Characterization of Freshwater Macroplastic-Associated Bacterial Communities Using 16S rDNA Sequencing

by
Shreya Sharma

A Dissertation
Submitted in Partial Fulfillment of the Requirements for
The Degree of Master of Science
In Biology

Keywords: microbiome, 16S rDNA, plastisphere, freshwater

University of Central Oklahoma
Edmond, Oklahoma

Dr. Matthew Parks, Major Advisor and Committee Chair, Associate Professor of Biology, Biology Department
Dr. Jyotisna Saxena, Committee Member, Assistant Professor of Biology, Biology Department
Dr. Sean Laverty, Committee Member, Professor of Mathematics, Department of Mathematics and Statistics

Characterization of Freshwater Macroplastic-Associated Bacterial Communities using 16S rDNA sequencing

Shreya Sharma

July 23, 2026

Jackson College of Graduate Studies at the University of Central Oklahoma

A THESIS APPROVED FOR

THE COLLEGE OF MATHEMATICS AND SCIENCE

By

Thesis Advisor _____

Committee member _____

Committee member _____

ACKNOWLEDGEMENTS

This endeavor would not be possible without the support of my thesis advisor Dr. Matthew Parks. His insightful guidance, consistent encouragement, and unwavering trust in my potential over the past two years have been fundamental to the successful completion of this research. I could not have undertaken this journey without my defense committee, Dr. Jyotisna Saxena and Dr. Sean Laverty, who generously provided knowledge and expertise throughout the project. I'm deeply grateful to Dr. Jenna Messick for recognizing the scientist in me long before I recognized it in myself by accepting me as a graduate student at the University of Central Oklahoma. I would like to extend my sincere gratitude towards UCO Office of Research and Sponsored Programmes for funding this project. I am grateful to Nicole Offutt at the Arcadia lake facility for allowing us to conduct research work at Arcadia Lake. I am also indebted to my friends for their constant motivation and making me feel at home away from home. I couldn't have done without them. Lastly, I would be remiss if I did not express my heartfelt gratitude to my family, especially my parents and my brother, for their endless love, encouragement, and sacrifices. Their unwavering support and belief in me made it possible for me to pursue my studies in the USA.

TABLE OF CONTENTS

List of Figures

List of Tables

ABSTRACT OF THESIS

University of Central Oklahoma

Edmond, Oklahoma

NAME: Shreya Sharma

TITLE OF THESIS: Characterization of Freshwater Macroplastic-Associated Bacterial Community Using 16S rDNA Sequencing

DIRECTOR OF THESIS: Dr. Matthew Parks

PAGES: ix-x

ABSTRACT:

Plastic pollution has become a major concern across all ecosystems, especially in freshwater environments. Freshwater bodies such as rivers, lakes, and streams serve as hotspots for accumulation of plastic waste, providing an interface for colonization and further succession of a self-sustaining microbial consortium known as the "plastisphere". In this study, plastisphere communities were sampled from individual and rafted, purposefully deployed and pre-existing polyethylene (PET), polypropylene (PP), polystyrene (PS) substrates during June, July and August 2025 at Arcadia Lake, Edmond, OK, USA. The microbial communities collected were further characterized using 16S rDNA metabarcoding. Wood substrates were also sampled to represent naturally occurring microbial communities. Our research supports primary influences of substrate type and sampling time on the progression of the plastisphere. Alpha diversity analysis revealed

that PET and PP showed similar levels of microbial diversity while PS was similar to wood. Family Leptolyngbyaceae was the most abundant family on all substrates across the sampling duration. Substrate preference was observed in families Rhodobacteraceae and Nostocaceae for PET and PP when compared to PS and wood. Additionally, Cyanobiaceae and Deinococcaceae were abundant on PS compared to other plastic substrates. Wood was enriched with Beijerinckiaceae for all three months. Across the sampling period, the abundance of Leptolyngbyaceae on PET and PP was highest in June and lowest in August while Nostocaceae was recovered to be abundant in July and August. Rhodobacteraceae was abundant in June on all the samples compared to June and July. Beta diversity analysis further supported our alpha diversity findings. Weighted UniFrac ANOVA analysis revealed significant results for individual factors such as substrate, collection date and combination factors substrate+collection date and substrate+single/rafts. Additionally, ANOVA analysis for combination factor substrate+artificial/naturals was recovered to be significant for all beta diversity measures including Bray-Curtis, Jaccard, UniFrac and wUniFrac. Finally, PERMANOVA analysis demonstrated significant results for substrate and collection date (time) establishing the importance of time in shaping plastisphere microbial communities along with substrate. Although numerous studies have shown the influence of a variety of factors on plastisphere succession, there is still a need to characterize the “global” plastisphere community while simultaneously determining the driving factors involved in shaping microbial succession among these communities.

Abbreviations: PET-polyethylene terephthalate; PP-polypropylene; PS-polystyrene; ANOVA-analysis of variance; PERMANOVA- permutational multivariate analysis of variance.

INTRODUCTION TO THE PROJECT

Plastics have become an integral part of everyday life because of their cost-effectiveness, lightweight characteristics, and long-lasting polymeric structure (Andrady & Neal, 2009). However, inefficient water management practices, residential and domestic littering, industrial waste, tourism and agricultural farms contribute significantly to plastic pollution (Blettler et al., 2018; Thushari & Senevirathna, 2020). Consequently, the high influx of plastics in the market coupled with improper waste management has resulted in widespread plastic pollution, emerging as one of the most pressing issues globally.

Plastic litter has become ubiquitous in every major ecosystem. Approximately 8% of plastic waste generated end up in aquatic ecosystems including rivers, lakes and oceans (Jambeck et al., 2015; L. Wang et al., 2021). Numerous studies have focused on microplastics pollution in marine environments neglecting freshwater macroplastic pollution (Azevedo-Santos et al., 2021; Blettler et al., 2018). Almost 87% of aquatic plastic pollution research has focused on the marine environment while only 13% has focused on freshwater systems (Azevedo-Santos et al., 2021; Blettler et al., 2018). Freshwater is only 0.01% of the Earth's water reserve, however, it supports approximately 100,000 species (6%) out of 1.8 million named species (Dudgeon et al., 2006). Additionally, macroplastic pollution in freshwater bodies represents perhaps 100 times more input in terms of weight than microplastics due to the large amount of macroplastic litter generated through multiple land-based sources (Lebreton et al., 2017; Schmidt et al., 2017).

In many cases, floating waste accumulates during a span of time, giving rise to vast buoyant solid plastic garbage "patches" or "rafts" (Amaral-Zettler et al., 2020). The increase in the volume of these patches has been reported in ocean gyres (Didier et al., 2017; Dris et al., 2015). These rafts

may serve as a small self-sustaining ecosystem harboring diverse microorganisms (Wright et al., 2020). Previous research has considered plastics as the novel habitat for microbial communities, in the terrestrial and aquatic ecosystems. These plastic-associated microbial communities are termed as the “plastisphere” and comprises of diverse community of microorganisms including bacteria, fungi, algae and protists. These organisms colonize plastic surfaces, forming complex biofilms that influence plastic degradation, nutrient cycling, and the transport of microorganisms, including potential pathogens. Scientists have reported that the development of the plastisphere microbial communities is influenced by various abiotic and biotic factors discussed briefly in my thesis review chapter (Chapter 1).

My research article (Chapter 2) primarily focuses on the role of substrate and time on development of plastisphere microbial communities in a freshwater environment. The major goal of my project is to assess the taxonomic differences among the microbial communities using 16S rDNA metabarcoding. Three plastic substrates, namely polyethylene terephthalate (PET), polypropylene (PP) and polystyrene (PS), were used based on ubiquity in the plastic waste in freshwater environments. We sampled biofilms from artificially deployed and previously existing singles and rafts plastics of the specific type on site to characterize patterns in microbial taxa diversity and composition. Overall, we found that substrates along with sampling duration affect microbial diversity and composition. Taxonomically, Leptolyngbyaceae is the most abundant family on all the substrates for June, July and August. Substrate specificity was observed in the family Rhodobacteraceae, which were abundant on PET and PP plastic types when compared to PS and wood across all sampling months. Families such as Cyanobiaceae and Deinococcaceae, known to play a key role in carbon and nitrogen regulation, were discriminant against PS. Our study also

revealed families specific to wood substrate compared to plastics, including JG30-KF-CM45, Nostocaceae and Micromonosporaceae. These families have been reported to be more prevalent on high-cellulose substrates. Furthermore, July and August revealed abundance of Comamonadaceae and Nostocaceae especially on PET and PP respectively, highlighting the influence of sampling time. As per our alpha diversity results, substrate was the governing factor in characterizing the microbial communities. PET and PP showed similar levels of microbial diversity when compared to PS and wood which were similar to each other. Also, PS and wood collectively showed higher diversity than PET and PP. These findings are novel and have not been reported before. Finally, beta diversity analyses including ANOVA and PERMANOVA further confirmed the influence of sampling time along with substrate in microbial abundance and composition.

Ever-changing dynamics of the Plastisphere: A brief review highlighting the effect of various spatio-temporal factors on plastisphere succession

Shreya Sharma, Dr. Matthew Parks

Chapter 1: Review article

INTRODUCTION

Plastics have become indispensable in modern society owing to their affordable, lightweight and durable polymeric properties (Andrady & Neal, 2009). The invention of Bakelite in 1907, officially marked the beginning of the "plastic age". Since then, plastics have brought numerous technological advances across diverse sectors including manufacturing, agriculture, construction and consumer products (Huang et al., 2022; Rhodes, 2018). According to Geyer et al., (2017), a total of 8300 Mt of virgin plastic has been generated thus far globally which is predicted to reach up to 1.1 billion tons annually by 2050 (Geyer, 2020). However, reuse of plastic is still limited to 6-26% of the plastic produced leading to a significant amount of plastic waste generation (Haque et al., 2024). Inefficient water management practices, residential and domestic littering, industrial waste, tourism and agricultural farms (Blettler et al., 2018; Thushari & Senevirathna, 2020) contribute to plastic pollution. Consequently, the high influx of plastics in the market coupled with improper waste management has resulted in widespread plastic pollution, emerging as one of the most pressing global issues.

Plastic pollution can be categorized into three main categories based on plastic size, microplastic (≤5 mm), mesoplastic (5 mm to 2.5 cm) and macroplastics (≥2.5 cm) (Lippiatt et al., 2013). Eventually, macroplastics and mesoplastics, due to various environmental factors such as

irradiation, wind, waves, water chemistry, surface erosion, abrasion and microbial degradation, progressively break down into micron plastics and nanoplastics (< 1 um) (Nguyen et al., 2021, 2023). Microscopic particles readily biomagnify and bioaccumulate through marine food chains via ingestion and entanglement by numerous species including zooplankton, mollusks, crustaceans, fishes and mammals (De Tender et al., 2015; Frère et al., 2018; A. McCormick et al., 2014), posing a critical threat to marine ecosystems.

Considerable amounts of research have been carried out to identify the extent of damage incurred by microplastics on marine environments, while largely neglecting freshwater macroplastic pollution despite its significant role in the generation of secondary microplastics and potential ecological impacts (Barboza et al., 2019; Gebreyohanes Belay et al., 2026; González-Pleiter et al., 2021). It has also been reported that freshwater bodies such as rivers, lakes, estuarine and streams act as plastic sinks from various land-based point sources, subsequently contributing approximately 80% of plastic pollution found in marine environments (Barboza et al., 2019; Blettler et al., 2017; Blettler & Wantzen, 2019). Therefore, detailed investigations into the impacts of macroplastic pollution in freshwater ecosystems are essential to address existing knowledge gaps and improve our understanding of their ecological consequences (Nguyen et al., 2021).

Plastics have also been recognized as a substrate on which microorganisms may hitchhike, inhabit, and eventually constitute a self-sufficient sustainable ecosystem (Didier et al., 2017; Nguyen et al., 2023). Zettler et al. (2013) first described the “plastisphere” as the artificial “microbial reef” where microbial communities colonize plastics debris in the aquatic environment. Microbial guilds in the plastisphere are aggregates of various primary producers (e.g., diatoms), predators (e.g., choanoflagellates, hydroid colonies), symbionts and saprotrophs (e.g., fungi) (Amaral-Zettler et

al., 2020). Plastisphere composition and spatiotemporal distribution is affected by several factors including substrate type, seasonal variation, depth in water column, polymer type (Miao et al., 2019), particle size (Frère et al., 2018), seasons and buoyancy (X. Chen et al., 2019), topography (Oberbeckmann et al., 2016), movement of plastics, and hydrodynamics of water (De Tender et al., 2017). The extent to which the microbial community is influenced by these factors is still debated. This review provides a detailed insight of the influence of numerous factors on development of plastisphere microbial communities and its further secondary succession.

Substrate-Driven Variation In Plastisphere Communities

A significant amount of research conducted has supported the impact of substrate on colonization of specific bacterial communities (Amaneesh et al., 2023; Amaral-Zettler et al., 2020; Coons et al., 2021; Oberbeckmann et al., 2018; Zettler et al., 2013; Zhai et al., 2023). According to Bhagwat et al. (2021), the family Crocinitomicaceae has been reported to be abundant on High-density polyethylene (HDPE) and polypropylene (PP). Genera Peredibacter (Bacteriovoracaceae) and Gemmatimonas (Gemmatimonadaceae) were found to be prevalent only on poly- β-hydroxy butyrate Co-β-hydroxyvalerate type (PHBV) and polylactic acid (PLA) however, previous research have indicated their presence on HDPE as well (Bhagwat et al., 2021). Furthermore, family Actinobacteria has been specifically found to be enriched on polyethylene terephthalate (PET) and PLA (Nguyen et al., 2021; Zhai et al., 2023). Family Flavobacteriaceae (Flavobacterium) was reported to be abundant on PHBV at approximately twice the level as it was on HDPE and PLA. In contrast, family Oxalobacteraceae was prevalent on HDPE and PLA

(Nguyen et al., 2023). Family Comamonadaceae was also prominent on HDPE, PLA and PHBV in freshwater (Nguyen et al., 2021). Other taxa, namely, Hyphomonadaceae and Erythrobacteraceae have also been found to be prevalent specifically on HDPE and polystyrene (PS) microplastics however, in marine environments (Oberbeckmann et al., 2018; Zettler et al., 2013). Moreover, organic substrates have shown to host unique taxa, for example, natural substrates such as wood and cobblestone seemed to influence the development of biofilms as phyla Acidobacteria, Chloroflexi, and Actinobacteria were found to be more prevalent on wood and cobblestone than on microplastics in freshwater (Miao et al., 2019). Hence, it has been generally accepted that there is no specific "global" plastisphere microbial community in aquatic environments but diverse group of microbes whose composition is primarily governed by local influences (Coons et al., 2021).

Influence Of Nutrients And Dissolved Oxygen (DO) On Microbial Communities

Gebreyohanes Belay et al. (2026) has reported the considerable impact of dissolved oxygen, light and nutrients on shaping plastisphere communities on microplastics in freshwater. A previous study conducted by Arias-Andres et al. (2018), showed positive correlation between biofouling and microbial respiration. In contrast, Leflaive et al. (2008) did not show any visible impact of nutrient concentration on planktonic or sessile microbes. Some researchers reported microplastics as a hub for heterotrophic microorganisms in aquatic ecosystems, which aid in the degradation of organic matter. Moreover, the impact of oxygen has been confirmed by Andrady et al. (2003) where accelerated degradation of microplastics in the presence of oxygen was observed in aquatic

environments. Occurrence of nitrifiers and ammonia-oxidizing bacteria such as genera *Nitrosomonas* and *Nitrosospira* belonging to Class Betaproteobacteria have been reported on polyvinyl chloride (PVC) plastic in chloraminated drinking water distribution systems (Lipponen et al., 2004). However, existing publications still do not delineate the impact of dissolved oxygen and nutrients on succession of plastisphere on various plastic types. Therefore, further research is needed to investigate the composition, diversity, and functional characteristics of plastisphere communities associated with different plastic polymers.

Effect Of Depth On Plastisphere Communities

Numerous studies have reported the sedimentation property of plastic pollution in aquatic environments. As of 2020, 3-11 million metric tons of plastic was estimated to be residing on the ocean floor, supporting the possibility of the deep sea serving as the final repository for plastics (Liu et al., 2025; Omura et al., 2024). However, characterization of plastisphere communities has been limited to epipelagic or shallow benthic waters with little attention paid to mesopelagic, bathypelagic, abyssopelagic and hadal zones (Choy et al., 2019; Liu et al., 2025). Whitman et al. (1998) reported open oceans to have approximately 1.2×10^{29} microbes below 200 m, while the specific concentration follows a depth gradient. Additionally, Vidal-Verdú et al. (2022) reported depth as a potential factor in plastisphere community development rather than substrate type. He reported the abundance of classes such as Gammaproteobacteria, Alphaproteobacteria, Acidimicrobiia and Bacteroidia on sediment-associated PET bottles derived from Mediterranean waste from the deep sea (Vidal-Verdú et al., 2022). Another study conducted by Omura et al. (2024)

confirmed the abundance of Rhodobacteraceae, Colwelliaceae, Campylobacteraceae, and Flavobacteriaceae on polyethylene (PE), PET, PS and PP plastics at depth ranging from 757 to 5552 m in the Pacific Ocean near Japan. Several polyhydroxyalkanoates (PHA)-degrading bacteria were also found belonging to classes Gammaproteobacteria, Alphaproteobacteria, and Deltaproteobacteria (Omura et al., 2024). In contrast to previous studies, Tu et al. (2020) reported the negative impact of depth on plastisphere microbial communities supported by Omura et al. (2024). Specifically, biodegradable plastics, particularly PHA, exhibited greater degradation at the seashore than at increasing water depths or on the deep-sea floor, indicating an inverse relationship between depth and degradation (Omura et al., 2024). No degradation was reported at all for common plastics including PE, PP, PS and PET either onshore or at the deep-sea floor, irrespective of depth (Omura et al., 2024). Substrates except plastics such as aluminum, titanium, glass, shale and limestone are colonized by microbial communities including Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria, Gram positive bacteria, Flexibacter/Cytophaga/Bacteroides, Leptospirillum/Nitrospira and Cyanobacteria at all depths except for cyanobacteria at 3500 m (Bellou et al., 2012). Hence, future research should focus on investigating the polymer role in different layers of the water column and identifying plastic-associated microbial communities.

Impact Of Time On Plastisphere Microbial Communities

Research has shown that microbial diversity on plastics increases over time, however, the question persists as to how important is time as a driving factor in plastisphere succession? Jacquin et al.

