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Biofilm Structural and Functional Features on Microplastic Surfaces in Greenhouse Agricultural Soil

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Abstract: Microplastics (MPs) enter the soil through a variety of pathways, including plastic mulching, sludge, and organic manure application. In recent years, domestic and foreign experts and scholars have been concerned about the residues and contamination of MPs in the soil of greenhouse agriculture. In this investigation, five types of MPs including low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET), and two concentrations (1% and 5%, *w*/*w*) were used in a 30-day external exposure test. Evidence of microbial enrichment was found on the surface of the MPs. The total amount of biofilm on the surface of MPs increased dramatically with increasing exposure time and MP concentrations. The polysaccharide content of extracellular polymers (EPS) in biofilms was significantly different, and the maximum PS1 (1% (*w*/*w*) PS) concentration was 50.17 mg/L. However, EPS protein content did not change significantly. The dominant bacteria on the surface of MPs with different types and concentrations were specific, and the relative abundance of *Patescibacteria* was significantly changed at the phylum level. At the genus level, *Methylophaga*, *Saccharimonadales*, and *Sphingomonas* dominated the flora of LDPE1 (1% (*w*/*w*) LDPE), PS1, and PET5 (5% (*w*/*w*) PET). The dominant bacteria decompose organic materials and biodegrade organic contaminants. According to the FAPROTAX functional prediction study, chemoheterotrophy and aerobic chemoheterotrophyplay a role in ecosystem processes such as carbon cycle and climate regulation. The application of LDPE1 has a greater impact on the carbon cycle. Plant development and soil nutrients in greenhouse agriculture may be influenced by the interaction between MPs and microorganisms in the growing area, while MP biofilms have an impact on the surrounding environment and pose an ecological hazard.

Keywords: microplastics; biofilm formation; EPS; biodegradation; microbial community composition

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1. Introduction

Plastic mulching technology is the fourth most important agricultural production method in China after seeds, fertilizers, and pesticides [1]. As China consumes nearly 75% of the world's plastic film, plastic film residues in agricultural soils are a major pollution problem in the country [2]. China utilizes nearly 1.5 million tons of plastic film per year, covering over 20 million hm^2 and making a significant contribution to maintaining a safe supply of agricultural products in the country, although residual film in the soil is estimated at 71.9–259 kg/hm^2 [2,3]. In Jiangsu, the amount of agricultural film utilized is at an intermediate level in the country. The annual amount of agricultural film utilized is approximately 116,000 tons. Mulch film consists mainly of ultra-thin films less than 0.008 mm thick, which are easily damaged and difficult to recycle [4]. However, the sustainability of Chinese agriculture depends on the proper recycling or centralized disposal of used mulch, sludge, and organic fertilizer. Whole plastics are broken up or dissolved, producing larger plastic fragments that break down into smaller microplastics (<5 mm).

Sludge and organic fertilizers contain high levels of plant nutrients and organic carbon and are essential agricultural fertilizers [5]. According to several studies and analyses of wastewater treatment plants, up to 90% of MPs are separated from the effluent and collected in the sludge after sewage treatment [6]. This sludge containing MPs is used as a fertilizer and when this reaches the soil, it increases the amount of MPs there. In the current study, the concentrations of MPs in organic fertilizers was found to be 2.38–1200 mg/kg. If MPs are taken into account, their concentrations will be considerably higher [7]. As a result of the application of sludge and organic fertilizer, MPs will accumulate in agricultural soils.

It is well known that MPs pollution is a complex problem. The chemical composition, additives, persistence, surface properties, size, and shape of the MPs themselves all have an impact on the properties of the MPs. MPs can have different properties in the environment and different effects on terrestrial ecosystems. Machado et al. (2018) showed differences in the impact of MPs of different sizes. The effect of microfibers on soil physical properties appears to be stronger than that of microspheres [8]. According to several studies, MPs reach the soil environment and accumulate to a certain extent, affecting soil characteristics, soil processes, and biodiversity [9,10]. MPs that accumulate in the soil have an impact on soil structure and properties and can also cause changes in soil structure [8,11]. In contrast to MPs in aqueous environments, soil MPs can provide sorption sites for soil microorganisms, establish unique microbial communities and influence the ecological role of soil microorganisms [12,13]. Microorganisms play an important role in the development and stabilization of soils and in the regulation of numerous biochemical processes and parent transformations and energy transfers in soils [14]. According to certain research, plastic films that remain in agricultural fields and decompose into MPs have a considerable impact on enzymatic activity and microbial diversity in the soil [9,15], as well as adversely affect water, nutrients, and crop growth [16,17].

Soil microorganisms can disrupt natural and man-made compounds in the environment and they play an important role in the nutrient cycling of soil ecosystems. Biofilms are mostly composed of microbial symbionts and the EPS they release [18,19]. The development of biofilms on the surface of MPs can alter their physical and chemical characteristics [20,21], as well as facilitate the movement of MPs in the environment [22,23], and subsequently participate in the adsorption of MPs and their destruction by microorganisms [24,25]. Moore–Kucera et al. (2014) discovered that the quantity of *A. flavus* [26] on the surface of biodegradable agricultural land films was much higher than that in the surrounding soil. Microorganisms concentrated on the surface of MPs would be prospective species for MP biodegradation, according to Huang et al. (2019) and Yi et al. (2020) [27,28]. Covering the cultured soil with MPs also helps to enhance the microorganisms involved in the breakdown of MPs [13].

