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Effects of Plastic Debris on the Biofilm Bacterial Communities in Lake Water

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Abstract: Increasing discharge of plastic debris into aquatic ecosystems and the worsening ecological risks have received growing attention. Once released, plastic debris could serve as a new substrate for microbes in waters. The complex relationship between plastics and biofilms has aroused great interest. To confirm the hypothesis that the presence of plastic in water affects the composition of biofilm in natural state, in situ biofilm culture experiments were conducted in a lake for 40 days. The diversity of biofilm attached on natural (cobble stones (CS) and wood) and plastic substrates (Polyethylene terephthalate (PET) and Polymethyl methacrylate (PMMA)) were compared, and the community structure and composition were also analyzed. Results from high-throughput sequencing of 16S rRNA showed that the diversity and species richness of biofilm bacterial communities on natural substrate (observed species of 1353~1945, Simpson index of 0.977~0.989 and Shannon–Wiener diversity index of 7.42~8.60) were much higher than those on plastic substrates (observed species of 900~1146, Simpson index of 0.914~0.975 and Shannon–Wiener diversity index of 5.47~6.99). The NMDS analyses were used to confirm the taxonomic significance between different samples, and Anosim (p = 0.001, R = 0.892) and Adonis (p = 0.001, R = 808, F = 11.19) demonstrated that this classification was statistically rigorous. Different dominant bacterial communities were found on plastic and natural substrates. Alphaproteobacterial, Betaproteobacteria and Synechococcophycideae dominated on the plastic substrate, while Gammaproteobacteria, Phycisphaerae and Planctomycetia played the main role on the natural substrates. The bacterial community structure of the two substrates also showed significant difference which is consistent with previous studies using other polymer types. Our results shed light on the fact that plastic debris can serve as a new habitat for biofilm colonization, unlike natural substrates, pathogens and plastic-degrading microorganisms selectively attached to plastic substrates, which affected the bacterial community structure and composition in aquatic environment. This study provided a new insight into understanding the potential impacts of plastics serving as a new habitat for microbial communities in freshwater environments. Future research should focus on the potential impacts of plastic-attached biofilms in various aquatic environments and the whole life cycle of plastics (i.e., from plastic fragments to microplastics) and also microbial flock characteristics using microbial plastics in the natural environment should also be addressed.

Keywords: biofilm; plastic debris; high-throughput sequencing; biodiversity; community structure

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1. Introduction

With the acceleration of industrialization and population increase, the production and utilization of plastics have risen sharply in the last decades. Global plastic produc-

tion reached 360 million tons in 2018 [1]. Millions of tons of plastic waste are constantly released into environment from industrial products and domestic use, and the fragmentation of plastics, microplastics (MPs, diameter less than 5 mm) widely spread around the world [2]. Plastic debris are released into the aquatic system by incineration, wastewater, surface runoff and others [3]. The migration, settling, biofouling, and degradation of (micro) plastics occur under both biotic and abiotic conditions [4]. Plastic debris can act as long-lasting reactive surfaces and absorb highly recalcitrant pollutants including organic matter and chemical substances, such as heavy metals, antibiotics, pesticides, and other xenobiotics [5,6].

Despite some progress, more studies are still needed to analyze the potential ecological effects of plastic debris in aquatic environment. Plastic debris can also provide new colonial substrates to biofilms as a new substance in water. The relationships between plastic debris and biofilms have aroused great attention. In general, bacterial communities affected by plastic debris are different from biofilms in natural freshwater ecosystems [7,8]. In natural water, microbial communities assemble as biofilm with an assortment of colonies and cellular and extracellular polymers, attaching to natural solid surfaces (such as rock and wood) [9]. Biofilms enable to biodegrade and remove organic pollutants at different levels, promoting the metabolism, mineralization, and circulation of essential nutrients in aquatic ecosystems. The biofilm is mainly formed by different microbial species [10] embedded in the extracellular polymeric substances (EPS). Biofilms have mechanical stability and can exist on the surface of solid–liquid, solid–gas, liquid–gas [11]. Many factors can affect the colonization and community composition of biofilm, including substrate type, hydrodynamic force, nutrient concentration, and temperature [9,10,12]. The biofilm formation process is also influenced by various environmental factors, including conductivity, TOD (total oxygen demand) and salinity [13]. Studies have confirmed that the biofilm in different temperatures, lighting conditions or seasons showed various growth characteristics [14,15]. In addition, the sewage contamination and water stagnation in river can affect biofilm [16–18]. On the other hand, the biofilm can influence environment at different levels. The periphytic biofilms increase the removal of Cu in wastewater and immobilized it into fibers [19], and they can be a new way to degrade MPs in aquatic environment [20]. Thus, the complex relationship between biofilm and plastic debris are far from clearly understood.