(2019) reported the initial colonization of diverse plastic types with Gamma and Alphaproteobacteria. In general, Rhodobacteriaceae was the most abundant group within 2-4 weeks of biofilm development on HDPE (Amaral-Zettler et al., 2015, 2020) while Rhodospirillaceae, Flavobacteriaceae and Xanthomonadales have been reported prevalent at 8-16 weeks (Jacquin et al., 2019). Similar classes of bacteria were observed as the primary colonizers on PE belonging to families Flavobacteriaceae (Bacteroidia), Rhodobacteraceae (Alphaproteobacteria), and Microtrichaceae (Acidimicrobiia) (Morohoshi et al., 2018). Rhodobacter also includes purple sulfur bacteria, known to fix N_2, providing a critical nitrogen source for the biofilm succession. This research aligned with Tu et al. (2020) who reported the development of families Flavobacteriaceae (Bacteroidia), Rhodobacteraceae (Alphaproteobacteria), and Microtrichaceae (Acidimicrobiia) on PE within 30 days of incubation, overtaken by Moraxellaceae (Gammaproteobacteria) and Bacillaceae (Bacilli) groups after 75 days. After 135 days, dominance shifted to the Flavobacteriaceae (Bacteroidia), with a significant increase of Microtrichaceae (Acidimicrobiia), Pirellulaceae (Planctomycetes), and Rhodobacteraceae (Alphaproteobacteria) (Dey et al., 2022). According to Gebreyohanes Belay et al. (2026) the initial plastisphere on PE, PS, HDPE, PP and PS was dominated by phyla Proteobacteria, Planctomycetes, Bacteroidota, Actinobacteriota and Cyanobacteria in freshwater. This trend shifted to Verrucomicrobiota and Cyanobacteria with decrement in Proteobacteria and Firmicutes across all plastic samples from week 8 to week 12. Substrate specificity was observed in several PS, PLA and HDPE samples that were found to be abundant with Desulfobacterota between weeks 4 and 8. Finally, pathogenic strains such as *Vibrio* have been reported by Xu et al. (2019) on microplastics only during February, April and July suggesting the impact of optimum

growth conditions and explaining the absence of *Vibrio* in other studies (Didier et al., 2017; Myers et al., 2007) which were consistent with studies by Kirstein et al. (2019) and Zettler et al. (2013). Classes such as Alphaproteobacteria and Betaproteobacteria, often described as plastisphere initial colonizers, were predominantly found on PP, PVC, PS, polycaprolactone (PCL), wood and water in aquatic ecosystems (Bhagwat et al., 2021; W. Li et al., 2023; Nguyen et al., 2023; Oberbeckmann et al., 2018). For example, members of family Rhodobacteraceae, have been consistently reported as initial colonizers across diverse plastic types such as low-density polyethylene (LDPE), PET, PE and PS. This trend could be explained by the metabolic flexibility of Rhodobacteraceae to utilize various carbon substrates as energy and carbon source, enabling it to thrive in both plastisphere and free-living planktonic environments (Dang et al., 2008; Salta et al., 2013a; Tan & Parales, 2019; P. Wang et al., 2022; Zettler et al., 2013).

Influence Of Site In Shaping Plastisphere Microbial Communities

Numerous studies so far have suggested a pertinent role of site characteristics including water chemistry, temperature and others playing a critical role in biofilm growth and activity (Vincent et al., 2022). Site has also been reported to play a more important role than polymer type in shaping the plastisphere community development in freshwater and marine ecosystems (Chaudhary et al., 2022; Coons et al., 2021; Hoellein et al., 2017; Parida et al., 2026; Salta et al., 2013a; Zhao et al., 2024; Zhu et al., 2023). Past research has suggested that plastisphere abundance on freely floating microplastics in multiple streams varied from upstream and downstream located in the Chicago metropolitan area of Illinois, USA, receiving treated effluent from wastewater treatment plant

(Hoellein et al., 2017; A. R. McCormick et al., 2016). At the family level, Flavobacteriaceae, unclassified Actinomycetales, and Cytophagaceae dominated upstream waters whereas downstream waters were characterized by the predominance of Flavobacteriaceae, unclassified Betaproteobacteria, and unclassified Actinomycetales, and Cytophagaceae (A. R. McCormick et al., 2016). Another study conducted characterized the difference of bacterial assemblage occurrence across different inter-continental-scale freshwater bodies namely, Higgins Creek (Illinois), North Branch Chicago River (Illinois), Springbrook Creek (Illinois), Santa Cruz River (Arizona), Gwynns Falls (Maryland) and Thornton Creek (Washington) (Hoellein et al., 2017). Overall, Higgins Creek had the highest operational taxonomic unit (OTU) richness, whereas Thornton Creek had the lowest. Subsequently, Thornton Creek did show higher abundance of Comamonadaceae along with Rhodobacteraceae and Sphingomonadaceae when compared to other sites. Family Rhizobiales was prominent in the Santa Cruz River along with unclassified Actinomycetales which were significantly lower at all the remaining sites. Family Methylophilaceae was found to be abundant in Gwynns Falls. At Higgins and Springbrook Creeks, unclassified Gammaproteobacteria, Pseudomonadaceae, and Campylobacteraceae were abundant. Hence, site-specific variability is another critical factor that requires comprehensive investigation to better understand its role in shaping the plastisphere.

Prevalence Of Antibiotic Resistance Genes (ARGs) And Bacteria (ARBs) In Plastisphere Communities

According to numerous studies, plastics may act as an important interface supporting microbial hitchhiking, eventually providing an ambient environment for dissemination of antibiotic-resistance genes (ARGs) via horizontal gene transfer (transformation, conjugation and transduction) (Hu et al., 2022). Out of all freshwater bodies, lakes have been regarded as a major reservoir for the selection process of ARGs in opportunistic pathogens (Nnadozie & Odume, 2019). The presence of extended-spectrum β-lactamase and carbapenemase-producing Enterobacteriaceae (CPE), such as *E. coli*, *K. pneumoniae*, *E. cloacae*, *C. frendii*, *Enterobacter asburiae* and *K. oxytoca* isolates has been documented in streams and rivers (Nnadozie & Odume, 2019). *Methicillin-resistant Staphylococcus aureus* (MRSA) in recreational water poses another major public health concern (Nnadozie & Odume, 2019; Pourmand et al., 2017). Hu et al. (2022) confirmed the impact of plastic size as a determining factor in horizontal gene transfer process. For example, PS particles of ≤100 nm induced nanopore formation on the cell membrane which further enhanced membrane permeability while larger particles (1000 and 10000 nm) showed no measurable effect. It has also been confirmed that PS microplastics enhanced HGT by the contact distance between donor and recipient cells (Cheng et al., 2022). Another study conducted by Wu et al. (2019) confirmed the presence of 103 harmful pathogens in surrounding water environment along with 73 diverse pathogens within the plastisphere belonging to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. The study also revealed that biodegradable PHA and PLA microplastics were more selectively enriched with opportunistic pathogens (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enterica*, *Mycobacteroides abscessus*,

Burkholderia pseudomallei, and *Klebsiella pneumoniae*), ARGs (evgS, smeR, NmcR, mtrA, oleB, bacA, mecA, qacA, TolC, dfrA1, mphA, mphB and evgA), mobile genetic elements (MGEs) and virulence factors (VFs) compared to PE, PP and PS microplastics and the surrounding water (Wu et al., 2019). PHA had specifically higher abundance of *Pseudomonas aeruginosa* compared to PE, PP, PHA and PS. Additionally, Wu et al. (2019) reported the higher abundance of ARGs such as sulfonamide and macrolide resistance, on LDPE, PET, PS, PVC, glass and rocks when compared to planktonic bacteria in freshwater at the sites which were located downstream of wastewater treatment plant. In conclusion, more comprehensive studies are needed to elucidate the factors influencing the prevalence of ARGs in plastisphere microbial communities.

CONCLUSION

This review focuses on the impacts of numerous abiotic and biotic factors in shaping plastisphere microbial communities in aquatic ecosystems. Research has shown that substrates play a decisive role in colonization by microbial communities; however, inclusion and role of common plastics such as PET, PP, PS, PE and PVC, ubiquitous in plastic pollution, in characterizing plastic-specific microbial communities needs to be researched further. Future research is also needed to measure the effect of nutrients and dissolved oxygen on ecologically critical metabolic processes such as nitrogen cycling and carbon degradation governed by plastisphere communities. It has also been shown that exposure time affects the secondary colonizers in the plastisphere. Further research is needed to study the extent of impact of migration, sinking, weathering and degradation of macroplastics and eventually microplastics. Hence, plastisphere research is still in its early stages,

and further investigations across different environmental settings, plastic types, water column depth, and exposure durations are needed to determine whether a “globally” conserved plastisphere microbial community exists.

Characterization of Freshwater Macroplastic-Associated Bacterial Communities Using 16S rDNA Sequencing

Shreya Sharma, Dr. Matthew Parks

Chapter 2: Research article

INTRODUCTION

Plastics are ubiquitous in everyday life, owing to their affordable, lightweight, and durable polymers (Andrady & Neal, 2009). During the 1850s-1860s, extensive research was conducted to create the first semi-synthetic plastic called "celluloid" or Parkesine, utilizing cellulose, a natural polymer (Geyer, 2020). However, the invention of the first fully synthetic plastic, Bakelite in 1907, ("the material of a thousand uses"), revolutionized manufacturing industries (Kaufmann & Baekeland, 2011) thereby initiating a "plastic age" (Thompson et al., 2009). This immensely reduced dependency on scarce and valuable natural materials such as rubber, horn, shellac, Gutta Percha, Bois Durci, bitumen materials, cellulose and casein (Walker, 1994). A variety of plastic polymers have been developed since then, employed in day-to-day life in innumerable ways (Geyer, 2020;Desidery & Lanotte, 2022). However, due to their high production rate, the disposal of these plastics has become one of the most pressing environmental issues (Gregory, 2009). According to Geyer et al., (2017), 8300 Mt of virgin plastic has been generated thus far globally, which is predicted to reach up to 1.1 billion tons annually by 2050 (Geyer, 2020). Reuse of plastic is still limited to 6-26% which leads to a significant amount of plastic waste generation (Haque et al., 2024). Approximately 8% of plastic waste generated end up in aquatic ecosystems including rivers, lakes and oceans (Jambeck et al., 2015; L. Wang et al., 2021).

Plastic pollution can be grouped into three categories based on size, comprising microplastic (≤5 mm), mesoplastic (5 mm to 2.5 cm) and macroplastics (≥2.5 cm) (Lippiatt et al., 2013). Microplastics are artificially employed in petrochemical industries (Deng et al., 2023), cosmetics (Anderson et al., 2016) and paint industries (Gaylarde et al., 2021) and also arise from the degradation of macroplastic waste due to biotic and abiotic factors including UV-B light, temperature, and abrasion (Y. Chen et al., 2024). However, macroplastic pollution in freshwater bodies represents perhaps 100 times more input in terms of weight than microplastics due to the large amount of macroplastic litter generated through multiple land-based sources (Lebreton et al., 2017; Schmidt et al., 2017). In addition, macroplastics may affect their surrounding environment including microorganisms, which thrive on them in comparatively different manners to microplastics through processes still in need of full characterization (Blettler et al., 2018a).

Plastic litter has become ubiquitous in every major ecosystem (Williams & Rangel-Buitrago, 2022) including freshwater bodies (Azevedo-Santos et al., 2021). Substantial amounts of plastic waste in freshwater bodies emerge from inefficient urban water management practices, littering, industrial waste, and agricultural farms (Blettler et al., 2018a). Freshwater is only 0.01% of the Earth's water reserve, however, it supports approximately 100,000 species (only 6%) out of 1.8 million species (Dudgeon et al., 2006). Plastics, along with other pollutants such as antibiotics and metals (nickel, cadmium, and lead), amplify harmful impacts on freshwater biodiversity (Azevedo-Santos et al., 2021; J. Li et al., 2018; J. Wang et al., 2017). In many cases, floating plastic waste accumulates during a span of time, giving rise to vast buoyant solid plastic garbage "patches" or "rafts" (Amaral-Zettler et al., 2020). The increase in the volume of these patches has been reported in ocean gyres (Didier et al., 2017a; Dris et al., 2015). These rafts may serve as small self-sustaining

ecosystem harboring diverse microorganisms (Wright et al., 2020). Research by Azevedo-Santos et al. (2021) and Blettler et al. (2018) suggest that 87% of aquatic plastic pollution research has focused on the marine environment while only 13% has focused on freshwater systems.

Plastic waste associated with aquatic habitats is colonized by microbial communities irrespective of water body and plastic type (Awari V. G. et al., 2023). This transition from free-living or planktonic state to sessile state is regulated by various genes governing chemotaxis, communication, transport of nutrients, and substrate (Amaral-Zettler et al., 2020). Bacteria forming surface biofilms collectively contribute to the "plastisphere" (Zettler et al., 2013). The plastisphere comprises of abiotic and biotic components. Abiotic components refer to plastic components of different types (Barros & Seena, 2021a; Dey et al., 2022; Kirstein et al., 2019). Biotic components include bacteria, diatoms, algae, and fungi (Bocci et al., 2024), including potential pathogens being transported through long distances (Zettler et al., 2013). Various factors may impact the formation of the plastisphere, including polymer type (Miao et al., 2019), particle size (Frère et al., 2018), season, buoyancy (X. Chen et al., 2019), topography (Oberbeckmann et al., 2016), movement of plastics, and hydrodynamics of water (De Tender et al., 2017). The extent to which the microbial community is influenced by these factors is debated. Some studies have demonstrated the selective impact of these factors while others have not documented significant correlations. For example, plastic substrate specificity may or may not impact the microbial community in diverse environments. Alpha and beta diversity may vary significantly among different plastic substrates for bacterial communities (Kirstein et al., 2019). According to Bhagwat et al. (2021), bacterial phyla Proteobacteria, Bacteroidetes, Cyanobacteria, and Thaumarchaeota dominated the community composition across all plastic substrates. However, the planktonic

environment was found to be dominated by oligotrophic *Prochlorococcus* (Bryant et al., 2016). Actinobacteria, Planctomycetes, and Chloroflexi were found to a lesser extent on polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polycaprolactone (PCL) (Bhagwat et al., 2021). In contrast, clades Deltaproteobacteria, Hyphomonadaceae and Desulfobacterales were predominantly found on microplastic PCL compared to PP, PS and PVC microplastics (Bhagwat et al., 2021). Also, biodegradable plastics may host different communities than non-biodegradable ones (Bhagwat et al., 2021). Deployment location and seasonal variations have been shown to influence the bacterial taxa growing on plastics (Oberbeckmann et al., 2016; Vincent et al., 2022) with communities significantly differing from the surrounding water communities (W. Li et al., 2023). Primary colonizers may differ from the secondary colonizers also highlighting the impact of time. Initial colonizers of biofilms may include gamma-Proteobacteria followed by an increase in alpha-Proteobacteria after initial incubation (Lee et al., 2008). Other studies (Dang et al., 2000a; Dang & Lovell, 2002; Lee et al., 2008) have shown opposite trends that could be attributed to sample collection time frame difference (Lee et al., 2008). For example, a study conducted in Korea revealed Gammaproteobacteria abundance on acryl plastic, steel, and glass substrate after 0-9 hours of incubation in the pioneer community, in contrast with Lee et al. (2008) who found that Alphaproteobacteria was abundant on all substrates within 12 hours of incubation indicating that latter could be a secondary microflora. Finally, numerous studies have reported the accumulation of antibiotic-resistant bacteria (ARBs) and genes (ARGs) in plastisphere communities (Aminov, 2009; J. Li et al., 2018a; Nnadozie & Odume, 2019). Pathogenic strains such as *Vibrio* and *Pseudomonas* have been isolated from plastisphere microbial communities

specifically on polyethylene (PET), PP and PS, raising the likelihood of health risks linked to aquatic ecosystems (Cholewińska et al., 2025; Kirstein et al., 2019; Wu et al., 2019).

The main purpose of this study was to identify the role of substrate and time on development of plastisphere microbial communities in a freshwater environment. Taxonomic differences among microbial communities were assessed using 16S rDNA metabarcoding. Plastic substrates polyethylene (PET), polypropylene (PP) and polystyrene (PS) were chosen for their ubiquity in plastic waste in freshwater ecosystems. Samples of those plastics from both artificially deployed single plastic waste samples and rafts, and previously existing single plastic waste and rafts on site were used to characterize patterns in microbial community diversity and composition. We predicted substrate and collection date as leading factors, influencing microbial communities diversity and composition. Furthermore, we also anticipated significant differences between microbial communities on purposefully deployed rafts and singles when compared to previously existing rafts and singles plastics.

METHODS

Sampling Site Description

A systematic sampling was conducted at Arcadia Lake near Edmond, Oklahoma, USA (**Figure 1**), to assess microbial community diversity across plastic types. Arcadia lake stores inflow from the Deep Fork River, with a surface size of 7 km^2 (1,820 acres) and ca. 42 km of shoreline. This reservoir serves as a key source of freshwater for the city of Edmond, and additionally hosts recreational activities including boating, fishing and swimming. Arcadia lake has substantial amounts of pre-existing plastic waste due to inflow from the Deep Fork river, which has its headwaters in nearby Oklahoma city, as well as localized litter and windblown trash. Three replicate sample locations were utilized in a protected southwest bay of the lake **(Figure 1)**: **Site 1** (35.60909500°N, -97.40899389°W), **Site 2** (35.60798806°N, -97.40951083°W) and **Site 3** (35.60854806°N, -97.41014472°W). Sampling depth ranged from ca. 1 m to 3 m across the sampling period and was consistent between the sites.

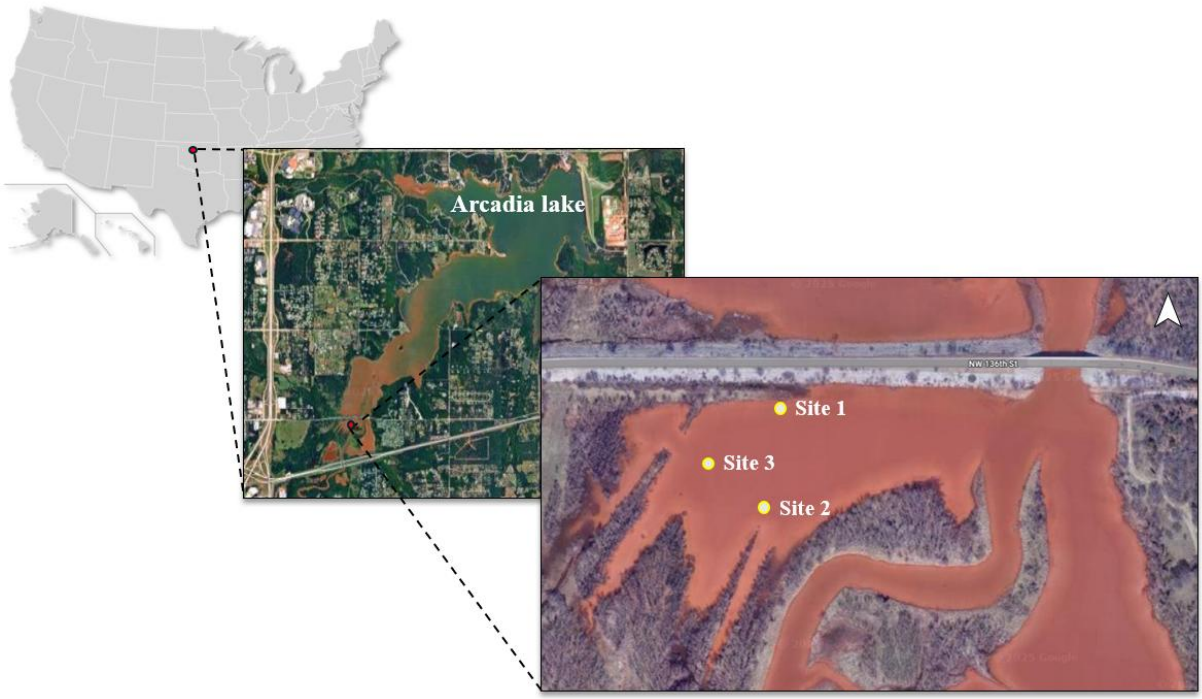


Figure 1. Sampling locations at Arcadia Lake, Edmond, Oklahoma, USA.