Despite the many studies carried out on MP biofilms in soil and microbial degradation of MPs, there has been little investigation into greenhouse agricultural soil. The mechanisms of interaction between biofilm and MP degradation, as well as the environmental impacts and ecological aspects of biofilms, need further study (Figure 1). Furthermore, at varied exposure times, the dynamic effects of different kinds and concentrations of MPs on the overall quantity of biofilms and extracellular polymers remain unknown. In this study, five different types of MPs and two different concentrations of MPs were used to investigate the association between MP surface biofilms and associated microbial community assemblages by using exposure studies in soils from planted areas of the greenhouse farmland. This study could help researchers to better understand the properties of soil microbial communities on the surface of MPs, which could provide essential information on MP contamination and, to some extent, represent the ecological risks that MPs may pose.

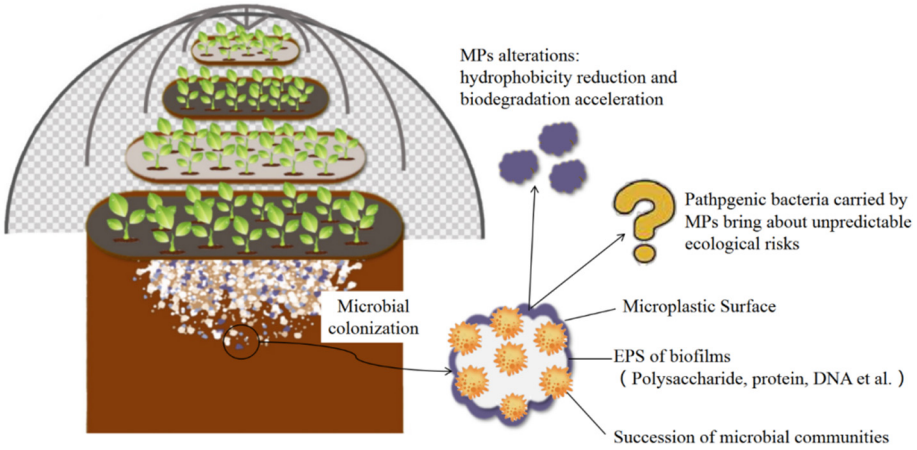

Figure 1. Biofilm and MP degradation interaction mechanism.

2. Materials and Methods

2.1. Sample Pretreatment

Five different species of MPs, including low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET), and two different concentrations (1%, 5% of soil dry weight) were used in this study. LDPE1, LDPE5, HDPE1, HDPE5, PS1, PS5, PP1, PP5, PET1 and PET5 represent 1% (*w/w*) LDPE, 5% (*w/w*) LDPE, 1% (*w/w*) HDPE, 5% (*w/w*) HDPE, 1% (*w/w*) PS, 5% (*w/w*) PS, 1% (*w/w*) PP, 5% (*w/w*) PP, 1% (*w/w*) PET and 5% (*w/w*) PET, respectively. LDPE is a milky white waxy crystalline resin with a density of 0.91–0.93 g/cm^3 that is mostly utilized in plastic bags, agricultural films, and other similar applications. HDPE is a white wax-like synthetic resin having a density of 0.94–0.965 g/cm^3. It's mostly utilized in blow molding, injection molding, and other applications. PS is a colorless, transparent thermoplastic with a density range of 1.02–1.05 g/cm^3, akin to a glass-like brittle substance. PP is a milky white, highly crystalline thermoplastic polyester polymer with a density of 0.9–0.92 g/cm^3 that may be used to make expanded polystyrene (polystyrene foam). PET is a pale yellow, highly crystalline thermoplastic polyester polymer having a density of 1.37 g/cm^3. MPs were purchased from Usoft Chemical Technology Co., Ltd. (Linyi, China). The MP particles utilized had a diameter of 1–3 mm. Before the experiment, MPs were soaked in 75% ethanol and then disinfected using UV light.

2.2. Microcosm Design

The test soil was taken from a farm in Changzhou City (31.977510° N, 119.776667° E) for greenhouse cultivation, which had not been mulched. The soil was initially air-dried to remove stones and root debris then passed through a 100-mesh sieve and properly mixed, providing deionized water to maintain a field water volume of 60%. Finally, the soil was pre-cultured in an artificial incubator for one week. One kilogram of test soil was placed in pots for each treatment and various MPs were added in proportion. The soil was incubated in an artificial incubator (temperature 27 °C, relative humidity 80%) in a diurnal cycle every 12 h. Proper stirring and mixing can be carried out with a stainless-steel spoon in order to allow sufficient exchange of substances between the MPs and the external environment [29]. Throughout the experiment, 150 mL of water was added daily. The soil samples were obtained on days 15 and 30 after the addition of the MP and the first sample was obtained 24 h after the addition of the MP. At the time of sampling, three replicate samples were obtained from each culture vessel for examination.

2.3. Biofilm Quantification by Use of Crystal Violet Assay

Samples of MP were obtained from the test soil, placed in sterile Petri dishes and the surface of the MP was washed three times with sterile water. In each group, three

parallel lines were established. At the same time, MPs that had not been placed in the test soil were used as a blank control group. MPs were washed and air-dried for at least 45 min. MPs were stained with 1% crystal violet solution (National Pharmaceutical Group Chemical Reagent Co., Ltd., Shanghai, China) for 45 min at room temperature. MPs were washed three times with sterile water to remove any remaining colors. The MP samples were cleaned and stored at room temperature for 45 min. MPs were placed in a centrifugal tube and decolorized with 2 mL of 95% ethanol solution (*V*/*V*) for 10 min. The absorbance value of the decolorization solution was measured in a cuvette with an absorbance value of 595 nm and the treatment with the decolorized solution only was used as a blank [30].