Some preliminary studies indicated that biofilms changed the absorption behaviors of tetracycline and copper onto polyethylene (PE) plastics [21]. The growing process of biofilms affected the sinking and floating of plastics, and decreased the hydrophobic property of plastics [22,23]. In addition, the biofilms could shorten the lifetime of plastics [23]. Some studies have compared the aquatic plankton in water with microbial communities on the plastic surface [14,24]. The results indicated that the biodiversity of aquatic plankton was either higher [25,26] or lower [24,27] than those on plastic-attached biofilms. These inconsistent results might be caused by the different environmental conditions or the various types of plastics used for the biofilm cultivation [26–28]. Besides, plastic biofilms can gather pathogen bacteria selectively [14,28], which increased the residence of pathogens in aquatic ecosystem. Since plastic debris can serve as a substance for biofilm to colonize, the control substrates, such as rock or glass, were recently recommended to use when studying the potential impacts of the plastic-biofilm in aquatic environment [29,30]. Miao et al. [8] investigated the bacterial diversity of biofilms on microplastic substrates (polyethylene, PE and polypropylene, PP) and natural substrates (cobblestone and wood) and the alpha diversity was found to be lower in the microplastic-associated communities than those on the natural substrates. Kettner et al. [31] explored the diversity and community composition of fungi attached to polyethylene (PE) and polystyrene (PS) particles incubated in different aquatic systems and the MP-associated communities were reported to be distinct from fungal communities in the surrounding water and on the natural substrate wood. Very recent studies found that biofilms on the plastic surface had a lower carbon metabolism rate compared with biofilms on the natural surface, such as rock [30,32]. In terms of types

of plastics, previous studies focused mainly on the most detectable plastic types, including polyethylene (PE), polypropylene (PP) and low-density polyethylene (LDPE) plastics in freshwater system [21,22,33], however, other plastics are also detected in aquatic environment, and their effects of the colonized biofilm are largely unknown. In addition, many recent studies comparing the differences of microbial communities between plastics and natural substrates were conducted in lab or in simulated natural environments [8,27,28], and more field experiments are needed to analyze the potential impacts of plastic-attached biofilm in aquatic environment.

To close the knowledge gap, in this study, different types of substrate were used for in situ biofilm culture experiment in the natural environment (Xuanwu lake, Nanjing, China). The impacts of polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA) plastics on biofilms were investigated by comparing with natural substrate (cobble stones (CS) and woods). All the experiments were performed in the natural water environment to simulate the forming of biofilms under natural conditions. Biofilm bacterial differences were analyzed by using 16 s rRNA amplicon sequencing. The main research aims of the study are to (1) investigate the bacterial community composition and structure on plastic debris in lake water; (2) compare the differences of bacterial communities between plastic debris and natural substrates (cobble stone and wood) in terms of alpha and beta diversities; (3) estimate the impacts of PET and PMMA on biodiversity of biofilm communities. This study could provide a new insight to understanding the potential impacts of plastics serving as a new habitat for microbial communities in freshwater environments.

2. Materials and Methods

2.1. Experimental Design and Setup

The cultivation experiment of biofilms on plastic substrates and natural substrates was performed at Xuanwu Lake (32.075° N, 118.794° E), which is a mesotrophic to eutrophic urban lake located in Nanjing, East China (Figure 1). The surface area of the lake is 5.02 km^2, and the average water depth is 1.5 m.

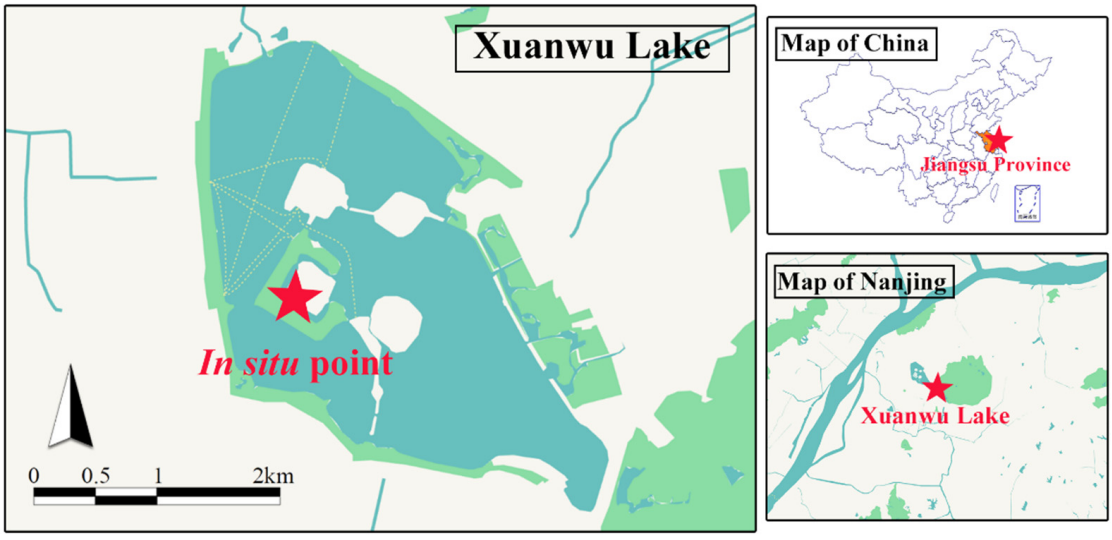

Figure 1. Location of study area and in situ cultivation site at Xuanwu Lake, Nanjing, East China.