Assembly And Deployment Of Artificial Plastic Rafts And Single Plastic Items

Macroplastics utilized consisted of three distinct plastic materials ubiquitous in consumer goods: polyethylene terephthalate (PET) (single-use drinking water bottles, Great Value™, 500 ml), polystyrene (PS) (single-use drinking cups, Hefty®, 532 ml), and polypropylene (PP) (Tornado tubes, Midsci™, 50 ml). Plastic rafts were custom-built using four of each type of macroplastic secured with monofilament fishing line (Berkeley Trilene™, 0.40 mm diameter/ 6.4 kg strength) and leaving a free tag end of ca. 0.5-1 m **(Figure 2)**. PET water bottles and PP Tornado tubes were halfway filled with distilled water to keep them partially submerged during deployment. On-site, the tag end of each sample or raft was knotted to roughly 4 m length of the same monofilament and tethered to approx. 20 cm x 10 cm x 10 cm brick using heavier nylon line to anchor samples in place in the reservoir. The 4 m length of monofilament was used to keep samples on the surface while accounting for

fluctuations in water levels over the course of the sampling period. Small plastic fishing floats were attached to the monofilament roughly 0.5-1 m distant from each sample to facilitate locating samples and to maintain PS cups near the surface of the water. Additionally, wood branches (approximately 1.5 m length, 5 cm diameter) were collected on-site from dry flotsam and deployed at the same locations to allow comparison between plastic substrates and an organic substrate.

Microbial biofilm from macroplastic samples was collected at one-month intervals in June, July and August 2025. The outer surface of substrates was scraped using a stainless-steel dental scraper followed by preservation in 100% ethanol, before returning to the lab where samples were stored at −20 °C until DNA extraction. The scraper was sterilized with 100% ethanol and wiped with clean Kimwipes® between each sample. In total, 139 samples were collected over the summer with 33 naturally existing plastic litter and 97 artificially deployed macroplastics, respectively **(Table 1)**. Over the sampling period, several individual PET bottles and PP falcon tubes were lost and most of the deployed PS cups were submerged due to flood conditions in early June and mid-August at all the three sampling sites. However, new respective samples were then replenished evenly to make up for the lost numbers and sampled for the remaining ca. 2-3 months. Additionally, seven lost PS samples

were recovered in August, due to decrement in water level and were sampled during the total sample collection.

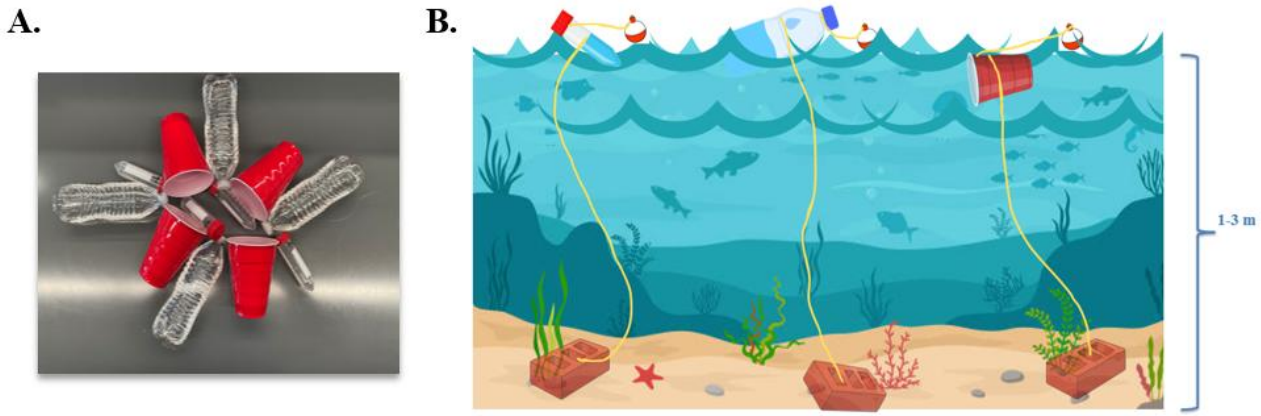


Figure 2. A. Example of an artificial raft, assembled in the laboratory, used to conduct this experiment. Four of each PP (red-capped falcon tubes), PS (red cups) and PET (transparent water bottles) items were used in each raft. **B.** Sampling strategy depicting the single plastic items tied to the bricks and bobber using fishing line (yellow) keeping them afloat.

Table 1. Distribution of samples collected in June, July, and August 2025.

	Natural (litter) Rafts	Sample Count	**Artificial (placed) Rafts**	Sample Count
	Polypropylene (PP)	2	Polypropylene (PP)	14
	Polyethylene terephthalate (PET)	3	Polyethylene terephthalate (PET)	16
	Polystyrene (PS)	4	Polystyrene (PS)	20
Total rafts		9		50
	Natural Singles		**Artificial Singles**	
	Polypropylene (PP)	0	Polypropylene (PP)	19
	Polyethylene terephthalate (PET)	4	Polyethylene terephthalate (PET)	15
	Polystyrene (PS)	2	Polystyrene (PS)	13
	Wood	18	Blanks (Negative controls)	9
Total singles		24		47
		33		97
Total samples				**139**

Laboratory Strategies

DNA Isolation And PCR Amplifications

DNA was isolated from collected samples using QIAGEN DNeasy PowerSoil Pro Kits following manufacturer's recommendations. Samples were amplified for the V3-V4 segment of the 16S ribosomal RNA locus through PCR. This amplification consisted of two PCR reactions (Parks et al., 2020, 2024). Step 1 PCR used the Pro341F/Pro805R primers (Takahashi et al., 2014), with primer tails consisting of variable in-line indices of 2–7 base pairs in length and additional sequence complementary to Illumina sequencing primers. Step 1 amplifications were composed of 1.5 µl millipore-filtered water, 1.0 µl BSA (New England Biolabs), 7.5 µl 2× Taq MasterMix (New England Biolabs), 0.5 µl each forward and reverse 10 µM primers, and 4 µl full or diluted DNA extraction (representing ca. 10–100 ng DNA), with amplification proceeding as follows: 95°C for 2 minutes, 25 cycles of 95°C for 15 seconds/65-55°C for 30 seconds (1°C decrease per cycle for first 10 cycles)/68°C for 30 seconds, followed by a final extension at 68°C for 5 minutes. All step 1 PCR amplifications were performed in triplicate with five negative control samples (PCR grade water replacing input DNA extraction).

For each sample, 5 µl of product from each of the three replicate step 1 amplifications was pooled and used as an input source for step 2 PCR amplifications. Step 2 PCR amplifications utilized primers complementary to step 1 primer tails and containing sequence complementary to Illumina flowcell-bound oligonucleotides (Parks et al., 2020, 2024). These reactions were composed of 7.5 µl 2× PCR MasterMix, 1 µl each of 10 µM forward and reverse primers, 4.5 µl millipore-filtered water, and 1 µl product from pooled step 1 PCR amplifications. PCR conditions for step 2 amplifications were: 95°C for 2 minutes, 10 cycles of 95°C for 15 seconds/55°C for 30 seconds/72°C for 30 seconds, followed

by a final extension at 72°C for 5 minutes. Finally, PCR products were checked for size and intensity using a 1% agarose gel stained with ethidium bromide. Step 2 PCR reactions were pooled ca. equimolarly and purified using Agencourt Ampure XP beads (www.beckman.com), at a ratio of 0.8:1 product:beads to remove residual primers and potential primer-dimers. Sequencing was performed at the University of Central Oklahoma on an Illumina MiSeq platform, using standard procedures with a MiSeq Reagent Kit V3 (600 cycle) (www.illumina.com) to produce 300 base pair paired-end reads.

Data Analysis

Bioinformatic processing and taxonomic assignments

Raw sequence data in fastq format was demultiplexed using custom Unix scripting and trimmed of adapter and primer sequences using Cutadapt v. 2.10 (Martin M., 2011) with minimum length cutoff of 100 bp for forward and reverse reads. Subsequent visualization, denoising and taxonomic assignment were carried out using QIIME2 v. 2021.4 (Bolyen et al., 2019) and largely followed the 'moving pictures' tutorial guidelines ("Moving Pictures" Tutorial — QIIME 2 2024.10.1 Documentation, 2019). Samples were first denoised using DADA2 (Callahan et al., 2017). Minimum cutoffs for feature frequency (100) and presence (≥ 2 samples) were applied. Taxonomy was assigned through the Naïve Bayes classifier (classify-sklearn) and the feature-classifier QIIME2 plugin. Assignments were based on the 16S rRNA gene Silva v. 138.1 SSU Ref NR99 database (Quast et al., 2013), which was trimmed to targeted V3V4 amplicons (not including primer sequences) following the 'microbiome_helper'guidelines(https://github.com/LangilleLab/microbiome_helper/wiki/Creating-QIIME-2-Taxonomic-Classifiers). Any features recovered as mitochondria, chloroplast, or Eukaryota were removed from 16S rRNA gene assignments. 16S rRNA gene samples were also filtered for

laboratory contamination based on feature counts in associated negative controls using recommended procedures in microDecon (McKnight et al., 2019). Rarefaction curves were used in QIIME2 to visually assess impact of sequencing depth on recovery of taxonomic diversity across samples.

Diversity and statistical analysis

Subsequent statistical analyses and visualization were performed in RStudio (v. 2022.07.02), primarily with sample metadata, microbial taxon names and abundance data, and corresponding phylogenetic tree loaded as objects in *phyloseq* v. 1.42.0 (McMurdie & Holmes, 2013). Complete R markdowns for statistical analyses were generated and are provided as supplementary files available through Zenodo (link will be provided). Sample read depth was included as a factor in statistical testing to assess any impact read depth might have on results. Read counts were transformed to proportions per sample (i.e. total sum normalization) for abundance-based diversity metrics. We initially visualized microbial community composition through two methods 1) stacked barplots for taxonomy were prepared using *microViz* v. 0.10.6 (Barnett et al., 2021); 2) co-occurrence patterns with the aid of *bipartite* package in R (Dormann & Fründ, 2026).

Alpha diversity describes richness, evenness and diversity in microbial communities for a variable. Specifically, three different measures of alpha diversity (Richness, Shannon diversity, Faith's phylogenetic diversity) were estimated using the R package *phyloseq* v. 1.42.0. Multiple regression modelling followed by ANOVA was performed in R to assess the significance and contribution of various factors to alpha diversity, with model assumptions checked visually through residual plots generated in *easystats* v. 0.6.0 (Lucecake, 2022). Two rounds of outlier identification and removal were completed using standardized residuals in *olsrr* v. 0.5.3 (Hebbali A. 2022). Regression and ANOVA analyses were performed both with and without inclusion of identified outlier samples.

Significance of pairwise comparisons was assessed with *p*-values adjusted using the R package *emmeans* v. 1.8.2 (Lenth R. 2022) through post-hoc Tukey's HSD correction. In initial models, alpha diversity measures were each regressed against single factors including collection date, site, replicates, substrate (plastic type), singles/rafts, naturals/artificials, sequencing read depth, and interaction factors including substrate+collection date, substrate+site, substrate+singles/rafts and substrate+naturals/artificials. Our preliminary analysis depicted results for substrate, collection date and substrate+collection date as significant interaction factors; hence, these were the primary focus of subsequent analyses. Information about the remaining variables has been provided in the supplementary material.

Beta diversity refers to the difference in composition of microbial communities for a variable. Trends in beta diversity were visualized using both heatmaps and ordination plots assembled in phyloseq v. 1.42.0 and vegan v. 2.6.4 (Oksanen J, 2022), respectively. Beta diversity was quantified through four measures: Bray-Curtis, Jaccard, UniFrac, and weighted UniFrac metrics. Beta diversity measures were subsequently tested for significant differences primarily for substrate, collection date and substrate+collection date along with substrate+artificial/naturals and substrate+singles/rafts using vegan v. 2.6.4. Variables were tested for differences in: 1) centroid location (the location of the centre of a cluster of samples), 2) dispersion (an estimate of homogeneity of variance), and 3) analysis-of-similarity (ANOSIM) (a metric ranging from 0 to 1 and indicating whether groups are discretely separated; values closer to one indicate more discrete clustering). Differences in species' centroid locations were tested using PERMANOVA using the adonis2 function with setting "by='margin'" to negate effects of model order, and with Benjamini-Hochberg correction for *p*-values (Benjamini et al., 2001). Beta dispersion effects were measured through ANOVA, with 999 permutations and with

post-hoc Tukey's correction for *p*-values. PERMANOVA analyses were conducted for all the variables however, for the sake of simplicity results have been discussed about plastic type, collection date, substrate+collection date substrate+artificial/naturals and substrate+singles/rafts. ANOSIM calculations were performed with 999 permutations.

RESULTS

Sequencing Output and Taxonomic Assignments

In total, forward and reverse raw read counts ranged from 1336 to 196749 (average = 114478.4 ± 43134.92 standard deviation) for all samples (excluding negative controls). Phylogenetic classification identified 3,832 taxonomic features or Operational Taxonomic Units (OTUs). Family level classification showed Leptolyngbyaceae (range = 13.3-17.8% abundance) as the most abundant family across all substrates followed by Rhodobacteraceae (range = 6.8-23.0% abundance), Nostocaceae (range = 1.9-24.2% abundance), Sphingomonadaceae (range = 4.0-8.7% abundance) and Comamonadaceae (range = 3.0-6.6% abundance) **(Figure 3A)**. Rhodobacteraceae, Sphingomonadaceae and Nostocaceae were relatively abundant on PET and PP compared to other substrates. However, family Exiguobactereraceae were only found on plastic substrates (PET, PP and PS). Wood was specifically enriched for Beijerinckiaceae and Nocardioidaceae for all three sampling months compared to plastics. Time variation influence was also apparent for certain taxa **(Figure 3B)**. For example, Leptolyngbyaceae was prominent on PET and PP in the month of July but decreased for June and August. Also, Rhodobacteraceae had higher abundance in June when compared to July and August for all samples. Unlike Rhodobacteraceae, Nostocaceae was abundant in July and August especially on PET and PP samples. Another family, Exiguobacteraceae, was only abundant during July for PET and PP, whereas on PS it was abundant during both July and August. Deinococcaceae largely followed a similar trend as Exiguobacteraceae. Nostocaceae was absent in June for all plastics but surged in the last two months for PET and PP. Lastly, Micromonosporaceae (Class= Chlorophyceae) was largely found

to be limited to wood (4.0% abundance) with a small presence on PS (1.25% abundance) **(Figure 3B)**. Similar patterns were observed at the Order level classification (supplementary material).

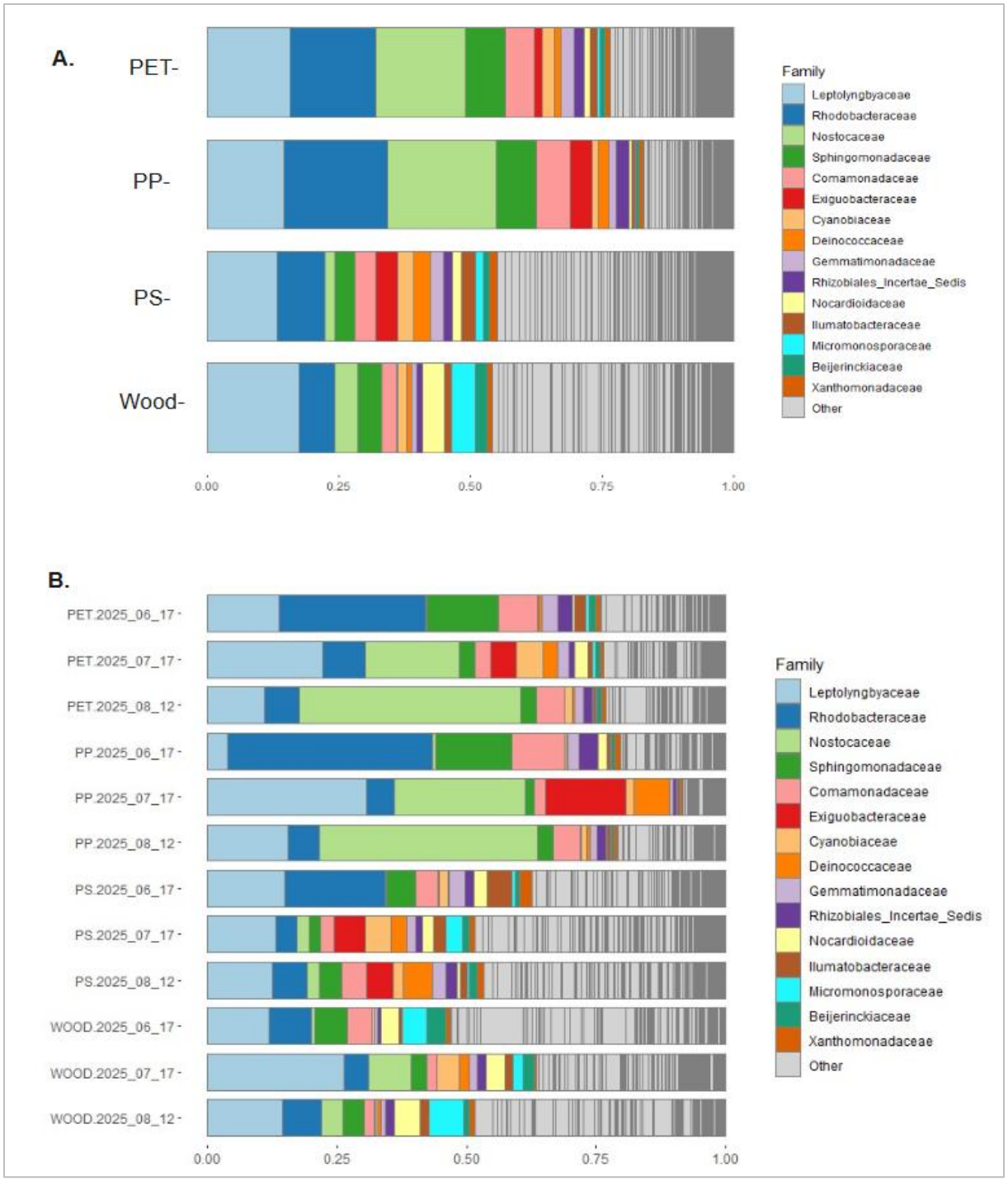


Figure 3. Stacked bar plots representing the abundance of different taxas at family level of taxonmoic classfication **A.** Based on substrate variable **B.** Impact of within-season variation (June, July and August) on the microbial communities adhered to different plastic types. Both sections shows the predominance of Leptolyngbyaceae at family level. Genus and order in present in the supplementary figure 1.