2.4. Determination of EPS in Biofilms on MP Surfaces

Biofilm bacteria on various MPs were obtained using a sterile EDTA technique [31], followed by rinsing off loose adhesions on the MPs with sterilized de-watering. Centrifuge tubes containing MPs received 10 mL of 0.5 M EDTA. Particles were removed after shaking and eluting for 30 min and then centrifuged at 8000 rpm for 20 min to obtain a bacterial suspension. Finally, the biofilm bacteria were removed from the supernatant and discarded. The phenol sulfate technique was used to assess the polysaccharide content of biofilm bacteria, with glucose as the standard [32]. Protein content was measured using the Coomassie Brilliant Blue technique using bovine serum albumin as a reference [33].

2.5. Characteristics of Microstructure

The MP particles were first treated with distilled water, 2.5% glutaraldehyde (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China), phosphate-buffered saline (PBS, National Pharmaceutical Group Chemical Reagent Co., Ltd., Shanghai, China), and ethanol, and then allowed to dry naturally. They were first treated with an SCD 500 ion sputtering device and then placed in the cavity of an S-4800 II field emission scanning electron microscope (SEM, S-4800 II, Hitachi Co., Ltd., Tokyo, Japan). Finally, the microstructure and surface attachments of the MPs were examined [34].

2.6. 16S rRNA Genes Sequencing

The E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract DNA from soil samples, and PCR was used to amplify the V4-V5 region of the bacterial 16S rRNA gene. 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were utilized for the primers. The Axygen DNA Gel Recovery Extraction Kit was used to purify the PCR results, which were then quantified using the Qubit® 3.0 Quantitative Detection Kit (Axygen Biosciences, Union City, CA, USA). Finally, the V3-V4 sections of the 16S rDNA gene sequences of soil microorganisms and bacteria were sequenced using the Illumina Miseq sequencing analysis platform.

2.7. Data Statistics and Analysis

Data on the total amount of biofilm on the surface of the MPs and the content of polysaccharides and proteins in EPS were counted using SPSS statistics 21 and Origin 9 software. Labels with 97% similarity from high-throughput sequencing results were used as operational taxonomic units (OTUs) and the relative formation abundance of each OTU was counted. The OTUs were analyzed taxonomically using the uclust method in Qiime software, and a genus-level microbial community structure map was created using R language tools. CIRCOS distribution maps at the bacterial-animal phylum level were created using the CIRCOS ONLINE program. PICRUSt2 was used to perform functional prediction of bacterial commonalities in prokaryotic taxonomic databases using the 16S rRNA gene sequences obtained (FAPROTAX).

3. Results and Discussion

3.1. MP Surface Characteristics

Based on microscopic examination of the SEM data, the MP samples contained complex surface morphological features that were closely related to the type of MP (Figure 2). These aggregated biofilms were largely absent on the initial surface of the material and even less on the surface of the MPs after 15 days of exposure. After 30 days of exposure, biofilms with high bacterial densities were covered in all MPs. This suggests that the bacteria attached to the MPs evolved an adaptive response to their new environment and acquired the ability to break down the polymer over time. On day 15, the microorganisms shown in the HDPE5 pictures were spherical bacteria. On day 30, no adsorption offsets were evident, showing a strong bond between the biofilm and the MPs. The surface structure of HDPE had an evident porosity structure, which would enhance microbe adsorption and colonization. On the first day, the bacterial coverage of PS1 and PS5 was in the shape of dots. The bacterial coverage was poor in PS1 and PS5. On days 15 and 30, the bacterial coverage increased significantly, showing a convex and cumulative colonization state. According to Sangale et al., several fungi isolated from plastic-contaminated mangrove soil have the capacity to break down PE [35]. In addition, several bacteria presented in mangrove sediments have been discovered to aid in the breakdown of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) [36,37]. On the one hand, this research adds to the growing body of data demonstrating the presence of biodegradable plastic functional flora in greenhouse agricultural soils. Plastic-contaminated greenhouse agricultural soils, on the other hand, might become a vast breeding ground for plastic-degrading bacteria.

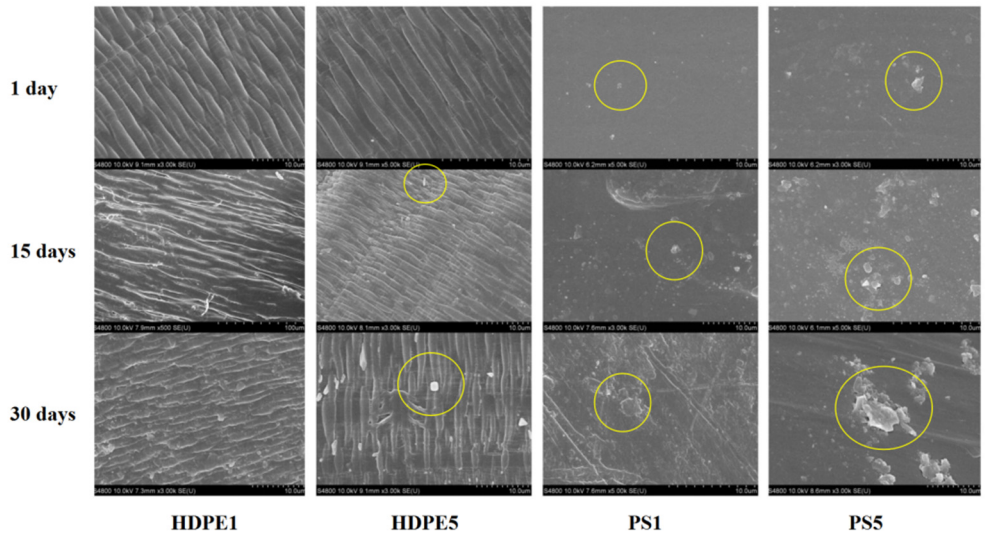

Figure 2. SEM images of various MP kinds and concentrations as a function of exposure duration.

