), and the specific biofilm communities might show different microbial functions, resulting in negative effects on the natural water ecosystems. Thus, in this study, we systematically evaluated the community structure and functional diversity of biofilms colonized on natural and MP substrates. Our findings provided evidence that the introduction of MP in fresh water results in distinct bacterial community composition and structure and decreases the richness and diversity of the biofilms compared with those colonizing natural

substrates. In addition, the functional diversity of biofilms colonizing MP substrates was significantly different from that of biofilms on the natural substrates. These results suggested that MP serves as a new substrate for microbial colonization, possibly altering microbial survival strategies and negatively affecting their ecological functions and further biogeochemical processes.

Previously, McCormick et al. (2014) reported that MP biofilms are significantly less diverse compared with those from the surrounding water in an urban river. A similar trend was also observed for fungal communities on PE and PS substrates from the River Warnow and the Baltic Sea (Kettner et al., 2017). Furthermore, Zettler et al. (2013) found that the average observed richness of bacterial assemblages was obviously lower on fragments of PE and PP compared with that in the seawater. These results indicated that the MP surfaces serve as a specific microhabitat for bacteria and fungi in both seawater and freshwater environments. However, there is limited information regarding the differences between biofilm communities developing on natural and MP substrates (Ogonowski et al., 2018), in spite of the fact that microbes in aquatic environments generally exist in the form of assemblages attached to solid surfaces (Battin et al., 2016). In this study, the alpha diversity (richness, evenness, and diversity) of bacterial communities on MP was significantly lower than that on natural substrates. These results suggested the occurrence of species sorting during biofilm development on MP, resulting in reduced capacity of MP-associated biofilm communities to withstand perturbation (Girvan et al., 2005) and maintain microbial activities (Philippot et al., 2013).

In the present study, a clear differentiation between the bacterial communities on the two types of substrates (natural and MP) was observed in terms of taxonomic composition and community structure. Several studies have demonstrated distinct microbial communities between plastic and the water column (Harrison et al., 2014; De Tender et al., 2017; Jiang et al., 2018). A very recent study (Ogonowski et al., 2018) reported that bacterial communities colonizing plastic were significantly different from those colonizing non-plastic substrates. The researchers found that *Alphaproteobacteria* and *Betaproteobacteria* were the two most dominant classes on plastic substrates, while Bacteroidetes and Actinobacteria showed higher abundances on the non-plastic substrates. In this study, *Alphaproteobacteria* and *Gammaproteobacteria* were found to be prevalent on the MP substrates, consistent with the research of Zettler et al. (2013), who investigated the bacterial communities growing on PE and PP debris in the Sargasso Sea. Similar results were also reported in other studies (McCormick et al., 2014; De Tender et al., 2015; Jiang et al., 2018). Moreover, among the significantly different OTUs between these two types of substrates, only a small fraction (six of the 40 OTUs) belonged to the biofilms colonizing MP, further indicating the specific preferences of bacterial communities colonizing different substrate types.

Distinct microbial communities colonizing MP might exhibit various microbial functions compared with those colonizing natural substrates. Herein, we observed higher abundance of *Pirellulaceae* and *Phycisphaerales*—belonging to Planctomycetes—on MP substrates, which was reported to be related to ammonium oxidation (Mohamed et al., 2010). The *Cyclobacteriaceae* family was enriched on the MP, and some species of this family are capable of degrading polysaccharides and other macromolecules like casein and lipids (McBride et al., 2014). The Verrucomicrobia genus *Luteolibacter* has been isolated from activated sludge (Park et al., 2013); however, its role in the environment remains largely unclear. The *Roseococcus* genus was only detected on MP and is known to use sodium thiosulphate as an additional source of energy (Liu et al., 2018). Collectively, the alterations in microbial communities were consistent with the prediction of the metabolic pathways, in which amino acid metabolism and metabolism of cofactors and vitamins were significantly different between the two types of substrates. Accordingly, these two metabolic pathways are strongly linked to the degradation of alanine, aspartate, glutamate and other carbohydrate (Neis et al., 2015), and the alterations of these functional properties might result in potential impacts on the carbon and nitrogen cycle in biofilm systems. Thus, these results indicated that MP, as a distinct microbial habitat, influence the metabolic performance and potentially the metabolism of nutrients of the specific communities in areas where MP accumulate (Arias-Andres et al., 2018b).

5. Conclusions

Although the investigations of microbial communities colonizing MP have attracted widespread attention, we focused on the less studied but equally important aspect of the comparison of natural and MP substrates in terms of community structure and microbial functions. We found that the introduction of MP into fresh water provides a new substrate for bacterial communities that not only alters the bacterial structure and composition but also changes the functional properties. This might result in notable ecological consequences for diversity and biogeochemical processes. With the accelerated release of MP into fresh water, the potential impacts of microbial MP colonization should be further investigated, especially those related to the ecological function of microbial communities in aquatic environments.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.378.

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