Co-Occurrence Patterns Of Microbial Communities Based On Substrate And Time

Based on the 20 most abundant families across all taxa combined, Leptolyngbyaceae was relatively evenly distributed across all samples for all collection months. Rhodobacteraceae was also abundant on all the samples but was more prevalent on PET and PP for all three months than on PS and wood **(Figure 4A)**. Nostocaceae followed a similar pattern to Rhodobacteraceae except it was most abundant during August. Comamonadaceae was also distributed on all samples, although was more prevalent on PET samples in July. At the genus level, Calothrix_PCC-6303 was the most abundant across all the samples followed by Rhodobacter, Porphyrobacter, Leptolyngbya_PCC-6306, Alkalinema_CENA528 and Exiguobacterium **(Figure 4B)**. Calothrix_PCC-6303 was specifically abundant on PET and PP sample collected in August. Rhodobacter and Porphyrobacter were most abundant on PET and PP, especially in August for PP. In addition, Leptolyngbya_PCC-6306 was most abundant in June for all the plastic types. Alkalinema was most abundant on PET June. PP and PS august collection showed Exiguobacterium abundance.

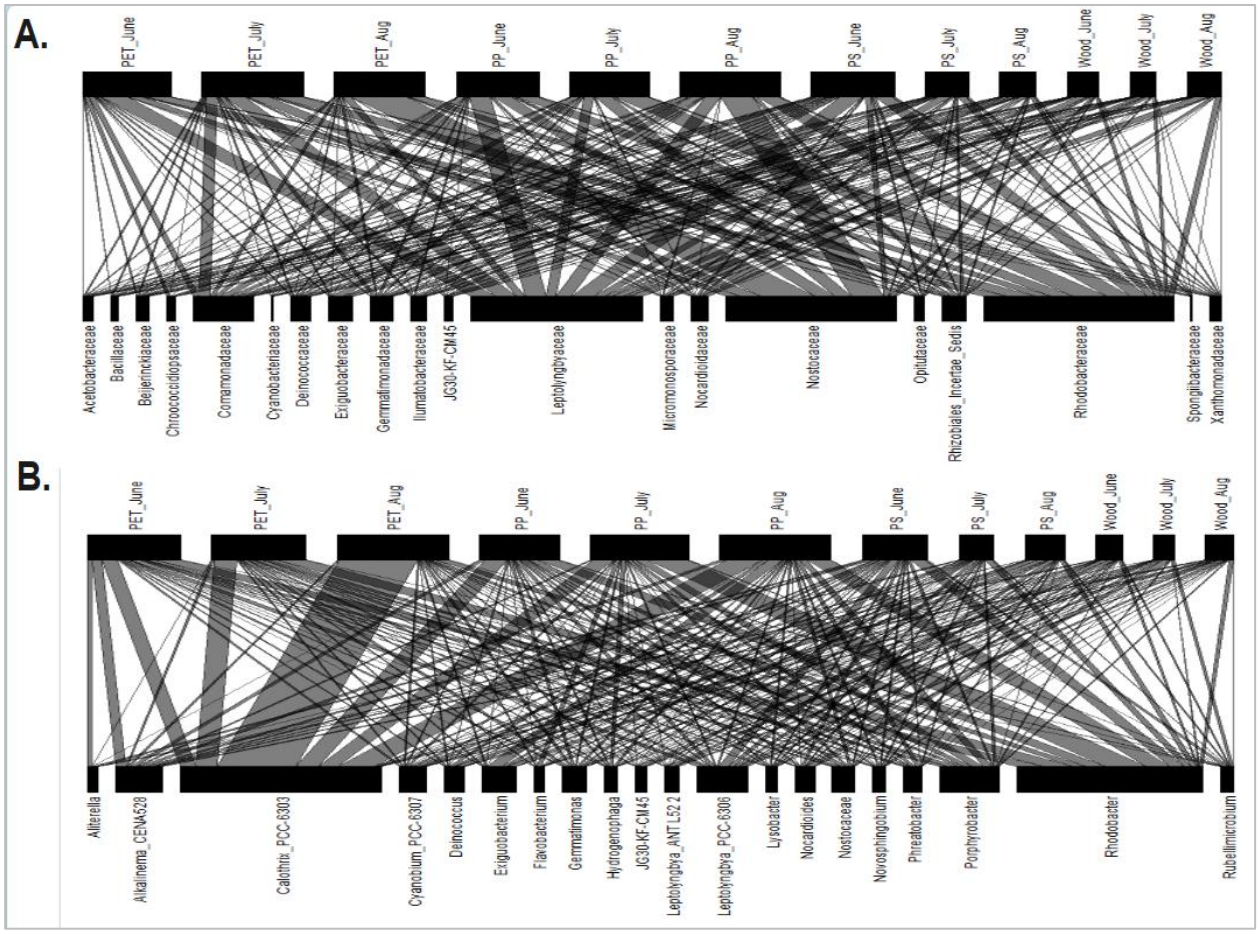


Figure 4. Co-occurrence patterns of 20 most abundant plastisphere microbial communities taxa based on substrate+collection date factor. **A**. At family-level, Leptolyngbyaceae was the most abundant on all the substrates, **B**. At genus level, Calothrix_PCC-6303 was the most abundant on substrate types.

	Regression Analysis (Alpha diversity)			PERMANOVA (Beta diversity)			
Variables	Shannon	Chao1	FPD	Bray-Curtis	Jaccard	UniFrac	wUniFrac
Substrate	■■■	■■■	■■■	■	■	■	■
Collection date	□□□	□□□	■■□	■	■	■	■
Singles/Rafts	□□□	□□□	□□□	■	■	■	■
Artificials/naturals	■■■	■■■	■■■	■	■	■	■
Substrate+collection date	■■■	■■■	■■■	■	■	■	■
Substrate+site	■■■	■■■	■■■	■	■	■	■
Substrate+singles/rafts	■■■	■■■	■■■	■	■	■	■
Substrate+artificials/naturals	■■■	■■■	■■■	■	■	■	■

Figure 5. Significant p-value shown for regression analysis and PERMANOVA analysis at ASV level. Filled rectangles indicate significant results at $p \leq 0.05$. Regression analysis three rectangles show significant results after two rounds of outlier samples removal. FPD= Faith's Phylogenetic Diversity.

General Regression Analysis/PERMANOVA Results For Alpha/Beta Diversity

At the ASV level, regression analysis for differences in alpha diversity showed significant results for the individual variable substrate and interaction variables such as substrate+collection date, substrate+site, substrate+singles/rafts and substrate+artificials/naturals for Shannon, Chao1 and FPD **(Figure 5)**. However, collection date was significant for only FPD and became insignificant after the second set of outlier samples removal. Additionally, singles/rafts did not show any significant result. PERMANOVA analysis for beta diversity revealed that all of the variables showed significant differences **(Figure 5)**. Below, we provide detailed results for these and related analyses.

Alpha Diversity

Regression analysis for substrate recovered significantly higher median diversity for PS and wood relative to PET and PP across all alpha diversity metrics (Shannon, Chao1 and Faith's phylogenetic diversity) at both ASV and family level **(Figure 6)**. Combination variables such as substrate+collection date, substrate+site, substrate+ naturals/artificials and substrate+singles/rafts also showed significant *p*-values ($p < 0.05$) for all the above metrics. Collection date, on the other hand showed statistical significance at the family level after second set of outlier samples removal for Shannon index (supplementary material). No significant differences were recovered at the genus level. Singles/rafts were significant for Shannon and further became significant for Chao1 and Richness after second set of outlier samples removal. However, sampling site did not show significant differences at ASV or family level.

Pairwise Tukey analysis also revealed significant differences between PET and PP versus PS and wood across samples collected in June, July and August at ASV and family level both. Pairwise comparisons for factors including substrate+collection date, substrate+singles/rafts, substrate+naturals/artificimmals and substrate+site, showed significant differences between groups PET+PP and PS+wood when compared across all the three consecutive months.

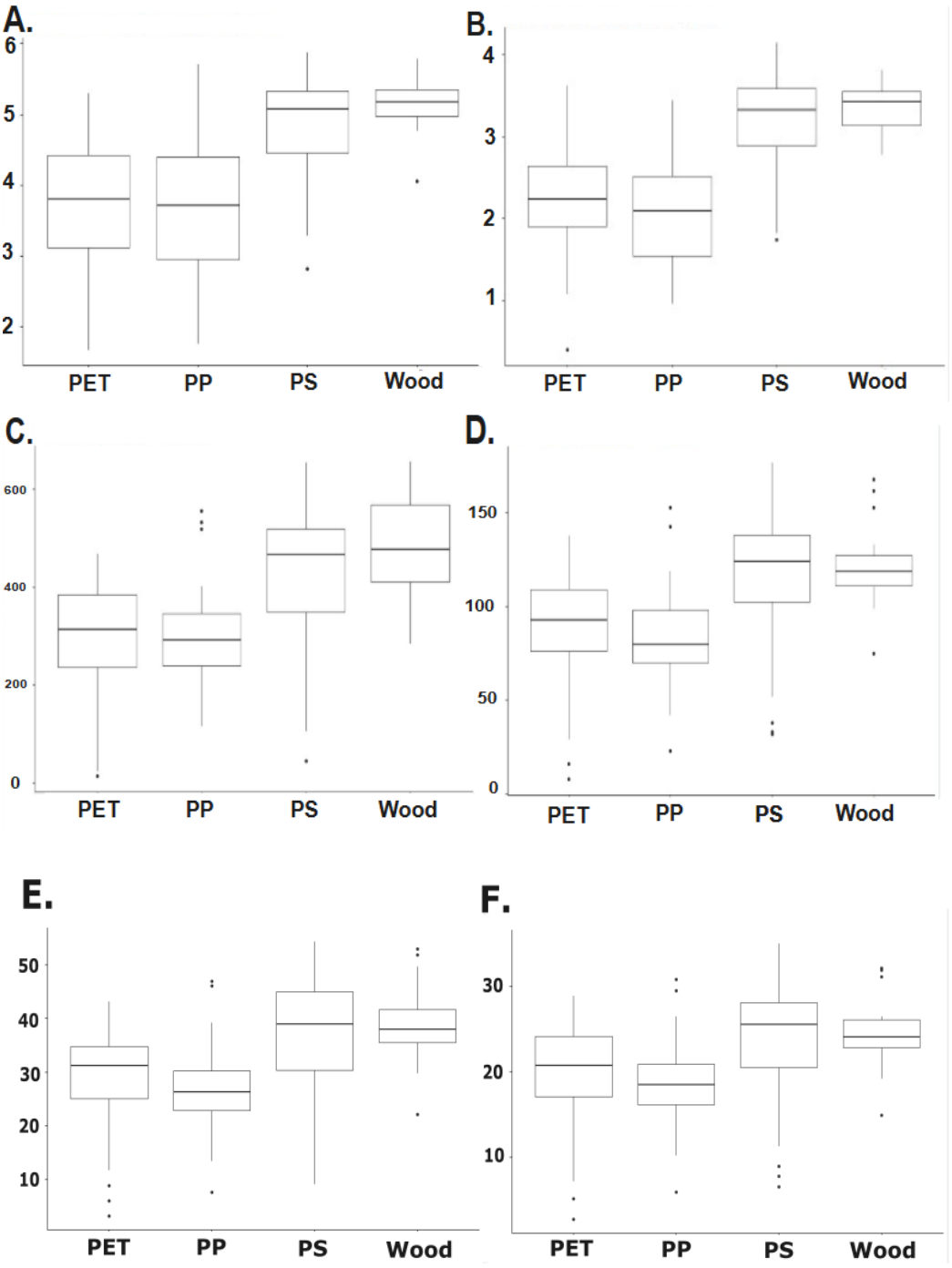


Figure 6. Shannon, Chao1 and Faith's Phylogenetic diversity (PD) indices to depict changes in the alpha diversity for PET, PP, PS and wood substrates. **A, C, E** shows ASV level of classification and **B, D, F** shows family level of classification.

Beta Diversity

Based on the heatmap via non-metric multidimensional scaling (NMDS) method, PET and PP substrates demonstrated similar overall OTU abundances for all three months at OTU level. However, OTU composition varied by time for both PET and PP as the OTU patterns visibly shifted for the three months illustrating the possibility of secondary colonization **(Figure 7)**. Simultaneously, OTUs differed between deployed plastic samples and pre-existing samples for both PET and PP across the sampling duration. In our study, single samples and rafts did not show much difference for any plastic type or month **(Figure 7)**. PS showed similar OTU patterns in June and August as PET and PP, however, July and August compositions were visually distinct for all the substrates. Unique OTU composition was observed in the case of wood **(Figure 7)**. At the genus level, similar trends were observed for all the substrates (supplementary material).

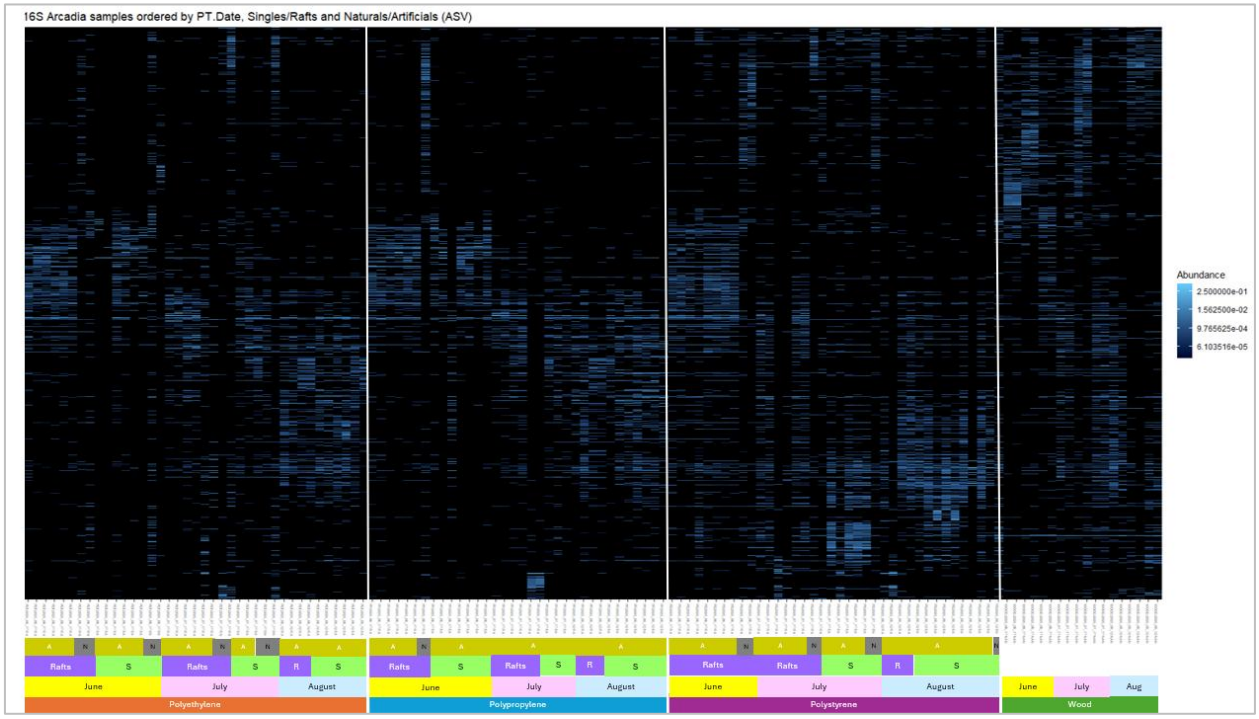


Figure 7. ASV level heatmap depicting PET, PP, PS and wood sampled in June, July and August with their corresponding singles/rafts and naturals/artificials. The genus level heatmap is provided in the supplementary figure 3.

Principal Coordinates Analysis (PcoA) plots based on Bray Curtis, Jaccard and UniFrac revealed clear spatial separation in microbial community composition driven predominantly by factors collection date, substrate, artificial/naturals, substrate+artificials/naturals and substrate+singles/rafts **(Figure 8)**. No statistical differences were obtained for weighted UniFrac **(supplementary figures 4-6)**. Clustering patterns for substrate analysis showed PET overlapping with PP, and PS strongly overlapping with wood **(Figure 8A)**. Based on time, microbial communities in June were significantly different from July and August **(Figure 8B)**. Also, artificially deployed samples displayed significant differences with pre-existing plastics **(Figure 8C)**. Distinct clustering patterns for substrate+collection date could be observed, for example our June collection of PP, PS and wood clustered together. Similarly, PET and PP samples in August shared a common distribution along with PP July. In addition, PS in August showed similar patterns as wood in July. However, PET collection in July remained separate from all other samples. Distinct microbial communities were also observed on PET, PS and wood in June, July and August respectively **(Figure 8D)**. On the basis of substrate+artificial/natural, PET and PP artificials did not differ significantly, but remained isolated from natural samples of all substrates and PS artificials as well **(Figure 8E)**. Both Bray-Curtis and Jaccard for substrate+singles/rafts ordination plot revealed overlapping between wood and PS singles; PET and PP singles and rafts microbial communities strongly overlapped. However, PS rafts remained isolated from either group except UniFrac analysis where PS singles and wood diverged **(Figure 8F and supplementary figures 4-6)**.