As two of the most detected plastic types in freshwater, PET and PMMA were selected as plastic substrates to cultivate biofilms. PET sheet and PMMA sheet were cut into 4 × 4 cm squares. The PET sheet has a thickness of 0.3 mm and a density of 1.38 g/cm^3 and the PMMA sheet has a thickness of 1 mm and a density of 1.2 g/cm^3. As the non-plastic control substrate, cobble stone (diameter 3–4 cm) and wooden segment (4 cm × 4 cm) were also incubated.

Stainless steel wire mesh (length of 50 cm, width of 50 cm, and depth of 30 cm) was made to culture biofilms (Figure 2). Twelve devices were installed in the water column and were separated into four parts, each part was packed separately with wood (refers to A), PET (refers to B), PMMA (refers to C), and CS (refers to D). All materials were sterilized by immersing in 70% ethanol for 30 min, then were rinsed in deionized water before being used for experiments. The devices were placed at 0.30 m below the surface water to receive the same light intensity and ensure that all devices were kept within the water with water level fluctuation, and then the natural culture experiment persisted for 40 days. Over incubation period, microorganisms attached to the different substrates and formed biofilms.

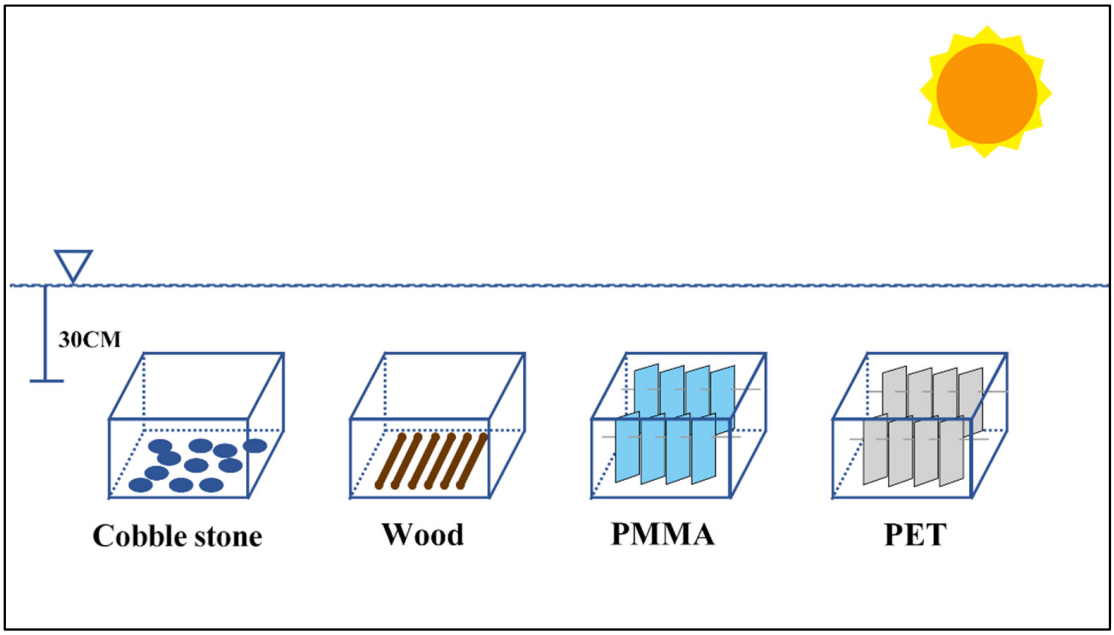

Figure 2. In situ biofilm incubation experiment on plastic (PET and PMMA) and natural (cobble stone and wood) substrates in lake water.

2.2. Analyses of Water Chemistry

In this study, water environmental parameters were measured at the beginning (day 0) and the end of the incubation (day 40). Temperature, pH, oxygen, and conductivity were measured in situ with a portable meter (HQ40d, Hach). Three 500 mL water samples were collected from the culturing sites for the measurement of total nitrogen (TN), total phosphorus (TP), nitrate nitrogen, ammonia nitrogen, and chemical oxygen demand (COD_{Mn}). The TN and TP was measured by using alkaline potassium persulfate digestion UV spectrophotometric method and molybdenum antimony photometric method, respectively. Nitrate nitrogen and ammonia nitrogen were measured by using a AA3 Continuous Flow Analytical System (SEAL AutoAnalyzer3, Norderstedt, Germany). COD_{Mn} was detected by using potassium permanganate oxidation method.