3.2. Differences in the Total Amount of Biofilms Enriched on the Surface of MPs

The total amount of biofilm formed on the surface of the MPs was quantified using crystal violet staining of five different MPs: LDPE, HDPE, PS, PP, and PET (Figure 3). Based on the results of the day one and day thirty exposure tests, the biofilms on the surfaces of these five MPs expanded over time. As a consequence, the results of biofilm enrichment on the surface of various forms of MPs changed considerably over time ($p < 0.05$). Due to the high concentration of microbial communities in the soil, the biomass attached to the surface of most MPs increased in the exposure experiments. According to Tu et al., biofilm content on MPs rose considerably with exposure duration in maritime settings [38]. Moreover, various microorganisms can adapt to the surface environment of MPs and colonize the surface MPs. However, this is not always the case due to differences in the surface structure of MP materials. On the one hand, HDPE surfaces contain more rough structures (Figure 2), making it easier for microorganisms to adhere. On the other hand, PS (Figure 2) and

PP have flat surfaces with less pore structure, making it difficult for microorganisms to bind to them. These phenomena suggest that the resulting biofilms may have altered the surface morphology and crystallinity of microplastics after long-term exposure to the soil environment [39]. Microbial adhesion and biofilm development have been found in other research to affect the hydrophilicity of MP surfaces [40].

Experiments were conducted with both 1% and 5% concentrations. According to the results of the study, biofilms on the surfaces of HDPE, LDPE, PS, and PP all showed an increasing trend with increasing concentrations. The effect of MP concentration on PET surface biofilm is not significantly different. After 30 days of exposure, HDPE5 increased 1.1 times more than HDPE1. When most of the MPs accounted for the high dry weight of the soil, the contact area between the MPs and the microorganisms in the soil was large and the total amount of biofilm attached to the surface of the MPs was also large. This suggests that biofilm formation is closely related to the different concentrations of MPs.

The total amount of biofilm varied with exposure time, with microbial biofilm growth rates being considerably higher at 15–30 days than at 1–15 days. In terms of coverage, thickness, and composition, mature biofilms differ from emerging biofilms [41]. According to Harrison et al. (2014), Microorganisms may colonize the surface of MPs in just a few hours, whereas full biofilm development takes only 14 days [42]. Therefore, the initial formation of biofilm was in the delayed phase in the first 15 days, with a huge accumulation of biofilms in the logarithmic phase during the next 15 days. Nevertheless, between 1 and 15 days, the overall number of biofilms in PET1 and PET5 decreased, probably due to competition and succession of microorganisms colonizing the MP surface. This type of succession and competition was more intricate, lasted longer, and was more visible in the soil environment. The interaction effects of different kinds and concentrations of MPs and varied exposure durations exhibited substantial changes in the enrichment of biofilms on the surface of MPs, according to the findings of the univariate analysis in SPSS ($p < 0.05$).

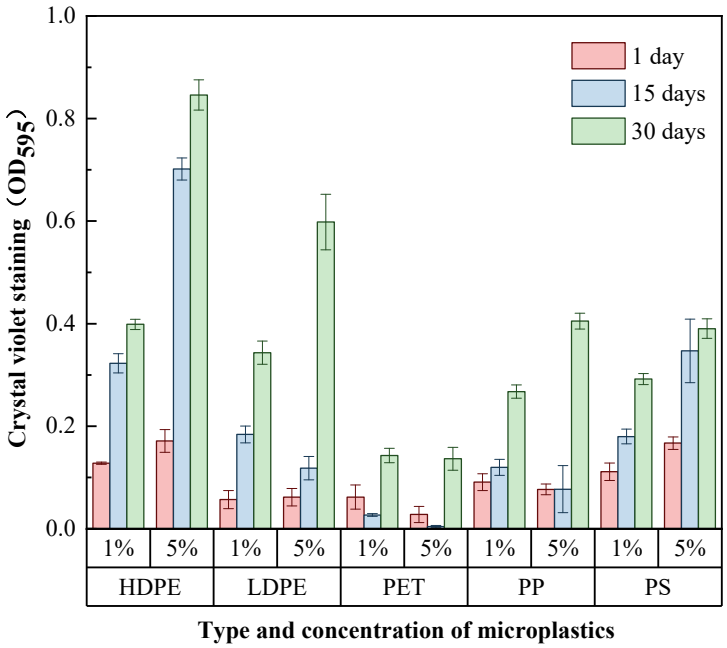

Figure 3. With exposure time, the biomass of various species changes, as does the quantity of MP biofilms.