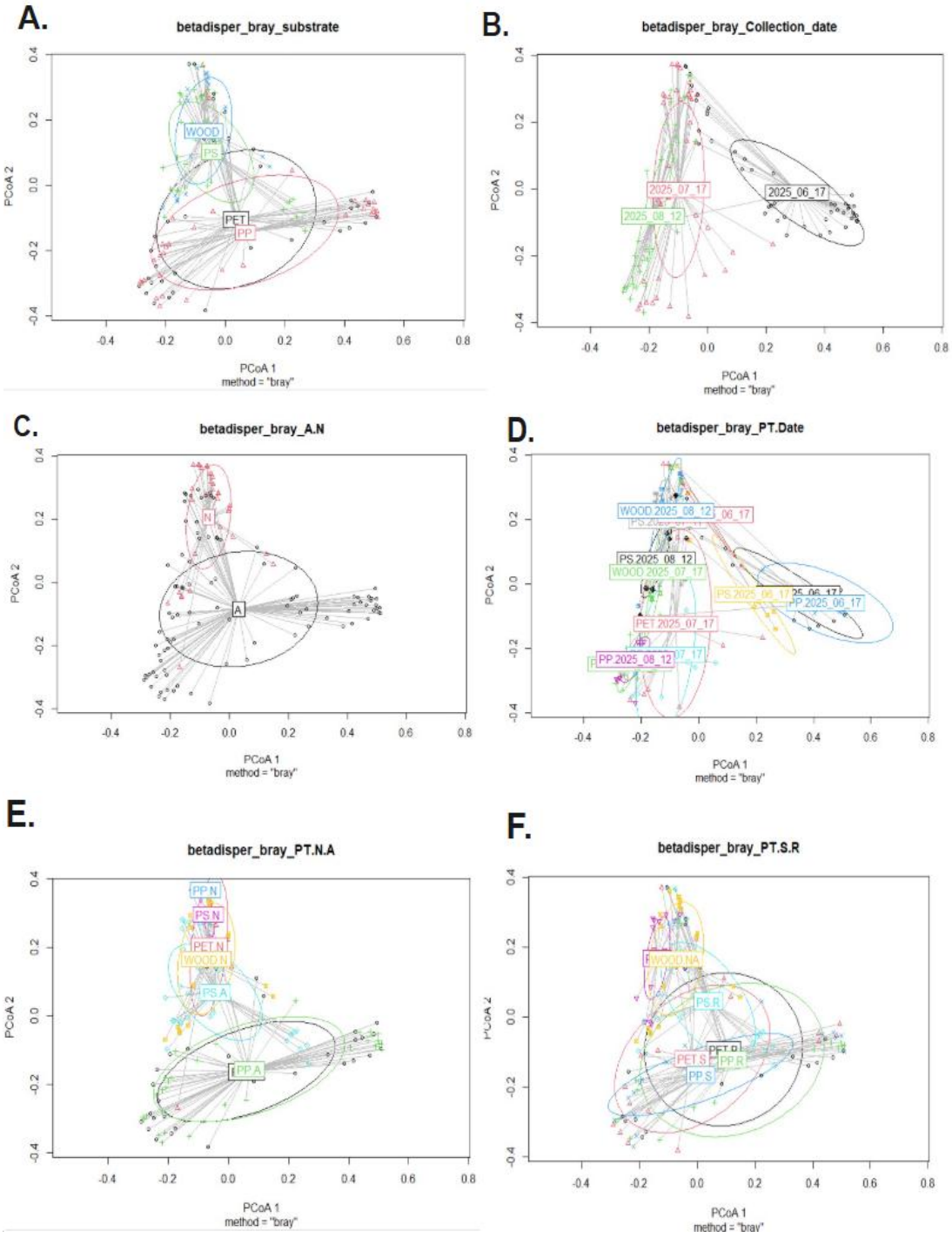


Figure 8. Bray-Curtis ordination plots for different variables at ASV level including **A.** substrate, **B.** collection date, **C.** artificials/naturals, **D.** substrate+collection date, **E.** substrate+artificials/naturals and **F.** substrate+singles/rafts. Jaccard, UniFrac and weighted UniFrac plots at ASV level are provided in the supplementary figures 4-6.

PERMANOVA Analysis

In PERMANOVA analysis for differences in centroid location, all factors tested (collection date, site, substrate, artificials/naturals, rafts/singles and $\log_{10}$[read depth], year) were recovered as significant for all comparisons (all *p*-values <0.010), with the exception of the factor $\log_{10}$ (read depth) for the weighted UniFrac metric. Collection date (time) consistently had the highest R^2 value across all comparisons followed by substrate and artificials/naturals. On average, R^2 for the collection date was 1.4875-fold higher than the substrate **(Table 2)**.

Table 2. PERMANOVA statistical analyses results showing collection date followed by substrate and artificials/naturals as the main factors influencing microbial communities.

	Df	Sum Of Sqs	R2	F	Pr(>F)
Substrate	**3**	**3.630**	**0.07555**	**4.4000**	**0.001**
Singles/Rafts	1	0.620	0.01291	2.2551	0.002
Artificials/Naturals	**1**	**2.132**	**0.04438**	**7.7539**	**0.001**
Collection_date	**2**	**5.815**	**0.12102**	**10.5727**	**0.001**
Site	2	1.135	0.02363	2.0643	0.001
Log10_read_depth	1	0.892	0.01856	3.2435	0.001
Residual	118	32.452	0.67534		
Total	128	48.052	1.00000		

	Adonis (Beta diversity)			
Variables	Bray-Curtis	Jaccard	UniFrac	wUniFrac
Substrate				
Collection date				
Singles/Rafts				
Artificials/naturals				
Substrate+collection date				
Substrate+artificials/naturals				

Figure 9. Significant results for PERMANOVA or centroid comparisons. Red boxes indicate significant results. All variables showed significant results for all the beta diversity measures.

Centroid Comparisons, Substrate

Centroid analysis for substrates gave significant results (*p*-value) across all beta-diversity measures. Pairwise comparisons for PET and PP, at ASV and genus level, showed significant differences with PS and wood. In addition, wood showed significant differences for PS **(Figure 9)**.

Centroid Comparisons, Collection Date

For all the beta measures, collection date was recovered to be significant. Tukey HSD pairwise comparisons revealed that three sampling months showed distinct microbial communities when compared to each other **(Figure 9)**.

Centroid Comparisons, Artificials/Naturals

Artificially deployed samples showed distinct microbial communities compared to pre-existing plastic substrates, also confirmed by Tukey analysis **(Figure 9)**.

Centroid Comparisons, Substrate+Collection Date

Combination factor substrate+collection date revealed significant differences for all beta diversity measures. Pairwise comparisons showed that PET June collections were significantly different from every other substrate irrespective of sampling month except PP June collection. PET samples in July were recovered as significantly different than PET and PP August collection along with PP June. PET August samples were significantly different PP August only. PP collections were different from each other for the three months. Furthermore, PS August was significant for all the samples for the sampling months except PET June. PS June collections showed significant differences with all wood samples **(Figure 9)**.

Centroid Comparisons, Substrate+Artificials/Naturals

Centroid comparisons for substrate+artificials/naturals revealed significant differences. Tukey HSD pairwise analysis demonstrated naturals were mostly significant when compared to artificial irrespective of the substrate type. In addition, PS artificials also showed significant differences with wood samples **(Figure 9)**.

	ANOVA (Beta diversity)			
Variables	Bray-Curtis	Jaccard	UniFrac	wUniFrac
Substrate				
Collection date				
Singles/Rafts				
Artificials/naturals				
Substrate+collection date				
Substrate+singles/rafts				
Substrate+artificials/naturals				

Figure 10. ANOVA significant results for different variables. Red boxes indicate significant results. Only wUniFrac measure recovered substrate, collection date, substrate+collection date, substrate+singles/rafts and substrate+artificials/naturals as significant.

Dispersion Comparisons, Substrate

Out of four beta diversity metrics, only weighted UniFrac ANOVA analysis recovered a significant *p*-value for substrate (*p*-value= 2.52×10^{-4}; F= 6.8778). Subsequently, pairwise comparisons for wUniFrac returned as significant for PET against all substrates except PP. Additionally, all PS and wood pairwise comparisons with PET and PP were recovered as significant for weighted UniFrac **(Figure 10)**.

Dispersion Comparisons, Collection Date

Collection date showed significant *p*-value only for weighted UniFrac measure (*p*-value= 0.04; F= 3.0967). Tukey HSD pairwise comparisons showed significant differences between June and July microbial communities irrespective of the substrate type **(Figure 10)**.

Dispersion Comparisons, Substrate+Collection Date

Only weighted UniFrac ANOVA analysis for substrate+collection date was recovered as significant (*p*-value= $1.2x10^{-4}$; F=3.7726). Tukey HSD pairwise comparisons for microbial communities showed significant differences between substrates PET and PS in August. Microbial communities on PS in August also differed with PP in June and August along with wood in June **(Figure 10)**.

Dispersion Comparisons, Substrate+Singles/Rafts

Only weighted UniFrac ANOVA analysis for substrate+singles/rafts was recovered as significant (*p*-value= $9.4x10^{-4}$; F=4.0644). Pairwise Tukey analysis revealed significant differences between PS (singles+rafts) and PET and PP singles **(Figure 10)**.

Dispersion Comparisons, Substrate+Aritificials/Naturals

Substrate coupled with artificials/naturals recovered a significant *p*-value (*p*-value= 0.001-$2.2x10^{-16}$; F=3.8-23.0) across all beta diversity measures. For instance, PP naturals were found to be different from PP artificials. In addition, PS and PET artificials and naturals also showed significant differences with PP naturals simultaneously. Specifically, for UniFrac pairwise comparisons, positive differences were observed between PP and PET artificials and naturals both with wood samples. Lastly, UniFrac analysis also showed significant differences between PS and PP naturals **(Figure 10)**.

ANOSIM

ANOSIM values were found to be significant (*p*-value<0.01) across all the measures for collection date, substrate, substrate+collection date, substrate+site, substrate+artificials/naturals and

substrate+singles/rafts. However, combination factor substrate+collection date was recovered to have the most coherent communities (average= 0.32) followed by artificials/naturals (average=0.25), collection date (average=0.24) and substrate (average=0.14) according to the Bray–Curtis, Jaccard, and UniFrac metrics. Additionally, artificials/naturals was not significant for weighted UniFrac. Finally, UniFrac and weighted UniFrac gave significant differences for singles and rafts **(Figure 11)**.

	ANOSIM (Beta diversity)			
Variables	Bray-Curtis	Jaccard	UniFrac	wUniFrac
Substrate	0.17	0.17	0.16	0.09
Collection date	0.28	0.28	0.27	0.13
Singles/Rafts	0.01	0.01	0.03	0.05
Artificials/naturals	0.34	0.34	0.28	0.04
Substrate+collection date	0.37	0.37	0.35	0.19
Substrate+singles/rafts	0.19	0.19	0.19	0.13
Substrate+artificials/naturals	0.31	0.31	0.27	0.16

Figure 11. The significant results for ANOSIM analyses. Red boxes indicate significant results. Combination factor of substrate and collection date showed the maximum coherent microbial communities composition followed by artificials/ naturals, collection date and substrate.

DISCUSSION

Carpenter et al. (1972) and Carpenter & Smith. (1972) first described the presence of microbial communities on plastic. Since then, numerous studies have focused on plastisphere colonization on diverse plastics. In our study, we investigated freshwater plastisphere communities colonizing PET, PP, PS and wood. Alpha and beta diversity results illustrate the impact of substrate (i.e., plastic type) as a driving factor in plastisphere communities succession. PS and wood showed similar levels of microbial diversity which was found to be higher when compared to PET and PP. The latter plastic groups showed similar levels of microbial diversity when compared to each other. Furthermore, beta diversity also revealed the influence of sampling time and highlighted the substantial potential for secondary colonization by plastisphere microbial communities. For instance, the microbial pattern gradually shifted from June to August for PET and PP. PS was relatively similar to PET and PP except for July. Finally, wood showed unique microbial community composition for all three sampling months.

Across 3,832 OTUs, Leptolyngbyaceae was found to be the most abundant family on all the substrates. These results were consistent with previous studies where Leptolyngbyaceae was described as one of the most abundant families on PET, polylactic acid (PLA) and Low-density polyethylene (LDPE) sampled in freshwater ecosystems (Bhagwat et al., 2021; Di Pippo et al., 2023; Johansen et al., 2011). According to Johansen et al., 2011, Leptolyngbyaceae, a polyphyletic assemblage, has been recovered from a plethora of sites including soils, lakes and ponds, thermal springs, saline inland waters, and marine habitats (De Philippis et al., 2002; Gkelis et al., 2016; J. Li et al., 2018; Y. Li et al., 2020). Previously, diverse strains of Leptolyngbyaceae were also isolated from polyhydroxy borate (PHB) surface in freshwater ecosystems (Lage et al., 2025).

Class Cyanobacteria, in general, is known to be among the primary colonizers, producing extracellular polymeric substance which aid in cell adhesion and secondary colonization (Di Pippo et al., 2023). Leptolyngbyaceae as a pathogen, has been identified in marine environments, from the coral black band disease (Bryant et al., 2016; Cholewińska et al., 2025). Hence, the pathogenic potential of Leptolyngbyaceae family is worth additional study.

Rhodobacteraceae was the most abundant family on all the plastic types in June which was found to be consistent with Salta et al., (2013) where Rhodobacterales was reported as the primary colonizer given its capability to survive on low-nutrient substrates (Salta et al., 2013; Aybar et al., 2022). Rhodobacter has also been associated with biodegradable polymers and might play a pertinent role in plastic degradation at deep sea level depending on incubation time, station and plastic type (Esposito et al., 2025; Liu et al., 2025; Vaksmaa et al., 2021). Nevertheless, more research needs to be conducted to validate the plastic-degrading ability of family Rhodobacteraceae. Additionally, the presence of Rhodobacter on PET and LDPE substrates indicates that plastisphere communities may harbor bacteria capable of hydrogen metabolism, photoheterotrophy, or even xenobiotic degradation (Sun et al., 2023).

Generally, Alphaproteobacteria is often recovered as the most predominant class especially associated with PE, PP, PCL and wood (Barros & Seena, 2021; Bhagwat et al., 2021; L. Wang et al., 2021; Zettler et al., 2013) which is consistent with our research. Another member of the Alphaproteobacteria class, Sphignomonadaceae, was also one of the most abundant families especially enriched on PET and PP. Sphignomonadaceae represent important microbial associations within the riverine plastisphere community reported previously on poly-β-hydroxy butyrate Co-β-hydroxyvalerate (PHBV) in freshwater bodies (Nguyen et al., 2021, 2023).

Members of the *Sphingomonadaceae*, such as *Erythrobacter* sp., and *Rhodobacteraceae* were regularly associated with plastics (Bryant et al., 2016; Didier et al., 2017). Additionally, this family is considered key members for biofouling on different surfaces in marine environments (Elifantz et al., 2013; Oberbeckmann & Labrenz, 2026). Family Rhodobacteraceae has also been reported to degrade PET in biofilms (Oberbeckmann et al., 2016). Furthermore, Nostocaceae, the third most abundant family in our study also belongs to Class Cyanobacteria like Leptolyngbyaceae. Consistent with previous studies, Nostocaceae was one of the most abundant phototrophic bacteria associated with plastisphere in freshwater (Zancarini et al., 2017; Zhai et al., 2023). Nostocales have been reported to play an important role in atmospheric nitrogen fixation; however, Nostocaceae has also been involved with toxic blooms in aquatic ecosystems (Galhano et al., 2011).

Comamonadaceae was also recovered to be abundant on all substrates in our research reflective of Comamonadaceae enrichment on PET and LDPE microplastics in both marine and freshwater studies (Parida et al., 2026). Comamonadaceae are associated with metabolic versatility and play an important roles in organic matter degradation and biofilm stability. High abundance of Comamonadaceae has also been observed on undefined surface organic materials (A. R. McCormick et al., 2016). Similar to Rhodobacteraceae, Comamonadaceae has shown to degrade PET however, further research is required to identify its role in degradation of plastics. In contrast, previous studies have reported that PET was abundant with Betaproteobacteria such as Burkholderiales and Rhodocyclales orders (Parida et al., 2026), diverging from our research where Alphaproteobacteria and Cyanobacteria were among the most abundant classes. Our research revealed Micromonosporaceae to be abundant especially on wood, aligning with previous

research, Micromonosporaceae (Actinobacteria) is usually found on organic material in freshwater due to their ability to degrade complex polysaccharides, such as chitin and cellulose thereby recycling organic matter (de Menezes et al., 2012). However, this is in contrast with Bhagwat et al., (2021) and Muthukrishnan et al., (2019) where wood hosted Gammaproteobacteria and Bacteroidetes as the most prevalent communities.

Regression analyses for alpha diversity conducted at ASV, family, and genus levels (**Figure 4.** and supp. material) showed differing levels of microbial diversity based on substrate. In our research, wood showed the maximum diversity followed by PS, PP and PET, which is in contrast with Gebreyohanes Belay et al., (2026) where plastic type did not influence the plastisphere colonization at all. Moreover, PET water bottles and PP falcon tubes revealed similar Shannon, Chao1 and Faith's phylogenetic diversity. PP plastic was abundant with classes Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Flavobacteriia, Acidimicrobiales, and Anaerolineae. At family level, Aaerolineae, Oscillatoriophycideae, Synechococcophycideae, Sphingomonadaceae, Rhodobacteraceae and Burkholderiaceae were prevalent on PP while PET hosted Comamonadaceae, Flavobacteriaceae, Rhodocyclaceae, Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, Oxyphotobacteria and Verrucomicrobiae. A significant amount of research has been done on PET (W. Li et al., 2023), PP (Miao et al., 2019; Tulloch et al., 2024; L. Wang et al., 2021; Zhang et al., 2022) and PS (Bhagwat et al., 2021; Didier et al., 2017; Kirstein et al., 2019) individually. Interestingly, our analysis revealed that PS and wood supported similar levels of microbial diversity, both of which were higher than those observed on PET and PP. To our knowledge, this pattern has not been previously reported in existing literature, although, wood has been characterized by earlier research to host

higher alpha diversity than plastics due to pits and cracks leading to greater surface heterogeneity (Miao et al., 2019; Parida et al., 2026; Shen et al., 2021).

Combination factors including substrate+collection date, substrate+naturals/artificials and substrate+singles/rafts showed significant results at ASV level (p-value <0.05). However, individual factors such as collection date, site, singles/rafts did not show much significance implying that substrate was the governing factor. In contrast, Vincent et al., (2022), conveyed that substrate is not a driving factor in shaping microbial communities. Hence, future studies should evaluate whether the microbial diversity pattern observed among PET, PP, PS, and wood are consistent across broader geographic regions, seasons, and environmental conditions. Incorporating longer deployment periods and naturally-aged substrates would provide greater insight into microbial succession and the role of substrate history in shaping plastisphere communities.