After 40 days of cultivation, the exposed materials were taken back to the laboratory and the biofilms were always kept wet using water collected from the Xuanwu Lake. Ultrasonic oscillometer was applied for the detachment of biofilm. The biofilm was then brushed into the centrifugal tube with a sterile brush for further determination.

2.3. DNA Extraction, Amplification and Sequencing

After the cultivation, 12 biofilm samples (three parallel samples per substrate in this study) were taken for DNA extraction. About 1 g of wet weight biofilm was collected, and 20 ng DNA was extracted by using the E.N.Z.A.® Tissue DNA kit (Omega Biotek, Norcross, GA, USA) following the instruction by the manufacturer. After that, 1% of agarose

gels were used to detect the integrity and purity of DNA. In the meantime, NanoDrop One was used to test the concentration and purity of DNA. Based on the template of genomic DNA, the 16 s rRNA primers (515F and 806R primer) in V4 region (which is used to identify the diversity of bacteria) with barcode and the Premix Tag (TaKaRa) were used for PCR amplification, and the product of PCR was examined by 1% of agarose gels. The samples within the length range of 290–310 bp main tape were used to further experiment. The concentration of PCR products was compared by GeneTools Analysis Software (Version4.03.05.0, SynGene, Frederick, MD, USA), and E.Z.N.A.® Gel Extraction Kit was used to recycle the mixture products of PCR. TE buffer elution was used to recover the target DNA fragment. Then, a database was created according to the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® procedure. At last, sequencing the PE250 to the amplified sublibrary on the IlluminaHiseq2500 platform.

2.4. Sequencing Processing and Data Analysis

The Trimmomatic software (V0.33, http://www.usadellab.org/cms/?page%20=%20trimmomatic) (accessed on 20 May 2021) was used to sift the raw reads data. The reads containing N element were sifted out. In addition, the ones with weights less than 20 or the filtered sequence length less than 100 bp were sifted out. All the high-quality clean reads were obtained. The paired-end clean reads were merged by using FLASH 1.2.11 software (https://ccb.jhu.edu/software/FLASH/) (accessed on 20 May 2021) according to the relationship of the overlap between the paired-end reads. When at least 10 of the reads overlap, the read was generated from the opposite end of the same DNA fragment. The maximum allowable error ratio of the overlap region is 0.1. The spliced sequences were called Raw Tags. Sequences were assigned to each sample based on their unique barcode and primer by using Mothur 1.35.1 software (http://www.mothur.org) (accessed on 20 May 2021), and the barcodes and primers were removed to obtain the effective Clean Tags.

For each representative sequence, the Unite (for 16S, chlorophyte, linomere, http://greengenes.secondgenome.com/) (accessed on 20 May 2021) database was used to annotate taxonomic information (the confidence threshold is set to be default $\geq$0.5) by using R package Qiime 1.9.0 [8]. The species taxonomies were divided into seven hierarches: kingdom, phylum, class, order, family, genus, and species. Multiple sequence alignment was conducted by using the FastTree software. The relative abundance of each OTU and the species annotation information of the representative sequence were combined by using R package ggtree for visual display. Subsequent analysis of alpha diversity and beta diversity were all performed basing on these normalized data.

R was used to analyze the relative abundance of each classification level and draw the species heatmap of relative abundance. At the same time the cluster analyses between samples and species were conducted [16]. Based on the homogenized-OTU abundance table, the script of alpha_diversity.py in R package Qiime was used to calculate two diversity indexes: Observed_species and Shannon–Wiener index. The script of make_rarefaction_plots.py on the Qiime was used to calculate the rarefaction of the six diversities above and draw the rarefaction plots. The differences between index groups of alpha diversity were analyzed by K-Sample Fisher–Pitman Permutation Test.

In terms of the beta diversity, the Bray Curtis, Unweighted Unifrac, and the functions of Anosim and Adonis were analyzed by using R. Based on Unweighted Unifrac distance matrix, the R packages Qiime and ggplot2 were used for nonmetric multidimensional scaling (NMDS) analysis and graphing.

2.5. Statistical Analysis

All index analyses of alpha diversity on natural and plastic substrates were performed in triplicate, and the values are presented as the mean $\pm$ standard deviation. The two diversity indices (observed species and Shannon–Wiener diversity index) of bacterial communities and the relative abundance of bacteria at different levels were compared by

using one-way analysis of variance (ANOVA) to evaluate the effects of different substrates. Alpha diversity was analyzed by using index differences, and the differences in diversity indices among different subgroups were evaluated by using Kruskal–Wallis test. $p < 0.05$ was taken as significant cut-off. Significant differences between biofilm samples were analyzed using Origin version 8.0 (OriginLab Corp, Northampton, MA, USA).