3.3. Differences of EPS in Biofilms on MP Surfaces

The production of EPS is the first step in promoting microbial adhesion to MP surfaces [43,44]. In this investigation, the content of polysaccharides and proteins in five different types of EPS was determined, as well as the concentrations of MPs in two types. Based on the experimental results (Figure 4a), it was found that the polysaccharide concentrations and the abundance of MP biofilms in the various types of EPS were sub-essentially

different ($p < 0.05$). The number of polysaccharides in EPS rose when the exposure time increased. After 30 days of incubation, the polysaccharide content of HDPE, LDPE, and PS increased, with PS1 having the largest amount at 50.17 mg/L and PET5 having the lowest amount at 14.21 mg/L. Wang (2021) et al. found the presence of glycosyl groups (including glucose and mannose) in EPS subjected to the dissolution of PE in soil and water using GC-MS techniques, which was comparable to the results of our investigation [45].

The protein concentration in the EPS was much greater than that of polysaccharides during the first 15 days of the exposure experiment, which was related to the high content of organic matter and complex organic matter in the soil [46]. During the first 15 days, the polysaccharide content of EPS increased rapidly, with a logarithmic growth rate of 1.08 in EPS on the surface of LDPE1. This is because viscous and hydrophilic polysaccharides induce cell aggregation and granulation [47]. They can stimulate biomes to form cross-network structures through bridging and promote the generation of biofilm matrix structures [48]. Figure 4b shows that there were no significant differences in the proteins in the EPS of different types and concentrations of MP biofilms ($p > 0.05$). As one of the components of the biofilm matrix, proteins play a crucial role in the generation of biofilms. On the one hand, they aid cell migration and early attachment to the surface of the medium and promote bacterial colonization of the culture medium surface. On the other hand, matrix proteins contribute to the structural stability of biofilms, and loss of proteins will alter the structure of biofilms and reduce their stability [49,50].

Polysaccharides and proteins in EPS are large organic molecules with complex chain or mesh-like structures. Microbial aggregates are mechanically stabilized by various interactions between macromolecules, including dispersion forces, electrostatic interactions, and hydrogen bonding. To form stable colonies, EPS produces a gel-like three-dimensional framework around the cell that holds the microorganisms together [51]. Proteins, in particular, play a larger role due to the attachment of their functional groups to metals. Cu^{2+} can be removed by carboxyl groups, Al^{3+} can be removed by amino and carbonyl groups, and Pb^{2+} may be complexed with hydroxyl, C-O-C, and carboxyl groups [52,53]. It's possible that the adsorption of metal ions by exocytic polymers occurs simultaneously with biofilm growth on the surface of the MP.

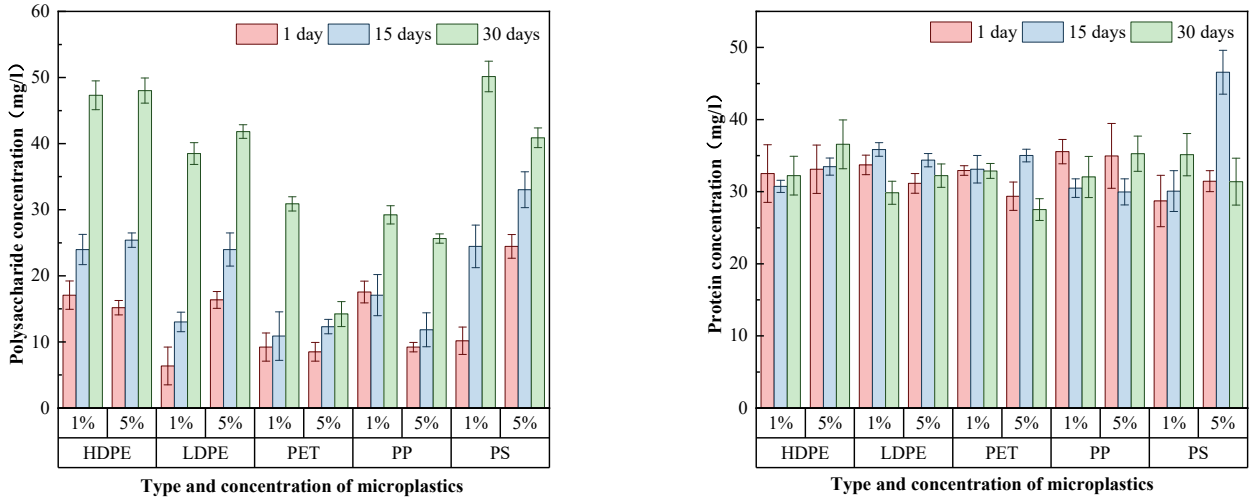

Figure 4. Differences of EPS in Biofilms on different types and concentrations of MPs. (**a**) Polysaccharide content in EPS; (**b**) Protein content in EPS.

3.4. Structural Diversity and Richness of Microorganisms Attached to MP Surfaces in Greenhouse Farmland

Bacterial species and abundance in the soil were high in the planting area of the greenhouse farmland. Bacteria colonized MP surfaces for 15 and 30 days, according to SEM