Preliminary beta diversity analysis **(Figure 7)** revealed that along with substrate, sampling time also influenced succession among plastisphere microbial communities. This can be attributed to adsorption of organic and inorganic materials and aging of plastics (Lee et al., 2008; Martínez-Campos et al., 2021; Silva et al., 2023). Based on impact of sampling time, many previous studies have supported Alphaproteobacteria and Betaproteobacteria as plastisphere initial colonizers predominantly on PP, PVC, PS and PCL (Bhagwat et al., 2021; Nguyen et al., 2023; Oberbeckmann et al., 2018; Yang et al., 2023). In general, Rhodobacteriaceae was the most abundant group within 2-4 weeks of biofilm development on HDPE, similar to our research (Amaral-Zettler et al., 2015, 2020), while Rhodospirillaceae, Flavobacteriaceae and Xanthomonadales have been reported prevalent at 8-16 weeks (Jacquin et al., 2019). In contrast,

Xu et al. (2019) reported Proteobacteria was abundant irrespective of sampling time and Bacteroidetes was abundant until four-months of sampling. Eventually, abundance of Rhodobacteraceae was reduced during week 4 on PVC. Consistent with our results, Silva et al. (2023) revealed a clear shift in microbial composition from week 1 to 4 for HDPE, LDPE, PP, PS, PVC and PET. Genera *Granulosicoccus*, *Thiogranum*, *Candidatus Amoebophilus*, *Sphingorhabdus* and an unclassified genus (family Ectothiorhodospiraceae) enriched through week 2 and 3 on HDPE, LDPE, PP and PS; however, their abundance decreased by week 4.. Rhodobacter also includes purple sulfur bacteria, known to fix N_2, providing a critical nitrogen source for the biofilm succession. Secondary colonization was also reported by Tu et al. (2020) where the development of families Flavobacteriaceae (Bacteroidia), Rhodobacteraceae (Alphaproteobacteria), and Microtrichaceae (Acidimicrobiia) on PE within 30 days of incubation was observed, which was overtaken by Moraxellaceae (Gammaproteobacteria) and Bacillaceae (Bacilli) groups after 75 days. After 135 days, the dominance shifted to Flavobacteriaceae (Bacteroidia), with a significant increase of Microtrichaceae (Acidimicrobiia), Pirellulaceae (Planctomycetes), and Rhodobacteraceae (Alphaproteobacteria) (Dey et al., 2022). According to Gebreyohanes Belay et al. (2026) the plastisphere on PE, PS, HDPE, PP and PS was initially dominated by phyla Proteobacteria, Planctomycetes, Bacteroidota, Actinobacteriota and Cyanobacteria in freshwater. In some cases, substrate specificity was observed on PS, PLA and HDPE samples that were found to be abundant with Desulfobacterota between weeks 4 and 8. This trend shifted to Verrucomicrobiota and Cyanobacteria with decrement in Proteobacteria and Firmicutes across all plastic samples from week 8 to week 12. It has been proposed that secondary colonization results due to alteration of the hydrophobicity of the surface of plastics (Dang et al.,

2000b; Zhu et al., 2023). However, further research needs to be conducted to characterize microbial succession coupled with elongated sampling durations in freshwater specifically.

In our study, both alpha and beta diversity results showed that pre-existing plastics were significantly different than artificially deployed samples. This has been consistent with the previous research conducted by Amaral-Zettler et al. (2020), Erni-Cassola et al. (2020) and Zettler et al. (2013), where the weathering process (cracks, pits and increased surface roughness) changes the plastic surface providing increased surface area and that directly correlates with greater diversity of microbial communities. However, most plastisphere studies have focused either on experimentally deployed plastics to investigate microbial succession or on naturally weathered environmental plastics to characterize mature biofilms. Therefore, direct comparisons between pre-existing environmental plastics and artificially deployed plastics under the same environmental conditions remain scarce, limiting our understanding of how microbial communities differ between newly colonizing and mature plastisphere assemblages. The beta diversity analysis including ANOVA and PERMANOVA further showed significant results for substrate impact on rafts versus isolated plastics, but to the best of our knowledge, this aspect presents a knowledge gap in existing literature because no comparisons have been made among plastisphere microbial communities based on individual sampling or accumulated plastic substrates. Lastly, our findings revealed that microbial communities on pre-existing and artificially deployed PP samples were highly variable and distinct from communities associated with other substrates. Previous studies have reported the most abundant taxa enriched on PP although these taxa were not exclusive to it (Di Pippo et al., 2022; Tu et al., 2020). Therefore, additional research is required to identify and characterize plastic-specific microbial communities.

CONCLUSION

Our freshwater plastisphere experiment presents comprehensive evidence suggesting the impact of substrate and time in shaping the microbial communities on diverse plastic types. Our application of high-throughput 16S rDNA sequencing revealed Leptolyngbyaceae as the most abundant family on all the substrates for June, July, and August 2025. Substrate specificity was observed in the family Rhodobacteraceae which were abundant on PET and PP plastic types when compared to PS and wood across all sampling months. Researchers have shown Rhodobacteraceae play a promising role in plastic degradation of PHA and PLA, which further needs to be characterized for additional common plastic types including PET, PP, PE and PS. Moreover, families such as Cyanobiaceae and Deinococcaceae, known to play a key role in carbon and nitrogen regulation, were discriminant against PS. Our study also revealed families specific to wood substrate compared to plastics, including JG30-KF-CM45, Nostocaceae and Micromonosporaceae. These families have been reported to be more prevalent on high-cellulose substrates. Furthermore, July and August revealed abundance of Comamonadaceae and Nostocaceae especially on PET and PP respectively, highlighting the influence of sampling time. Alpha diversity analysis revealed that PET and PP showed similar levels of microbial diversity while PS was similar to wood. Beta diversity analysis further supported our alpha diversity findings. Weighted UniFrac ANOVA analysis revealed significant results for individual factors such as substrate, collection date and combination factors substrate+collection date and substrate+single/rafts. Additionally, ANOVA analysis for combination factor substrate+artificial/naturals was recovered to be significant for all beta diversity measures including Bray-Curtis, Jaccard, UniFrac and wUniFrac. Finally, PERMANOVA analysis

demonstrated significant results for collection date (time) establishing the importance of time in shaping plastisphere microbial communities along with substrate. Future efforts should include longer incubation periods to more fully characterize the potential plastic-specific microbial communities. Since our samples all come from the same locale, our study did not reveal the vital impact of site on plastisphere succession which further needs to be investigated by future researchers.

SUMMARY

Our research supported the influence of substrate and time on the plastisphere microbial communities. Taxonomic characterization revealed various ecology important families playing pertinent roles in biofouling, nitrogen fixation, hydrogen metabolism and degradation of plastics. We found abundance of the family Leptolyngbyaceae on all plastic types and wood resonating with Bhagwat et al. (2021), Di Pippo et al. (2023) and Johansen et al. (2011). Leptolyngbyaceae was found to be the primary colonizer likely due to the ability to secrete of extra polymeric substance (EPS) (Johansen et al., 2011; Lage et al., 2025). Another family, Rhodobacteraceae, was among the primary colonizers attributed to its capability to survive in low-nutrient environments and was predominantly abundant in June consistent with Salta et al. (2013). Rhodobacteraceae has been reported to play an important role in biodegradation of plastics at deep levels in the aquatic ecosystems (Esposito et al., 2025; Liu et al., 2025; Vaksmaa et al., 2021). Family Sphignomonadaceae was found to be enriched on PET and PP. Sphignomonadaceae has been reported as key members for biofouling on various surfaces on marine environments (Elifantz et al., 2013; Oberbeckmann & Labrenz, 2026). Furthermore, Nostocaceae was abundant on PET and PP. According to (Galhano et al., 2011), Nostocaceae has been involved in nitrogen fixation in aquatic water bodies. Additionally, Comamonadaceae was also enriched on all plastic substrates. Comamonadaceae has been associated with organic matter degradation and biofilm stability (A. R. McCormick et al., 2016). Finally, Micromonosporaceae was specifically abundant on wood. This result aligned with De Menezes et al. (2008), attributed to its ability to degrade complex polysaccharide.

Our alpha diversity results revealed novel findings where PS and wood showed similar level of microbial diversity and higher when compared to PET and PP microbial diversity level which showed similar level of microbial diversity. Since this phenomenon has not been documented previously, determining the underlying cause of these patterns remains difficult. However, studies by Oberbeckmann et al. (2016, 2018) suggest that nutrient availability and adsorption onto plastic surfaces may influence surface-specific microbial attachment and community diversity, highlighting an important area for future research. Also, substrate surface area might play role in hosting higher microbial diversity due to pits and cracks leading to greater surface heterogeneity (Miao et al., 2019; Parida et al., 2026; Shen et al., 2021).

Beta diversity results revealed both substrate and time were the influencing factors in microbial composition. Through our research, we highlight the potential difference in initial and secondary colonizers. Classes Alphaproteobacteria and Betaproteobacteria as plastisphere initial colonizers predominantly on PP, PVC, PS and PCL (Bhagwat et al., 2021; Nguyen et al., 2023; Oberbeckmann et al., 2018; Yang et al., 2023). In general, Rhodobacteraceae was the most abundant group within 2-4 weeks of biofilm development on HDPE, similar to our research (Amaral-Zettler et al., 2015, 2020), while Rhodospirillaceae, Flavobacteriaceae and Xanthomonadales have been reported prevalent at 8-16 weeks (Jacquin et al., 2019).

Finally, both alpha and beta diversity analyses revealed that pre-existing plastics harbored significantly different microbial communities compared with artificially deployed samples. This finding is consistent with previous studies by Amaral-Zettler et al. (2020), Erni-Cassola et al. (2020), and Zettler et al. (2013), which reported that weathering processes, including the formation of cracks, pits, and increased surface roughness, alter the physical properties of plastic surfaces.

These changes increase the available surface area for microbial colonization, thereby promoting greater microbial diversity and shaping distinct community compositions. However, most plastisphere studies have focused either on experimentally deployed plastics to investigate microbial succession or on naturally weathered environmental plastics to characterize mature biofilms. Therefore, direct comparisons between pre-existing environmental plastics and artificially deployed plastics under the same environmental conditions remain scarce, limiting our understanding of how microbial communities differ between newly colonizing and mature plastisphere assemblages.

REFERENCES

Amaneesh, C., Anna Balan, S., Silpa, P. S., Kim, J. W., Greeshma, K., Aswathi Mohan, A., Robert Antony, A., Grossart, H. P., Kim, H. S., & Ramanan, R. (2023). Gross Negligence: Impacts of Microplastics and Plastic Leachates on Phytoplankton Community and Ecosystem Dynamics. In *Environmental Science and Technology* (Vol. 57, Number 1, pp. 5–24). American Chemical Society. https://doi.org/10.1021/acs.est.2c05817

Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. In *Nature Reviews Microbiology* (Vol. 18, Number 3, pp. 139–151). Nature Research. https://doi.org/10.1038/s41579-019-0308-0

Amaral-Zettler, L. A., Zettler, E. R., Slikas, B., Boyd, G. D., Melvin, D. W., Morrall, C. E., Proskurowski, G., & Mincer, T. J. (2015). The biogeography of the Plastisphere: Implications for policy. *Frontiers in Ecology and the Environment*, *13*(10), 541–546. https://doi.org/10.1890/150017

Aminov, R. I. (2009). The role of antibiotics and antibiotic resistance in nature. In *Environmental Microbiology* (Vol. 11, Number 12, pp. 2970–2988). https://doi.org/10.1111/j.1462-2920.2009.01972.x

Anderson, A. G., Grose, J., Pahl, S., Thompson, R. C., & Wyles, K. J. (2016). Microplastics in personal care products: Exploring perceptions of environmentalists, beauticians and students. *Marine Pollution Bulletin*, *113*(1–2), 454–460. https://doi.org/10.1016/j.marpolbul.2016.10.048

Andrady, A. L., & Neal, M. A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1977–1984. https://doi.org/10.1098/rstb.2008.0304

Arias-Andres, M., Kettner, M. T., Miki, T., & Grossart, H. P. (2018). Microplastics: New substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems. *Science of the Total Environment*, *635*, 1152–1159. https://doi.org/10.1016/j.scitotenv.2018.04.199

Aybar, C. A. V., Dantur Juri, M. J., Santana, M., Lizarralde De Grosso, M. S., & Spinelli, G. R. (n.d.). The spatio-temporal distribution patterns of biting midges of the genus Culicoides in Salta province, Argentina. In *Number 145 Journal of Insect Science* (Vol. 12). Retrieved http://www.insectscience.org/12.145

Azevedo-Santos, V. M., Brito, M. F. G., Manoel, P. S., Perroca, J. F., Rodrigues-Filho, J. L., Paschoal, L. R. P., Gonçalves, G. R. L., Wolf, M. R., Blettler, M. C. M., Andrade, M. C., Nobile, A. B., Lima, F. P., Ruocco, A. M. C., Silva, C. V., Perbiche-Neves, G., Portinho, J. L., Giarrizzo, T., Arcifa, M. S., & Pelicice, F. M. (2021). Plastic pollution: A focus on freshwater biodiversity. In *Ambio* (Vol. 50, Number 7, pp. 1313–1324). Springer Science and Business Media B.V. https://doi.org/10.1007/s13280-020-01496-5

Barboza, L. G. A., Cózar, A., Gimenez, B. C. G., Barros, T. L., Kershaw, P. J., & Guilhermino, L. (2019). Macroplastics pollution in the marine environment. In *World Seas: An*

Environmental Evaluation Volume III: Ecological Issues and Environmental Impacts (pp. 305–328). Elsevier. https://doi.org/10.1016/B978-0-12-805052-1.00019-X

Barnett, D., Arts, I., & Penders, J. (2021). microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software*, *6*(63), 3201. https://doi.org/10.21105/joss.03201

Barros, J., & Seena, S. (2021a). Plastisphere in freshwaters: An emerging concern. In *Environmental Pollution* (Vol. 290). Elsevier Ltd. https://doi.org/10.1016/j.envpol.2021.118123

Barros, J., & Seena, S. (2021b). Plastisphere in freshwaters: An emerging concern. In *Environmental Pollution* (Vol. 290). Elsevier Ltd. https://doi.org/10.1016/j.envpol.2021.118123

Bellou, N., Papathanassiou, E., Dobretsov, S., Lykousis, V., & Colijn, F. (2012). The effect of substratum type, orientation and depth on the development of bacterial deep-sea biofilm communities grown on artificial substrata deployed in the Eastern Mediterranean. *Biofouling*, *28*(2), 199–213. https://doi.org/10.1080/08927014.2012.662675

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. In *Behavioural Brain Research* (Vol. 125). www.elsevier.com/locate/bbr

Bhagwat, G., Zhu, Q., O'Connor, W., Subashchandrabose, S., Grainge, I., Knight, R., & Palanisami, T. (2021). Exploring the Composition and Functions of Plastic Microbiome Using Whole-Genome Sequencing. *Environmental Science and Technology*, *55*(8), 4899–4913. https://doi.org/10.1021/acs.est.0c07952

Blettler, M. C. M., Abrial, E., Khan, F. R., Sivri, N., & Espinola, L. A. (2018a). Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. In *Water Research* (Vol. 143, pp. 416–424). Elsevier Ltd. https://doi.org/10.1016/j.watres.2018.06.015

Blettler, M. C. M., Abrial, E., Khan, F. R., Sivri, N., & Espinola, L. A. (2018b). Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. In *Water Research* (Vol. 143, pp. 416–424). Elsevier Ltd. https://doi.org/10.1016/j.watres.2018.06.015

Blettler, M. C. M., Ulla, M. A., Rabuffetti, A. P., & Garello, N. (2017). Plastic pollution in freshwater ecosystems: macro-, meso-, and microplastic debris in a floodplain lake. *Environmental Monitoring and Assessment*, *189*(11). https://doi.org/10.1007/s10661-017-6305-8

Blettler, M. C. M., & Wantzen, K. M. (2019). Threats Underestimated in Freshwater Plastic Pollution: Mini-Review. In *Water, Air, and Soil Pollution* (Vol. 230, Number 7). Springer International Publishing. https://doi.org/10.1007/s11270-019-4220-z

Bocci, V., Galafassi, S., Levantesi, C., Crognale, S., Amalfitano, S., Congestri, R., Matturro, B., Rossetti, S., & Di Pippo, F. (2024). Freshwater plastisphere: a review on biodiversity, risks, and biodegradation potential with implications for the aquatic ecosystem health. In *Frontiers in Microbiology* (Vol. 15). Frontiers Media SA. https://doi.org/10.3389/fmicb.2024.1395401

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. In *Nature Biotechnology* (Vol. 37, Number 8, pp. 852–857). Nature Publishing Group. https://doi.org/10.1038/s41587-019-0209-9

Bryant, J. A., Clemente, T. M., Viviani, D. A., Fong, A. A., Thomas, K. A., Kemp, P., Karl, D. M., White, A. E., & DeLong, E. F. (2016a). Diversity and Activity of Communities Inhabiting Plastic Debris in the North Pacific Gyre. *MSystems*, *1*(3). https://doi.org/10.1128/msystems.00024-16

Bryant, J. A., Clemente, T. M., Viviani, D. A., Fong, A. A., Thomas, K. A., Kemp, P., Karl, D. M., White, A. E., & DeLong, E. F. (2016b). Diversity and Activity of Communities Inhabiting Plastic Debris in the North Pacific Gyre. *MSystems*, *1*(3). https://doi.org/10.1128/msystems.00024-16

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Carpenter, E. J., Anderson, S. J., Harvey, G. R., Miklas, H. P., & Peck, B. B. (1972). Polystyrene spherules in coastal waters. *Science*, *178*(4062), 749–750. https://doi.org/10.1126/science.178.4062.749

Carpenter, E. J., & Smith, K. L. (1972). Plastics on the Sargasso sea surface. *Science*, *175*(4027), 1240–1241. https://doi.org/10.1126/science.175.4027.1240

Chaudhary, A., Dunn, S. T., Kelly, J., & Hoellein, T. J. (2022). Plastic microbiome development in a freshwater ecosystem. *Science of the Total Environment*, *848*. https://doi.org/10.1016/j.scitotenv.2022.157697

Chen, X., Xiong, X., Jiang, X., Shi, H., & Wu, C. (2019). Sinking of floating plastic debris caused by biofilm development in a freshwater lake. *Chemosphere*, *222*, 856–864. https://doi.org/10.1016/j.chemosphere.2019.02.015

Chen, Y., Yan, Z., Zhou, Y., Zhang, Y., Jiang, R., Wang, M., Yuan, S., & Lu, G. (2024). Dynamic evolution of antibiotic resistance genes in plastisphere in the vertical profile of urban rivers. *Water Research*, *249*. https://doi.org/10.1016/j.watres.2023.120946

Cheng, Y., Lu, J., Fu, S., Wang, S., Senehi, N., & Yuan, Q. (2022). Enhanced propagation of intracellular and extracellular antibiotic resistance genes in municipal wastewater by microplastics. *Environmental Pollution*, *292*. https://doi.org/10.1016/j.envpol.2021.118284

Cholewińska, P., Wojnarowski, K., Moniuszko, H., Pokorny, P., & Palić, D. (2025). An Exploratory Review of Microplastic Pollution, Associated Microbiomes and Pathogens in Water. In *Applied Sciences (Switzerland)* (Vol. 15, Number 15). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/app15158128

Choy, C. A., Robison, B. H., Gagne, T. O., Erwin, B., Firl, E., Halden, R. U., Hamilton, J. A., Katija, K., Lisin, S. E., Rolsky, C., & S. Van Houtan, K. (2019). The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-44117-2

Coons, A. K., Busch, K., Lenz, M., Hentschel, U., & Borchert, E. (2021). Biogeography rather than substrate type determines bacterial colonization dynamics of marine plastics. *PeerJ*, *9*. https://doi.org/10.7717/peerj.12135

Dang, H., Li, T., Chen, M., & Huang, G. (2008). Cross-ocean distribution of Rhodobacterales bacteria as primary surface colonizers in temperate coastal marine waters. *Applied and Environmental Microbiology*, *74*(1), 52–60. https://doi.org/10.1128/AEM.01400-07

Dang, H., & Lovell, C. R. (2002). Numerical dominance and phylotype diversity of marine Rhodobacter species during early colonization of submerged surfaces in coastal marine waters as determined by 16S ribosomal DNA sequence analysis and fluorescence in situ hybridization. *Applied and Environmental Microbiology*, *68*(2), 496–504. https://doi.org/10.1128/AEM.68.2.496-504.2002

Dang, H., Lovell, C. R., & Program, M. S. (2000a). Bacterial Primary Colonization and Early Succession on Surfaces in Marine Waters as Determined by Amplified rRNA Gene Restriction Analysis and Sequence Analysis of 16S rRNA Genes. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 66, Number 2). http://www.pasteur.fr/letondal/Pise/

Dang, H., Lovell, C. R., & Program, M. S. (2000b). Bacterial Primary Colonization and Early Succession on Surfaces in Marine Waters as Determined by Amplified rRNA Gene Restriction Analysis and Sequence Analysis of 16S rRNA Genes. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 66, Number 2). http://www.pasteur.fr/letondal/Pise/

De Menezes, A. B., Lockhart, R. J., Cox, M. J., Allison, H. E., & McCarthy, A. J. (2008). Cellulose degradation by micromonosporas recovered from freshwater lakes and classification of these actinomycetes by DNA gyrase B gene sequencing. *Applied and Environmental Microbiology*, *74*(22), 7080–7084. https://doi.org/10.1128/AEM.01092-08

de Menezes, A. B., McDonald, J. E., Allison, H. E., & McCarthy, A. J. (2012). Importance of Micromonospora spp. As colonizers of cellulose in freshwater lakes as demonstrated by quantitative reverse transcriptase PCR of 16s rRNA. *Applied and Environmental Microbiology*, *78*(9), 3495–3499. https://doi.org/10.1128/AEM.07314-11

De Philippis, R., Sili, C., Faraloni, C., & Vincenzini, M. (2002). Occurrence and significance of exopolysaccharide-producing cyanobacteria in the benthic mucilaginous aggregates of the Tyrrhenian Sea (Tuscan Archipelago). In *Annals of Microbiology* (Vol. 52).