3. Results

3.1. Taxonomic Annotation and Alpha Diversity

During the biofilm incubation experiment, the water chemistry was measured, and the results were given in the Supplementary information (Table S1). Results showed that during the biofilm culture period, there is no significant change in the temperature, DO and the nutrient level of the water.

The biofilm samples were analyzed using high-throughput sequencing of 16S rRNA. After quality filtering, 349,854 sequences were detected. The rarefaction curve tends to be flat and it means that the sequencing data is large enough, reflecting the most bacteria diversity information (Figure S1).

This study applied the alpha components including the observed_species, Simpson, and Shannon–Wiener diversity indices to assess the bacterial community complexity on different substrates. There were significant differences in the observed_species ($p < 0.05$), Simpson ($p < 0.05$) and Shannon–Wiener diversity indices ($p < 0.05$), indicating obvious biofilm community differences between the four substrates. The largest species richness and diversity were observed on the wood substrate, while the values were much smaller on plastic substrates (Figure 3). The four substrates were grouped in two categories—natural substrates (CS and wood) and plastic substrates (PET and PMMA)—to determine the influence of substrate type. The indices indicated that Wood and CS had higher species richness and complex community structure than those on PET and PMMA ($p < 0.05$).

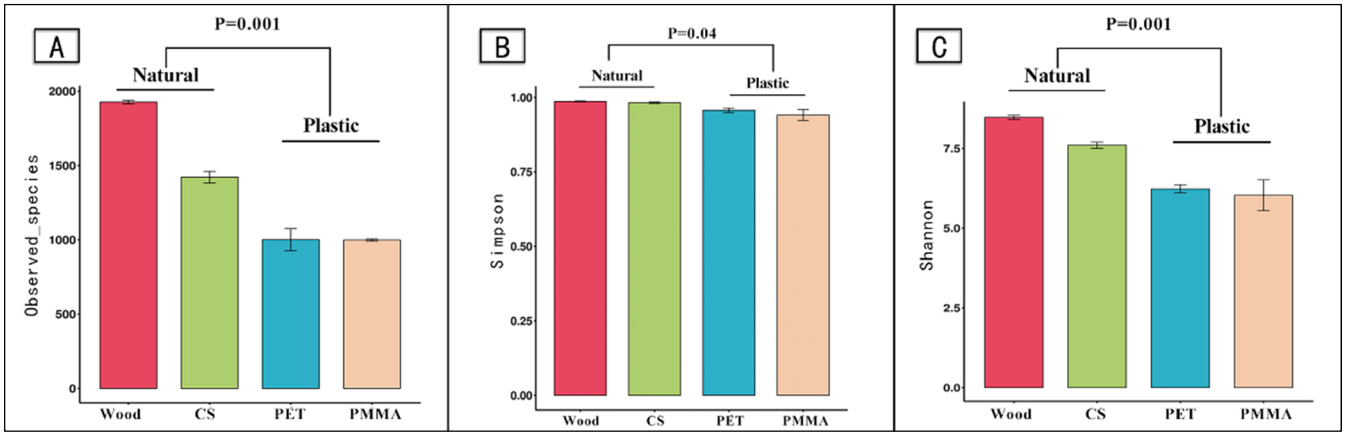

Figure 3. Different alpha diversities, including observed_species (**A**), varies from 999 to 1927, Simpson (**B**), varies from 0.941 to 0.986 and Shannon index (**C**), varies from 6.03 to 8.48 of biofilm bacterial communities on wood, cobble stones (CS), polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA).

3.2. Community Composition and Structure of Biofilm

Similarly, the biofilms on wood, CS and PET, PMMA were divided into two types, natural and plastic substrates (Figure 4). This classification was confirmed by the differences between the index groups, which showed the matrix type has a marked effect on the bacterial community structure.

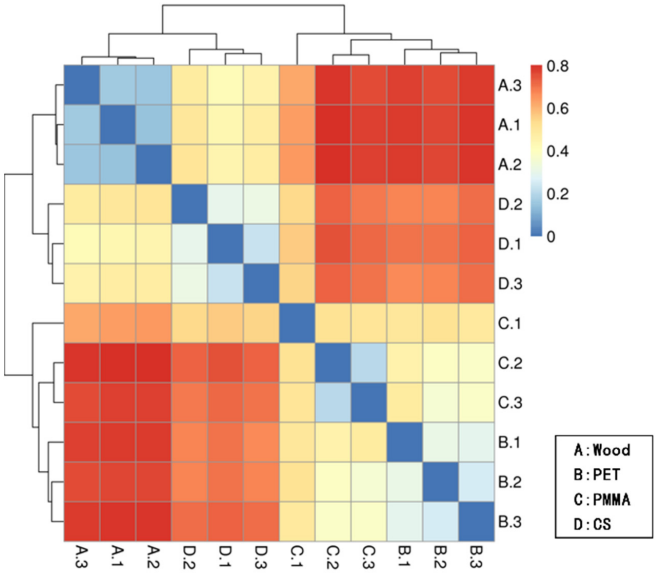

Figure 4. Heatmap of sample distance under Bray–Curtis distance formula. The bluer the color means the more similar the sample. The results confirm that the A, D and B, C have distinct differences.