images of HDPE1, HDPE5, PS1, and PS5 MP surfaces (Figure 2). The inter-rhizosphere soil on day 30 had more bacterial species diversity (Shannon index) and species richness (Chao1 index) compared to the inter-rhizosphere soil on day 15. The soil microbial community structure of the greenhouse agriculture changed significantly with the exposure period. As shown in Figure 5, on day 15, Proteobacteria were most abundant, followed by *Bacteroidota*, *Actinobacteriota*, and *Chloroflexi*. On day 30, the number of *Proteobacteria* decreased but was still the highest, while the number of *Bacteroidota*, *Actinobacteriota*, *Chloroflexi*, and other bacteria increased. After the addition of MPs, the relative abundance of *Patescibacteria* rose. The relative abundance of *Patescibacteria* in PS1 increased by 11.2% compared to soils in the greenhouse farming area, indicating that MPs had an effect on the soil microbiota. Furthermore, the quantity of MPs was related to the organization of the microbial community. Microbial communities vary in their vulnerability to MP disturbance, and different phyla of microbial communities may respond differently to increases in MP concentrations [54,55]. The relative abundance of *Patescibacteria* on MP surfaces differed in abundance from the major phyla of *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. HDPE5 showed a 5.1% increase over HDPE1, while PS5 showed a 10.9% decrease over PS1. *Patescibacteria* were discovered to have an important role in the biofilm microbiota formed on vinyl ester-based polymer composites that lead to molecular chain breaks in the polymer [56]. *Patescibacteria* were shown to be significantly enriched on PE and PVC MP particles in previous investigations [57,58]. As a result of this and the previous study's findings, *Patescibacteria* are probably the most important bacteria in MP contamination. Nevertheless, it has been observed that *Proteobacteria* may decompose the chemical molecule methyl tert-butyl ether [59].

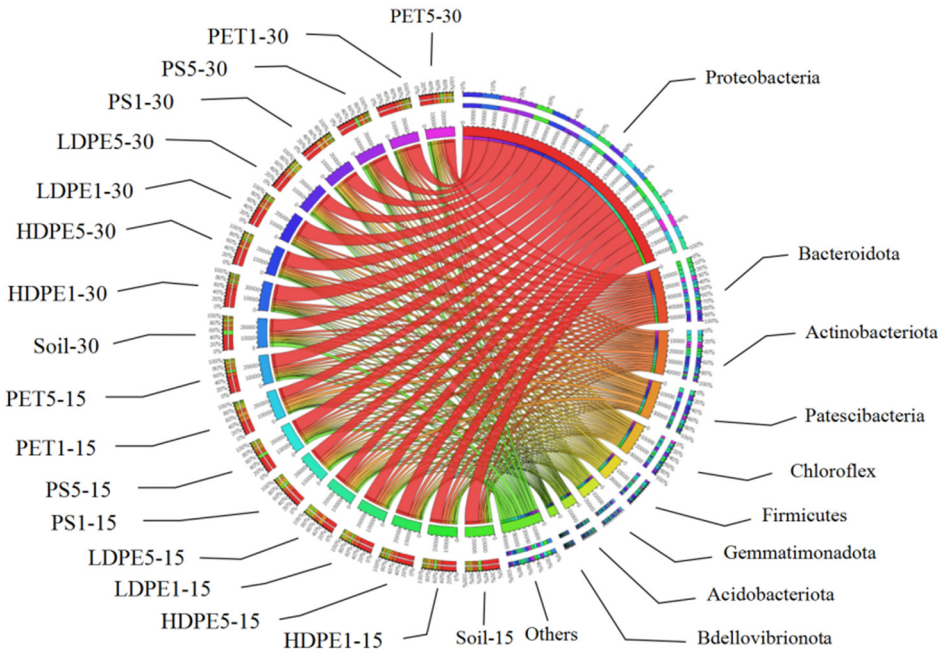

Figure 5. Effects of different types and concentrations of MPs on community relative abundance at the bacterial phylum level in soil.

Based on the community distribution of microplastics at the genus level by species and abundance, *Sphingomonas* accounted for the vast majority of LDPE1, while *Saccharimonadales* accounted for the vast majority of LDPE5 and PET1 at 15d (Figure 6). The relative abundance of *Methylophaga*, *Pseudomonas*, *Vibrionimonas*, and other bacteria grew dramatically after 30 days of cultivation. *Methylophaga* ranked on the surface of LDPE1, *Saccharimonadales* ranked first on the surface of PS1, and *Sphingomonas* ranked first on the surface of PET5. It implies that various MPs attract different microbes to inhabit their surfaces. The top genera of HDPE1, HDPE5, LDPE5, PS5, and PET1 biofilms were essentially the same as

the soil composition in the planted areas of the greenhouse. *Limnobacter* was the most common genus, accounting for between 7.7% to 26.4% of the total. The relative abundance of *Sphingomonas* increased with the addition of MPs due to its ability to degrade a variety of complex organic compounds, including polycyclic aromatic hydrocarbons [60], the plasticizer bisphenol A [61], and plastic monomers [62,63], along with the production of polysaccharides that promote the formation of biofilms on plastic surfaces [64]. *Methylophaga* is a carbon-containing chemical that can be used to aid the development of methylotrophic organisms. *Methylophaga* has a protein that degrades to produce methylamine [24], which may then be deaminated into formaldehyde and ammonium by mixing with methylamine dehydrogenase and crossing the membrane of the beetle [65]. *Methylotrophy* is an important component of the global carbon cycle and climate control. *Saccharimonadale* is a species of *Saccharibacteria* and it was found that *Saccharibacteria*, which feed on plant exudates, was isolated from the rhizosphere of wild oat. Trehalose-decomposing genes for starch/glycogen and glucose synthesis have also been identified in their genomes [66]. As a result, bacteria concentrated on the surface of MPs play an essential role in the biodegradation of organic contaminants and the decomposition of organic matter.