De Tender, C. A., Devriese, L. I., Haegeman, A., Maes, S., Ruttink, T., & Dawyndt, P. (2015). Bacterial Community Profiling of Plastic Litter in the Belgian Part of the North Sea. *Environmental Science and Technology*, *49*(16), 9629–9638. https://doi.org/10.1021/acs.est.5b01093

De Tender, C. A., Devriese, L. I., Haegeman, A., Maes, S., Vangeyte, J., Cattrijsse, A., Dawyndt, P., & Ruttink, T. (2017). The temporal dynamics of bacterial and fungal colonization on plastic debris in the North Sea. In *Environ. Sci. Technol., Just Accepted Manuscript • Publication Date*. http://pubs.acs.org

Deng, L., Xi, H., Wan, C., Fu, L., Wang, Y., & Wu, C. (2023). Is the petrochemical industry an overlooked critical source of environmental microplastics? *Journal of Hazardous Materials*, *451*. https://doi.org/10.1016/j.jhazmat.2023.131199

Desidery, L., & Lanotte, M. (2022). Polymers and plastics: Types, properties, and manufacturing. In *Plastic Waste for Sustainable Asphalt Roads* (pp. 3–28). Elsevier. https://doi.org/10.1016/B978-0-323-85789-5.00001-0

Dey, S., Rout, A. K., Behera, B. K., & Ghosh, K. (2022). Plastisphere community assemblage of aquatic environment: plastic-microbe interaction, role in degradation and characterization technologies. In *Environmental Microbiomes* (Vol. 17, Number 1). BioMed Central Ltd. https://doi.org/10.1186/s40793-022-00430-4

Di Pippo, F., Bocci, V., Amalfitano, S., Crognale, S., Levantesi, C., Pietrelli, L., Di Lisio, V., Martinelli, A., & Rossetti, S. (2023). Microbial colonization patterns and biodegradation of petrochemical and biodegradable plastics in lake waters: insights from a field experiment. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1290441

Di Pippo, F., Crognale, S., Levantesi, C., Vitanza, L., Sighicelli, M., Pietrelli, L., Di Vito, S., Amalfitano, S., & Rossetti, S. (2022). Plastisphere in lake waters: Microbial diversity, biofilm structure, and potential implications for freshwater ecosystems. *Environmental Pollution*, *310*. https://doi.org/10.1016/j.envpol.2022.119876

Didier, D., Anne, M., & Alexandra, T. H. (2017a). Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. *Science of the Total Environment*, *599–600*, 1222–1232. https://doi.org/10.1016/j.scitotenv.2017.05.059

Didier, D., Anne, M., & Alexandra, T. H. (2017b). Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. *Science of the Total Environment*, *599–600*, 1222–1232. https://doi.org/10.1016/j.scitotenv.2017.05.059

Dormann, C. F., & Fründ, J. (2026). *Using bipartite to describe and plot two-mode networks in R*.

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., & Laforsch, C. (2015). Beyond the ocean: Contamination of freshwater ecosystems with (micro-)plastic particles. *Environmental Chemistry*, *12*(5), 539–550. https://doi.org/10.1071/EN14172

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. In *Biological Reviews of the Cambridge Philosophical Society* (Vol. 81, Number 2, pp. 163–182). https://doi.org/10.1017/S1464793105006950

Elifantz, H., Horn, G., Ayon, M., Cohen, Y., & Minz, D. (2013). Rhodobacteraceae are the key members of the microbial community of the initial biofilm formed in Eastern Mediterranean coastal seawater. *FEMS Microbiology Ecology*, *85*(2), 348–357. https://doi.org/10.1111/1574-6941.12122

Environment, M. (2013). *Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the*.

Environmental effects of ozone depletion and its interactions with climate change : 2002 assessment. (2003). UNEP.

Erni-Cassola, G., Wright, R. J., Gibson, M. I., & Christie-Oleza, J. A. (2020). Early Colonization of Weathered Polyethylene by Distinct Bacteria in Marine Coastal Seawater. *Microbial Ecology*, *79*(3), 517–526. https://doi.org/10.1007/s00248-019-01424-5

Esposito, R., Federico, S., Amato, A., Viel, T., Caramiello, D., Macina, A., Miralto, M., Ambrosino, L., Chiusano, M. L., Cocca, M., Manfra, L., Libralato, G., Zupo, V., & Costantini, M. (2025). Isolation and Identification of Bacterial Strains Colonizing the Surface of Biodegradable Polymers. *Microorganisms*, *13*(3). https://doi.org/10.3390/microorganisms13030609

Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., Cassone, A. L., Lambert, C., Reveillaud, J., & Paul-Pont, I. (2018). Microplastic bacterial communities in the Bay of Brest: Influence of polymer type and size. *Environmental Pollution*, *242*, 614–625. https://doi.org/10.1016/j.envpol.2018.07.023

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., & Sarniguet, A. (2011). Bacterial-Fungal Interactions: Hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists. *Microbiology and Molecular Biology Reviews*, *75*(4), 583–609. https://doi.org/10.1128/mmbr.00020-11

Galhano, V., de Figueiredo, D. R., Alves, A., Correia, A., Pereira, M. J., Gomes-Laranjo, J., & Peixoto, F. (2011). Morphological, biochemical and molecular characterization of Anabaena, Aphanizomenon and Nostoc strains (Cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. *Hydrobiologia*, *663*(1), 187–203. https://doi.org/10.1007/s10750-010-0572-5

Gaylarde, C. C., Neto, J. A. B., & da Fonseca, E. M. (2021). Paint fragments as polluting microplastics: A brief review. In *Marine Pollution Bulletin* (Vol. 162). Elsevier Ltd. https://doi.org/10.1016/j.marpolbul.2020.111847

Gebreyohanes Belay, B. M., Koelmans, A. A., & de Senerpont Domis, L. N. (2026). The role of biofouling and microbial colonization in shaping macroplastic fate in freshwaters. *Nature Water*. https://doi.org/10.1038/s44221-026-00629-6

Geyer, R. (2020). Production, use, and fate of synthetic polymers. In *Plastic Waste and Recycling* (pp. 13–32). Elsevier. https://doi.org/10.1016/b978-0-12-817880-5.00002-5

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). *Production, use, and fate of all plastics ever made*. https://www.science.org

Gkelis, S., Ourailidis, I., Panou, M., & Pappas, N. (2016). Cyanobacteria of Greece: An annotated checklist. *Biodiversity Data Journal*, *4*(1). https://doi.org/10.3897/BDJ.4.e10084

González-Pleiter, M., Velázquez, D., Casero, M. C., Tytgat, B., Verleyen, E., Leganés, F., Rosal, R., Quesada, A., & Fernández-Piñas, F. (2021). Microbial colonizers of microplastics in an Arctic freshwater lake. *Science of the Total Environment*, *795*. https://doi.org/10.1016/j.scitotenv.2021.148640

Haque, A., Holsen, T. M., & Baki, A. B. M. (2024). Distribution and risk assessment of microplastic pollution in a rural river system near a wastewater treatment plant, hydro-dam, and river confluence. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-56730-x

Hoellein, T. J., McCormick, A. R., Hittie, J., London, M. G., Scott, J. W., & Kelly, J. J. (2017). Longitudinal patterns of microplastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. *Freshwater Science*, *36*(3), 491–507. https://doi.org/10.1086/693012

Hu, X., Waigi, M. G., Yang, B., & Gao, Y. (2022). Impact of Plastic Particles on the Horizontal Transfer of Antibiotic Resistance Genes to Bacterium: Dependent on Particle Sizes and Antibiotic Resistance Gene Vector Replication Capacities. *Environmental Science and Technology*, *56*(21), 14948–14959. https://doi.org/10.1021/acs.est.2c00745

Huang, S., Wang, H., Ahmad, W., Ahmad, A., Vatin, N. I., Mohamed, A. M., Deifalla, A. F., & Mehmood, I. (2022). Plastic Waste Management Strategies and Their Environmental Aspects: A Scientometric Analysis and Comprehensive Review. In *International Journal of Environmental Research and Public Health* (Vol. 19, Number 8). MDPI. https://doi.org/10.3390/ijerph19084556

Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., Barbe, V., Meistertzheim, A. L., & Ghiglione, J. F. (2019). Microbial ecotoxicology of marine plastic debris: A review on colonization and biodegradation by the "plastisphere." *Frontiers in Microbiology*, *10*(APR). https://doi.org/10.3389/fmicb.2019.00865

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.1260352

Johansen, J. R., Kovacik, L., Casamatta, D. A., Fučiková, K., & Kaštovský, J. (2011). Utility of 16S-23S ITS sequence and secondary structure for recognition of intrageneric and intergeneric limits within cyanobacterial taxa: Leptolyngbya corticola sp. nov. (pseudanabaenaceae, cyanobacteria). *Nova Hedwigia*, *92*(3–4), 283–302. https://doi.org/10.1127/0029-5035/2011/0092-0283

Kaufmann, C. B., & Baekeland, L. H. (2011). *Leo H. Baekeland*. https://pubs.acs.org/sharingguidelines

Kirstein, I. V., Wichels, A., Gullans, E., Krohne, G., & Gerdts, G. (2019). The plastisphere – Uncovering tightly attached plastic "specific" microorganisms. *PLoS ONE*, *14*(4). https://doi.org/10.1371/journal.pone.0215859

Lage, A., Berrendero Gómez, E., García-Abad, L., Martínez-Gutiérrez, C., Garcia, J., & Gonzalez-Flo, E. (2025). Assessment of Polyhydroxybutyrate Production by Cyanobacteria Strains Isolated from Environmental Water Sources Using a Secondary Effluent. *ACS ES and T Water*, *5*(12), 7267–7278. https://doi.org/10.1021/acsestwater.5c00677

Lebreton, L. C. M., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nature Communications*, *8*. https://doi.org/10.1038/ncomms15611

Lee, J. W., Nam, J. H., Kim, Y. H., Lee, K. H., & Lee, D. H. (2008). Bacterial communities in the initial stage of marine biofilm formation on artificial surfaces. *Journal of Microbiology*, *46*(2), 174–182. https://doi.org/10.1007/s12275-008-0032-3

Leflaive, J., Danger, M., Lacroix, G., Lyautey, E., Oumarou, C., & Ten-Hage, L. (2008). Nutrient effects on the genetic and functional diversity of aquatic bacterial communities. *FEMS Microbiology Ecology*, *66*(2), 379–390. https://doi.org/10.1111/j.1574-6941.2008.00593.x

Li, J., Zhang, K., & Zhang, H. (2018a). Adsorption of antibiotics on microplastics. *Environmental Pollution*, *237*, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050

Li, J., Zhang, K., & Zhang, H. (2018b). Adsorption of antibiotics on microplastics. *Environmental Pollution*, *237*, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050

Li, W., Miao, L., Adyel, T. M., Wu, J., Yu, Y., & Hou, J. (2023). Characterization of dynamic plastisphere and their underlying effects on the aging of biodegradable and traditional plastics in freshwater ecosystems. *Journal of Hazardous Materials*, *446*. https://doi.org/10.1016/j.jhazmat.2022.130714

Li, Y., Naman, C. B., Alexander, K. L., Guan, H., & Gerwick, W. H. (2020). The Chemistry, Biochemistry and Pharmacology of Marine Natural Products from Leptolyngbya, a Chemically Endowed Genus of Cyanobacteria. In *Marine Drugs* (Vol. 18, Number 10). MDPI AG. https://doi.org/10.3390/md18100508

Lipponen, M. T. T., Martikainen, P. J., Vasara, R. E., Servomaa, K., Zacheus, O., & Kontro, M. H. (2004). Occurrence of nitrifiers and diversity of ammonia-oxidizing bacteria in developing drinking water biofilms. *Water Research*, *38*(20), 4424–4434. https://doi.org/10.1016/j.watres.2004.08.021

Liu, R., Wei, G., Yang, Y., Wang, J., Zhao, S., Zhang, B., Hao, X., Liu, K., & Shao, Z. (2025). Discovery of potentially degrading microflora of different types of plastics based on long-term in-situ incubation in the deep sea. *Environmental Research*, *268*. https://doi.org/10.1016/j.envres.2025.120812

Martínez-Campos, S., González-Pleiter, M., Fernández-Piñas, F., Rosal, R., & Leganés, F. (2021). Early and differential bacterial colonization on microplastics deployed into the effluents of wastewater treatment plants. *Science of the Total Environment*, *757*. https://doi.org/10.1016/j.scitotenv.2020.143832

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. *Environmental Science and Technology*, *48*(20), 11863–11871. https://doi.org/10.1021/es503610r

McCormick, A. R., Hoellein, T. J., London, M. G., Hittie, J., Scott, J. W., & Kelly, J. J. (2016). Microplastic in surface waters of urban rivers: Concentration, sources, and associated bacterial assemblages. *Ecosphere*, *7*(11). https://doi.org/10.1002/ecs2.1556

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). microDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. *Environmental DNA*, *1*(1), 14–25. https://doi.org/10.1002/edn3.11

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S., & Li, T. (2019). Distinct community structure and microbial functions of biofilms colonizing microplastics. *Science of the Total Environment*, *650*, 2395–2402. https://doi.org/10.1016/j.scitotenv.2018.09.378

Morohoshi, T., Oi, T., Aiso, H., Suzuki, T., Okura, T., & Sato, S. (2018). Biofilm formation and degradation of commercially available biodegradable plastic films by bacterial consortiums in freshwater environments. *Microbes and Environments*, *33*(3), 332–335. https://doi.org/10.1264/jsme2.ME18033

Muthukrishnan, T., Al Khaburi, M., & Abed, R. M. M. (2019). Fouling Microbial Communities on Plastics Compared with Wood and Steel: Are They Substrate- or Location-Specific? *Microbial Ecology*, *78*(2), 361–374. https://doi.org/10.1007/s00248-018-1303-0

Myers, J. L., Sekar, R., & Richardson, L. L. (2007). Molecular detection and ecological significance of the cyanobacterial genera Geitlerinema and Leptolyngbya in black band disease of corals. *Applied and Environmental Microbiology*, *73*(16), 5173–5182. https://doi.org/10.1128/AEM.00900-07

Nguyen, N. H. A., El-Temsah, Y. S., Cambier, S., Calusinska, M., Hrabak, P., Pouzar, M., Boruvka, M., Kejzlar, P., Bakalova, T., Gutleb, A. C., & Sevcu, A. (2021). Attached and planktonic bacterial communities on bio-based plastic granules and micro-debris in seawater and freshwater. *Science of the Total Environment*, *785*. https://doi.org/10.1016/j.scitotenv.2021.147413

Nguyen, N. H. A., Marlita, M., El-Temsah, Y. S., Hrabak, P., Riha, J., & Sevcu, A. (2023). Early stage biofilm formation on bio-based microplastics in a freshwater reservoir. *Science of the Total Environment*, *858*. https://doi.org/10.1016/j.scitotenv.2022.159569

Nnadozie, C. F., & Odume, O. N. (2019). Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes. In *Environmental Pollution* (Vol. 254). Elsevier Ltd. https://doi.org/10.1016/j.envpol.2019.113067

Oberbeckmann, S., Kreikemeyer, B., & Labrenz, M. (2018). Environmental factors support the formation of specific bacterial assemblages on microplastics. *Frontiers in Microbiology*, *8*(JAN). https://doi.org/10.3389/fmicb.2017.02709

Oberbeckmann, S., & Labrenz, M. (2026). *Downloaded from www.annualreviews.org. Guest (guest) IP: 174.67.1.225 On: Wed*. https://doi.org/10.1146/annurev-marine-010419

Oberbeckmann, S., Osborn, A. M., & Duhaime, M. B. (2016). Microbes on a bottle: Substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0159289

Omura, T., Isobe, N., Miura, T., Ishii, S., Mori, M., Ishitani, Y., Kimura, S., Hidaka, K., Komiyama, K., Suzuki, M., Kasuya, K. ichi, Nomaki, H., Nakajima, R., Tsuchiya, M., Kawagucci, S., Mori, H., Nakayama, A., Kunioka, M., Kamino, K., & Iwata, T. (2024). Microbial decomposition of biodegradable plastics on the deep-sea floor. *Nature Communications*, *15*(1). https://doi.org/10.1038/s41467-023-44368-8

Parida, D., Dhali, S. L., Bala, K., & Nogueira, R. (2026). Early microbial colonization study of daily-use plastics exposed to river water. *World Journal of Microbiology & Biotechnology*, *42*(5). https://doi.org/10.1007/s11274-026-04907-z

Parks, M., Kedy, C., & Skalla, C. (2020). Consistent patterns in 16S and 18S microbial diversity from the shells of the common and widespread red-eared slider turtle (Trachemys scripta). *PLoS ONE*, *15*(12 December). https://doi.org/10.1371/journal.pone.0244489

Parks, M., Lee, J. S., Camua, K., & Hollender, E. (2024). Turtle species and ecology drive carapace microbiome diversity in three seasonally interconnected wetland habitats. *Access Microbiology*, *6*(1). https://doi.org/10.1099/acmi.0.000682.v3