As for the relative abundance of biofilm bacteria, Kruskal–Wallis test results showed that there was not a clear boundary line at the phylum level. Proteobacteria, Acidobacteria, Cyanobacteria and Bacteroidetes were the majority at two substrates. However, at the class level, there was a clear difference (Figure 5). Alphaproteobacterial, Betaproteobacteria and Synechococcophycideae dominated on the plastic substrates, while Gammaproteobacteria, Phycisphaerae and Planctomycetia played the main role on the natural substrates.

Figure 5. Relative abundance of bacteria at the class level. The figure shows bacteria in A (Wood), B (PET), C (PMMA), and D (CS) substrates with relative abundance greater than 0.01.

There were also marked differences in biodiversity and bacterial community structure between substrates. The NMDS, which based on the Unweighted Unifrac distance showed a strong gathering in two groups on NMDS1 dimension (Figure 6). The distance heatmap calculated by Bray–Curtis formula also proved that strong gathering (Figure 4). Both of them confirmed the biodiversity between different samples. The bacterial community structure of the two substrates also showed a significant difference. Anosim (Figure 7) (p = 0.001, R = 0.892) and Adonis (Table 1) (p = 0.001, R = 808, F = 11.19) confirmed that the classification was valid.

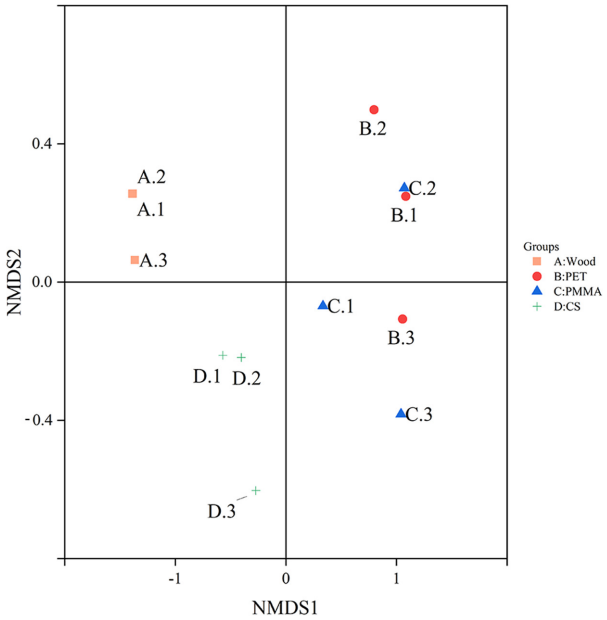

Figure 6. Unweighted nonmetric multidimensional scaling (NMDS) analysis of A (Wood), B (PET), C (PMMA), and D (CS) substrates. On NMDS1 dimension, the A, D and B, C were set apart clearly, which indicates the clear differences of species compositions between the two groups.

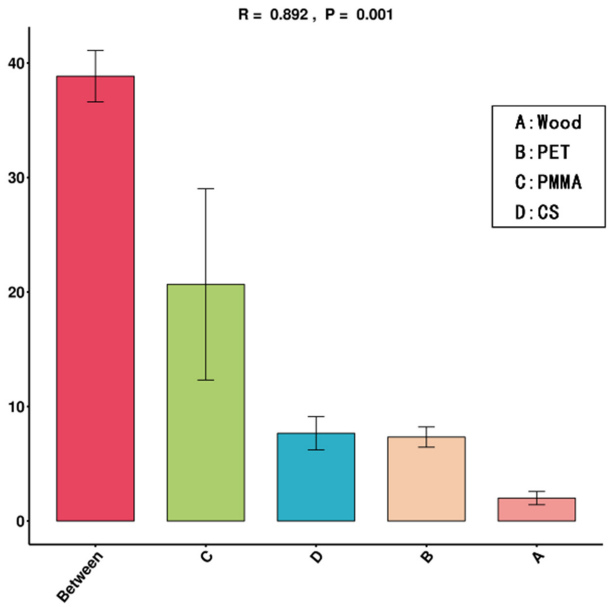

Figure 7. Anosim result of A (Wood), B (PET), C (PMMA), and D (CS) substrates. The ordinate stands for the ranks of distances between or inside the groups. The R value is 0.892, quite close to 1, indicating that distances between groups are larger than distances within groups. p value is 0.001, indicating that these differences are statistically significant.