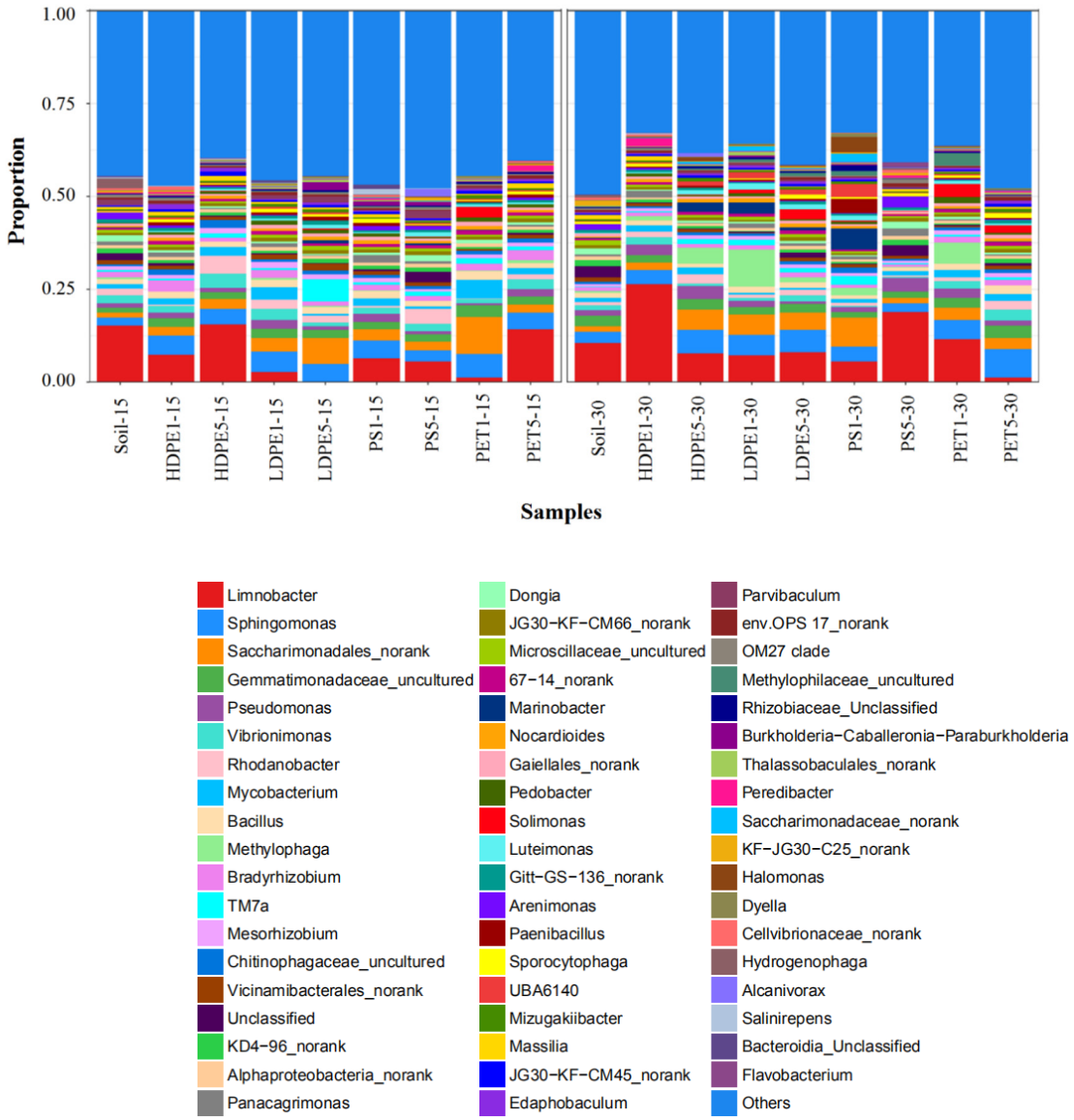

Figure 6. Effects of different types and concentrations of MPs on the relative abundance of bacterial communities in soil at the genus level.

3.5. Prediction of the Potential Functional Diversity of Microbes by MPs in the Planting Area of Greenhouse Farmland

FAPROTAX was used to annotate 16S rRNA sequencing data for functional diversity (Figure 7). Of the 91 functional taxa in the FAPROTAX database, 73 were found to have at least one example. The most common functional taxa in all soil samples were chemoheterotrophy and aerobic chemoheterotrophy. The relative abundance of chemoheterotrophy ranged from 26.4–34.3%, while the relative abundance of aerobic chemoheterotrophy ranged from 19.5–28.3%. On day 30, the MPs treatment greatly increased the proportion of methylation and methanol oxidation compared to day 15. Both methylotrophy and methanol oxidation of LDPE1 increased from 2.0 to 12.8%. Because methylotrophy and methanol oxidation are significant components of the carbon cycle, the application of LDPE1 has a stronger influence on the carbon cycle, according to the functional prediction findings. Nevertheless, nitrite denitrification, nitrogen respiration, nitrate reduction, photoheterotrophy, nitrate denitrification, nitrite respiration, chitinolysis, and nitrous oxide nitrification all decreased. Chemical heterotrophy and aerobic chemical heterotrophy are two important features that distinguish MP surface flora. The enrichment of heterotrophic bacteria on the surface of MPs was confirmed, and it was revealed that the bacteria mostly derived carbon and energy via oxidizing organic compounds [67]. Therefore chemoheterotrophy, aerobic chemoheterotrophy, phototrophy, and photoheterotrophy were important ecological activities associated with the carbon cycle [68]. MPs would contribute to the assimilation and use of carbon by soil bacteria, leading to increased greenhouse gas emissions. Zhang et al. (2021) found that large amounts of polyethylene particles (18%) significantly increased soil carbon dioxide emissions [67]. MPs have been shown to alter the activity of soil bacteria in two ways: community composition and functional group prediction.