Table 1. Adonis result of A (Wood), B (PET), C (PMMA), and D (CS) substrates.

Vs_Group	Df	SumsofSqs	MeanSqs	F.Model	R^2	Pr (>F)
A_B_C_D	3	1.584971838	0.528323946	11.18835851	0.807530724	0.001
A_B	1	0.849885112	0.849885112	32.26187121	0.889691296	0.1
A_C	1	0.708050946	0.708050946	13.15419172	0.766820841	0.1
A_D	1	0.275281725	0.275281725	10.38952391	0.72201999	0.1
B_C	1	0.15905402	0.15905402	2.340901059	0.369174828	0.1
B_D	1	0.64271035	0.64271035	15.82457228	0.798230199	0.1
C_D	1	0.534961524	0.534961524	7.855713948	0.662609943	0.1

Df represents the degree of freedom; SumsofSqs represents total variance; MeanSqs represents mean variance; F.model represents test value; R^2 represents degree of differences; Pr stands for *p* value, and less than 0.05 means high confidence.

4. Discussion

4.1. Biofilm Community Diversity on Microplastics

Due to the increased usage of plastics and the unreserved releasing, the plastic debris can be easily found in aquatic ecosystems, and the water pollution has aroused increasing attention worldwide, particularly in developing countries [34]. Although most of the existing studies have focused on the comparison between different plastic types [7,14] and between plastic and water environment [24], there is little comparison between plastic and natural materials. In this research, the difference of biofilm microbial community between plastic and natural substrates were compared through the in-situ culturing experiments. To explore the impacts of PET and PMMA plastics on biofilms, two types of substrates, plastic and natural substrates (as control group), were installed in natural freshwater system for 40 days and biofilms incubated on two substrates were analyzed using by 16S rRNA high-throughput sequencing and determined for the alpha and beta diversities.

The alpha indices showed that the species diversities on PET and PMMA were much smaller than those on cobblestones and wood, which meant plastic debris can alter the biofilm biodiversity. Previously, the surface roughness could serve as a factor influencing the attachment of microbial community and the substrate with a rough surface is likely to adhere more CFUs (colony-forming unit) [35,36]. Therefore, the biggest factor causing the difference in species diversity may be the difference between the natural substrates with a rough surface and plastics with a smooth surface in this study. In an urban river [25,26,37], MP biofilms diversity on MPs was also found much lower than that in the surrounding nature environment. Obviously, the decrease of biodiversity will weaken the ecosystem resilience to environmental change, including global warming and pollution.

Furthermore, the main bacteria species were compared between different substrates in Xuanwu Lake and also with published findings (Table 2). On class level, Alphaproteobacteria, Betaproteobacteria and Synechococcophycideae were found to be the main species on MPs. Scholars found that Alphaproteobacteria widely existed in the water system and played a key role on the filamentous expansion of sludge in sewage treatment process [38]. Erythrobacter (belonging to Alphaproteobacteria) was found to have a preference for PE and PS at monitoring sites along the Baltic coast, while Sphingopyxis (belonging to Alphaproteobacteria) appeared at a higher abundance in the combination of plastic set in freshwater [39]. Furthermore, compared with glass substrate and cellulose substrate, plastics attract proteobacteria to adhere to them, and the proteobacteria (mainly composed of Alphaprobacteria and Betaprobacteria) dominate the microbial community on microplastic surface [27].

Table 2. Comparison of biofilms on plastics and natural substrates.

Plastics and Natural Substrates	Dominant Species	Biodiversity	Environment (Field or Laboratory)	References
PP, PE, cobblestone and wood	Proteobacteria was the dominant followed by Bacteroidetes in MPs (at phylum level)	The order of total species richness was the same as Shannon index, which was Wood > Cobblestone > PP > PE	Laboratory environment for 21 days	[7]
Nine kinds of plastic materials including PP, PC, ABS, LDPE and others	α-Proteobacteria, γ-Proteobacteria, Bacteroidia and Acidimicrobiia (at class level)	The minimum Simpson index is 0.9955, and all the Shannon curves tend to flatten with the increase of the number of sequencing lines	Natural environment for 2 weeks. (Artificial coral culture areas and wild coral areas)	[14]
PVC, rocks and leaves	Proteobacteria in MPs and rock biofilm, and Bacteroidetes in leaf biofilm (at phylum level)	The order of observed biomass was leaf biofilm > microplastic biofilm > rock biofilm, while the order of Shannon index was microplastic biofilm > rock biofilm > leaf biofilm.	Laboratory for 2 weeks (the water was collected in the Haihe River)	[28]
Selected two kinds of PMD (plastic marine debris), PE and PP, compared with sargassum and seawater	The major ciliates were the genus Ephelota (Gammaproteobacteria), and the alphaproteobacterial family Hyphomonadaceae were unique to PMD	The order of mean Simpson evenness is plastics (0.95) > sargassum (0.90) > seawater (0.85)	Natural environment (collected from the North Atlantic)	[24]
PS, PP, PE, glass beads, native fibrous cellulose	Proteobacteria was higher on the plastics than on glass and cellulose (at the genus level)	All the Shannon evenness and Shannon–Wiener diversity were similarly low except glass substrate	Natural environment for 2 weeks (around the Baltic Sea)	[27]
PET, PMMA, cobble stones and wood	Alphaproteobacterial, Betaproteobacteria and Synechococcophycideae played the dominating roles on the MPs substrate	The Shannon–Wiener diversity of biofilm bacterial communities on natural substrates were much higher than those on MPs substrates	Lake water for 40-day cultivation	This study