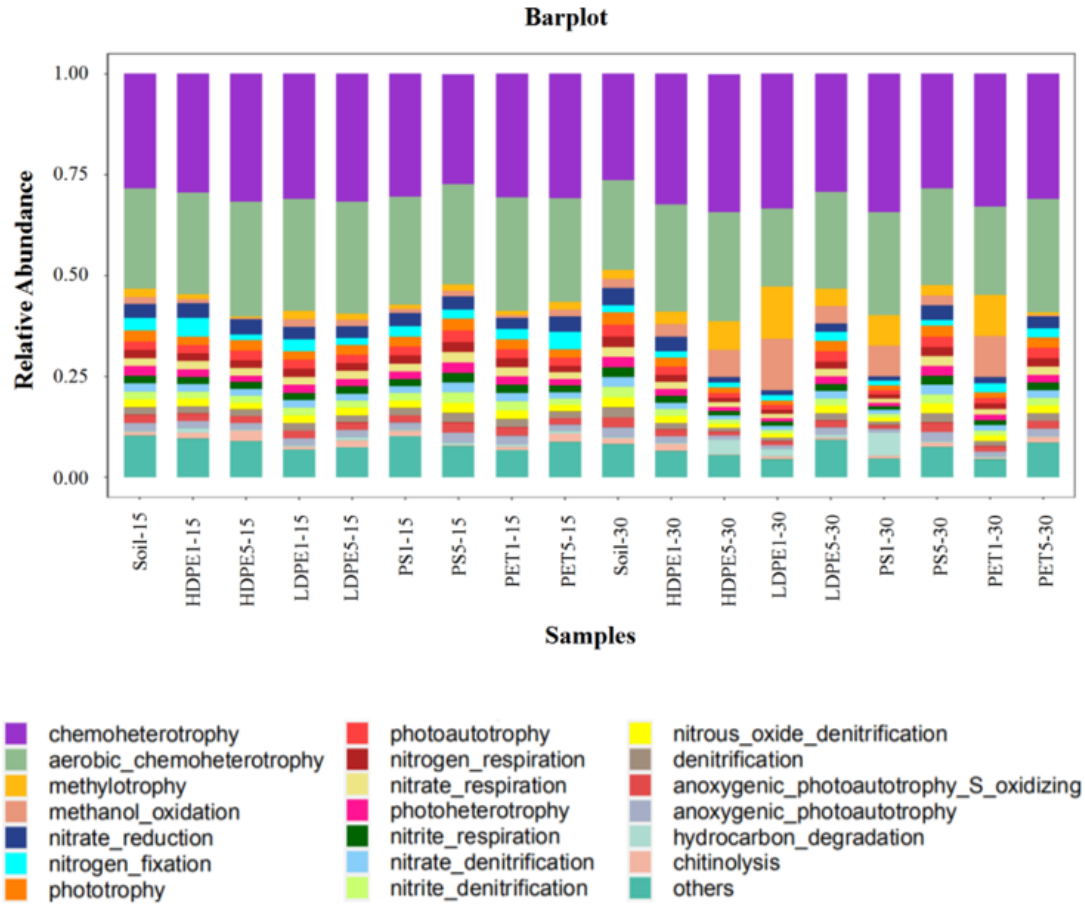

Figure 7. Functional groups of bacterial communities in MP samples from greenhouse farmland.

4. Conclusions

MPs provide a new biological habitat for microorganisms as well as a transmission medium. The primary goal of this research was to determine how biofilms generated on the surface of MPs affect the environment of MPs and soil microbial populations in greenhouse agriculture. At the same time, there is a dynamic process of biofilm development on the surface of MPs with different species and concentrations in greenhouse agricultural soils. In this study, five MPs (HDPE, LDPE, PS, PP, PET) and two concentrations (1%, 5%) of biofilms were investigated in the greenhouse farm of this study. After one month of contact incubation, microbial colonization of the HDPE and PS surfaces occurred, indicating that the MPs were enriched with microorganisms. The results revealed that the polysaccharide content of the EPS in the biofilms on the MP surfaces increased considerably over time, as did the enriched biofilms on the MP surfaces of different species and concentrations. The protein content did not change much. At the genus level, the relative quantity of microbes adhered to the surface of different kinds of MPs varied. *Methylophaga*, *Saccharimonadales*, and *Sphingomonas*, for example, ranked first in LDPE1, PS1, and PET5, respectively. The addition of MPs altered the structure of the bacterial community and significantly altered the relative abundance of *Patescibacteria* at the phylum level. Microorganisms concentrated on the surface of MPs can biodegrade organic contaminants and break down organic compounds, as well as play a significant part in the global carbon cycle and climate regulation. According to this study, the role of chemoheterotrophy and aerobic chemoheterotrophy may alter the carbon cycle. Because the application of LDPE1 resulted in a large increase in the two functional groups of methylotrophy and methanol oxidation, the carbon cycle was impacted more. MPs have possible effects on soil microbial community function. The interaction mechanisms between biofilm and MP degradation may affect plant depletion and soil nutrients in areas grown for greenhouse agriculture. In addition, the impact of biofilms on the surrounding environment and ecological issues should be further investigated in the future.

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