Alphaproteobacteria was also a parasite and resistant to most disinfectants, and some species of them even led to human etiology [40]. Betaproteobacteria was also a kind of pathogenic bacterium which vastly existed in the wastewater. Our result confirmed the result that plastic biofilms have a preference for pathogenic bacteria [24]. The beta diversity results indicated the evident difference between plastic and natural substrates.

Gasim et al. (2015, 2016) [41,42] evaluate the efficiency of plastic materials for water treatment. The similar method was also applied to explore the differences of microbial communities of biofilms on plastic substrates and natural substrates [28–30]. PE and LDPE have been applied to research on biofilm colonizing in previous studies [28,30], while PMMA and PET were used in this study to serve as new substrates for biofilm.

4.2. Practical Implications of This Study

The two kinds of plastics researched in the current study, PET and PMMA, are the plastics that are commonly used in daily life in many countries. Our results show the large impact of plastics on the aquatic biofilm, both biofilm bacterial species composition and structure. The findings led light on the importance and urgency of reducing, reusing and recycling waste [43], including plastic wastes, particularly in cites.

The results indicated that PMMA and PET can act as new habitats for biofilm colonization, which may further disturb biofilm communities and change biogeochemical processes in aquatic ecosystems, for example, carbon, nitrogen and phosphorus cycles [41,42]. Therefore, the massive plastic waste produced in the city should be managed efficiently and treated in a proper way to minimize the impact. At the same time, the production process of plastics should reduce the use of toxic and harmful additives, which will be released into the natural environment with the decomposition of plastics, and thus have a negative impact on natural organisms. The recent study found that plastic particles have already polluted drinking water and the mean concentration of microplastics in the tap water was around 440 particles L^{-1} in China [44]. Therefore, the inland water ecosystem could be susceptible by plastic pollution [45]. Clearly, more stringent policies on the treatment and disposal of pollutants, including plastic waste, should be enforced worldwide [46]. Given the increasing release of plastic debris into the aquatic environments, the biofilm microbial functions of plastics should be further explored. Future research should focus on the natural processes in which plastics are involved, including various environments (soil, atmosphere) and the impact of their presence on these processes. In addition, future studies should pay attention to the whole life cycle of plastics (i.e., from plastic fragments to microplastics) and microbial flock characteristics using microbial plastics in the natural environment. Besides, more comprehensive management policies should be developed for the generation and discharge of plastic waste on the global scale.

4.3. Limitation and Conclusions

Similar to many studies, there are some limitations in the current study. The biofilm community can be largely affected by different water environmental factors, while this research focused on the in-situ growth of biofilm in a single lacustrine environment. It is still unavailable to make comparisons between different regions and different hydrodynamic conditions, which may limit the wide application of the current results. As plastics contain different kinds of additives, the additives may leach from plastics during the incubation period (40 days), with some possible influence on aquatic organisms [45,47]. While the data of additive release from plastics are unavailable in the current study, more research on the release of plastic additives and their impacts on aquatic organisms are needed in the future studies.

Overall, in this study, the results showed that the species diversities on PET and PMMA were much smaller than those on cobblestones and wood, and the bacterial community structure of the two substrates (plastic and natural substrates) also showed a significant difference. The results indicate that PMMA and PET can act as new habitats for biofilm colonization, which result in altered bacterial community structure and composition. This can further disturb biofilm communities and change biogeochemical processes in aquatic ecosystems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13111465/s1, Figure S1: Rarefaction plots of Shannon index of A (Wood), B (PET), C (PMMA), and D (CS) samples. The abscissa represents numbers of sequences, while the ordinate represents biodiversity value. The rarefaction curve tends to be flat and it means the sequencing is large enough to represents the majority of species. Table S1: The variation of water chemistry at DAY0, DAY40.

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