Pourmand, A., Mazer-Amirshahi, M., Jasani, G., & May, L. (2017). Emerging trends in antibiotic resistance: Implications for emergency medicine. In *American Journal of Emergency Medicine* (Vol. 35, Number 8, pp. 1172–1176). W.B. Saunders. https://doi.org/10.1016/j.ajem.2017.03.010

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1). https://doi.org/10.1093/nar/gks1219

Rhodes, C. J. (2018). Plastic pollution and potential solutions. *Science Progress*, *101*(3), 207–260. https://doi.org/10.3184/003685018X15294876706211

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R., & Briand, J. F. (2013a). Marine biofilms on artificial surfaces: Structure and dynamics. In *Environmental Microbiology* (Vol. 15, Number 11, pp. 2879–2893). https://doi.org/10.1111/1462-2920.12186

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R., & Briand, J. F. (2013b). Marine biofilms on artificial surfaces: Structure and dynamics. In *Environmental Microbiology* (Vol. 15, Number 11, pp. 2879–2893). https://doi.org/10.1111/1462-2920.12186

Schmidt, C., Krauth, T., & Wagner, S. (2017). Export of Plastic Debris by Rivers into the Sea. *Environmental Science and Technology*, *51*(21), 12246–12253. https://doi.org/10.1021/acs.est.7b02368

Shen, C., Huang, L., Xie, G., Wang, Y., Ma, Z., Yao, Y., & Yang, H. (2021). Effects of plastic debris on the biofilm bacterial communities in lake water. *Water (Switzerland)*, *13*(11). https://doi.org/10.3390/w13111465

Silva, V., Pérez, V., & Gillanders, B. M. (2023). Short-term plastisphere colonization dynamics across six plastic types. *Environmental Microbiology*, *25*(12), 2732–2745. https://doi.org/10.1111/1462-2920.16445

Sun, Y., Wu, M., Zang, J., Du, L., Huang, M., Chen, C., & Wang, J. (2023). Plastisphere microbiome: Methodology, diversity, and functionality. In *iMeta* (Vol. 2, Number 2). John Wiley and Sons Inc. https://doi.org/10.1002/imt2.101

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., & Nishijima, M. (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0105592

Tan, W. A., & Parales, R. E. (2019). Hydrocarbon Degradation by Betaproteobacteria. In *Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes* (pp. 1–18). Springer International Publishing. https://doi.org/10.1007/978-3-319-60053-6_18-1

The Ubiquity, Importance and Harmful Effects of Microorganisms: an Environmental and Public Health Perspective. (2023). *International Journal of Progressive Research in Engineering Management and Science*. https://doi.org/10.58257/ijprems32308

Thompson, R. C., Swan, S. H., Moore, C. J., & Vom Saal, F. S. (2009). Our plastic age. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 364, Number 1526, pp. 1973–1976). Royal Society. https://doi.org/10.1098/rstb.2009.0054

Thushari, G. G. N., & Senevirathna, J. D. M. (2020). Plastic pollution in the marine environment. In *Heliyon* (Vol. 6, Number 8). Elsevier Ltd. https://doi.org/10.1016/j.heliyon.2020.e04709

Tu, C., Chen, T., Zhou, Q., Liu, Y., Wei, J., Waniek, J. J., & Luo, Y. (2020). Biofilm formation and its influences on the properties of microplastics as affected by exposure time and depth in the seawater. *Science of the Total Environment*, *734*. https://doi.org/10.1016/j.scitotenv.2020.139237

Tulloch, C. L., Bargiela, R., Williams, G. B., Chernikova, T. N., Cotterell, B. M., Wellington, E. M. H., Christie-Oleza, J., Thomas, D. N., Jones, D. L., & Golyshin, P. N. (2024). Microbial communities colonising plastics during transition from the wastewater treatment plant to marine waters. *Environmental Microbiome*, *19*(1). https://doi.org/10.1186/s40793-024-00569-2

Vaksmaa, A., Knittel, K., Abdala Asbun, A., Goudriaan, M., Ellrott, A., Witte, H. J., Vollmer, I., Meirer, F., Lott, C., Weber, M., Engelmann, J. C., & Niemann, H. (2021). Microbial Communities on Plastic Polymers in the Mediterranean Sea. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.673553

Vidal-Verdú, À., Latorre-Pérez, A., Molina-Menor, E., Baixeras, J., Peretó, J., & Porcar, M. (2022). Living in a bottle: Bacteria from sediment-associated Mediterranean waste and potential growth on polyethylene terephthalate. *MicrobiologyOpen*, *11*(1). https://doi.org/10.1002/mbo3.1259

Vincent, A. E. S., Chaudhary, A., Kelly, J. J., & Hoellein, T. J. (2022). Biofilm assemblage and activity on plastic in urban streams at a continental scale: Site characteristics are more important than substrate type. *Science of the Total Environment*, *835*. https://doi.org/10.1016/j.scitotenv.2022.155398

Walker, A. (1994). Plastics: The Building Blocks of the Twentieth Century. In *History* (Vol. 10). https://www.jstor.org/stable/41613731

Wang, J., Peng, J., Tan, Z., Gao, Y., Zhan, Z., Chen, Q., & Cai, L. (2017). Microplastics in the surface sediments from the Beijiang River littoral zone: Composition, abundance, surface textures and interaction with heavy metals. *Chemosphere*, *171*, 248–258. https://doi.org/10.1016/j.chemosphere.2016.12.074

Wang, L., Tong, J., Li, Y., Zhu, J., Zhang, W., Niu, L., & Zhang, H. (2021). Bacterial and fungal assemblages and functions associated with biofilms differ between diverse types of plastic debris in a freshwater system. *Environmental Research*, *196*. https://doi.org/10.1016/j.envres.2020.110371

Wang, P., Song, T., Bu, J., Zhang, Y., Liu, J., Zhao, J., Zhang, T., Xi, J., Xu, J., Li, L., & Lin, Y. (2022). Does bacterial community succession within the polyethylene mulching film plastisphere drive biodegradation? *Science of the Total Environment*, *824*. https://doi.org/10.1016/j.scitotenv.2022.153884

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). *Perspective Prokaryotes: The unseen majority* (Vol. 95). www.pnas.org.

Williams, A. T., & Rangel-Buitrago, N. (2022). The past, present, and future of plastic pollution. *Marine Pollution Bulletin*, *176*. https://doi.org/10.1016/j.marpolbul.2022.113429

Wright, R. J., Erni-Cassola, G., Zadjelovic, V., Latva, M., & Christie-Oleza, J. A. (2020). Marine Plastic Debris: A New Surface for Microbial Colonization. *Environmental Science and Technology*, *54*(19), 11657–11672. https://doi.org/10.1021/acs.est.0c02305

Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., & Wang, Y. (2019). Selective enrichment of bacterial pathogens by microplastic biofilm. *Water Research*, *165*. https://doi.org/10.1016/j.watres.2019.114979

Xu, X., Wang, S., Gao, F., Li, J., Zheng, L., Sun, C., He, C., Wang, Z., & Qu, L. (2019). Marine microplastic-associated bacterial community succession in response to geography, exposure time, and plastic type in China's coastal seawaters. *Marine Pollution Bulletin*, *145*, 278–286. https://doi.org/10.1016/j.marpolbul.2019.05.036

Yang, W., Li, Y., & Boraschi, D. (2023). Association between Microorganisms and Microplastics: How Does It Change the Host–Pathogen Interaction and Subsequent Immune Response? *International Journal of Molecular Sciences*, *24*(4). https://doi.org/10.3390/ijms24044065

Zancarini, A., Echenique-Subiabre, I., Debroas, D., Taïb, N., Quiblier, C., & Humbert, J. F. (2017). Deciphering biodiversity and interactions between bacteria and microeukaryotes within epilithic biofilms from the Loue River, France. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-04016-w

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "plastisphere": Microbial communities on plastic marine debris. *Environmental Science and Technology*, *47*(13), 7137–7146. https://doi.org/10.1021/es401288x

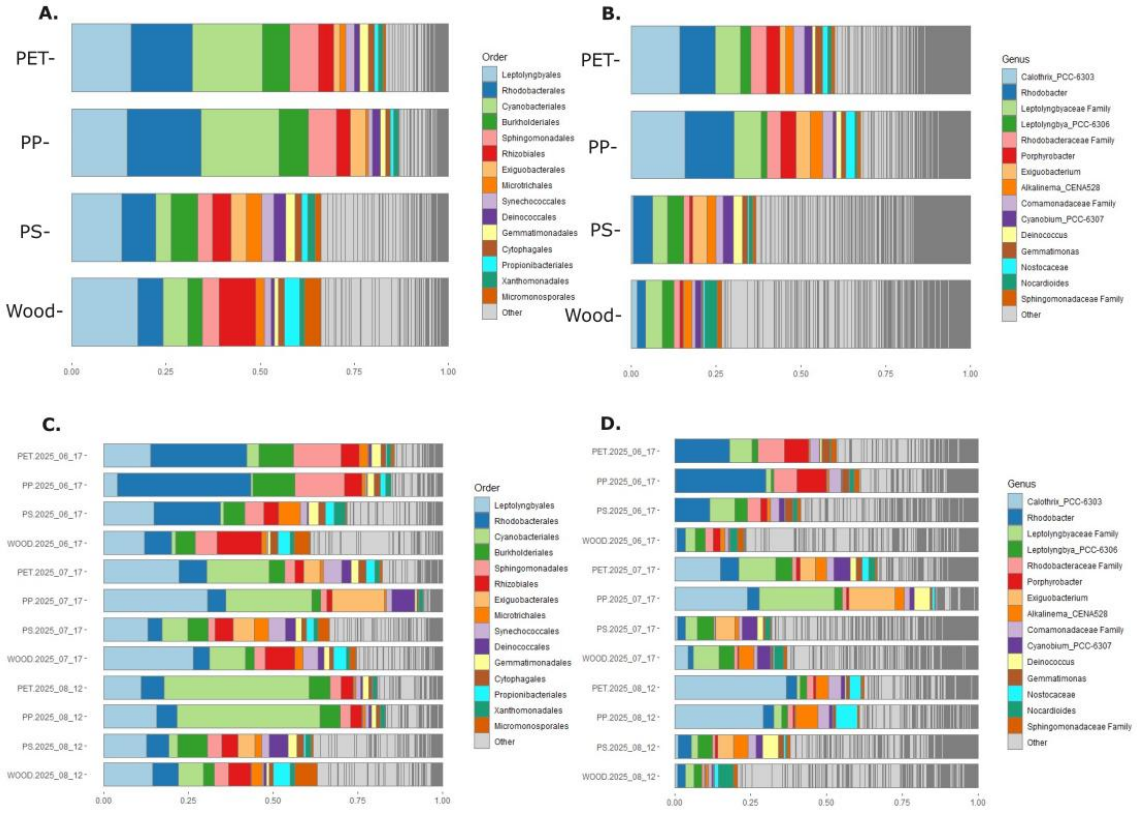
Zhai, X., Zhang, X. H., & Yu, M. (2023). Microbial colonization and degradation of marine microplastics in the plastisphere: A review. In *Frontiers in Microbiology* (Vol. 14). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2023.1127308

Zhang, S. J., Zeng, Y. H., Zhu, J. M., Cai, Z. H., & Zhou, J. (2022). The structure and assembly mechanisms of plastisphere microbial community in natural marine environment. *Journal of Hazardous Materials*, *421*. https://doi.org/10.1016/j.jhazmat.2021.126780

Zhao, Z., Wang, Y., Wei, Y., Peng, G., Wei, T., He, J., Li, R., & Wang, Y. (2024). Distinctive patterns of bacterial community succession in the riverine micro-plastisphere in view of biofilm development and ecological niches. *Journal of Hazardous Materials*, *480*. https://doi.org/10.1016/j.jhazmat.2024.135974

Zhu, M., Qi, X., Yuan, Y., Zhou, H., Rong, X., Dang, Z., & Yin, H. (2023). Deciphering the distinct successional patterns and potential roles of abundant and rare microbial taxa of urban riverine plastisphere. *Journal of Hazardous Materials*, *450*. https://doi.org/10.1016/j.jhazmat.2023.131080

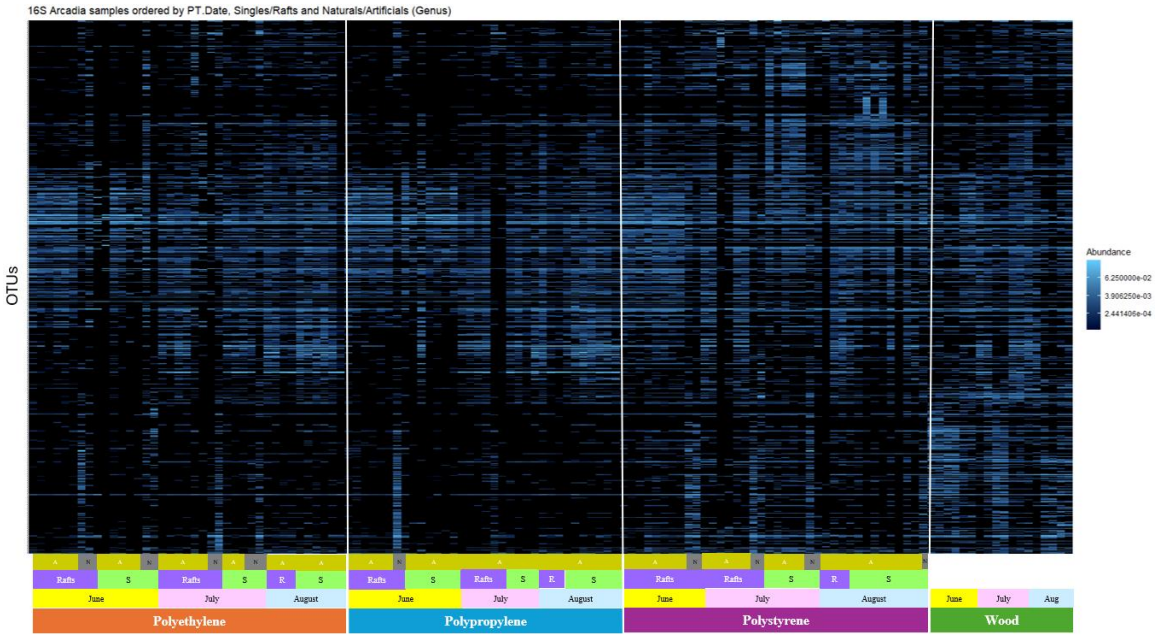
APPENDICES



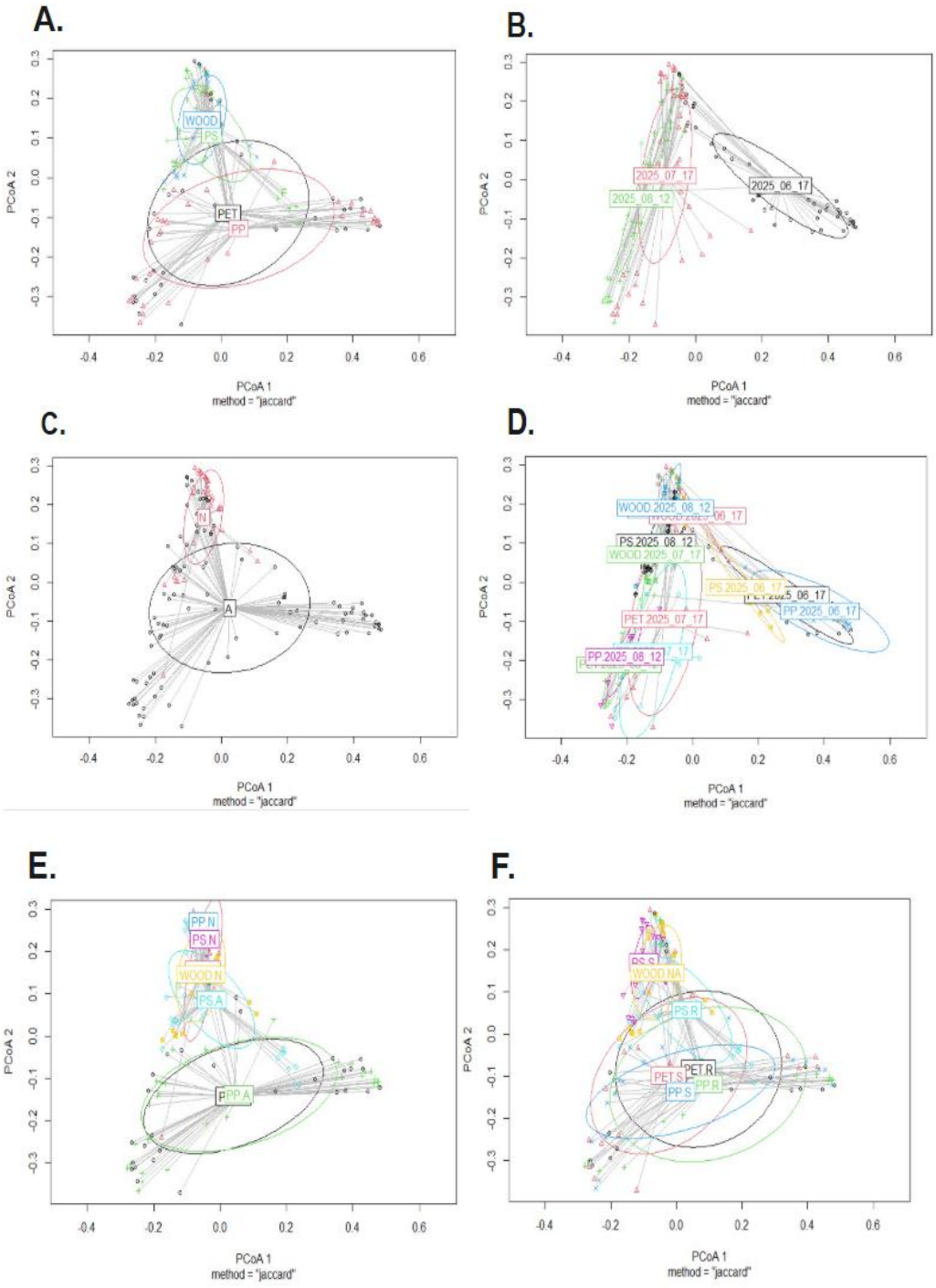
Supplementary figure 1. Stacked bar plots showing taxonomic asssignments. **A** and **B** depicts the substrate based taxonomic difference at the Order and Genus level respectively; **C** and **D** shows collection date based taxonomic differences at the Order and Genus level respectively.

	Regression Analysis (Alpha diversity)		
Variables	Shannon	Chao1	FPD
Substrate	■■■	■■■	■■■
Collection date	□□■	■■■	■■■
Singles/Rafts	□□□	□□■	□□□
Artificials/naturals	■■■	□■□	■■■
Substrate+collection date	■■■	■■■	■■■
Substrate+site	■■■	■■■	■■■
Substrate+singles/rafts	■■■	■■■	■■■
Substrate+artificials/naturals	■■■	■■■	■■■

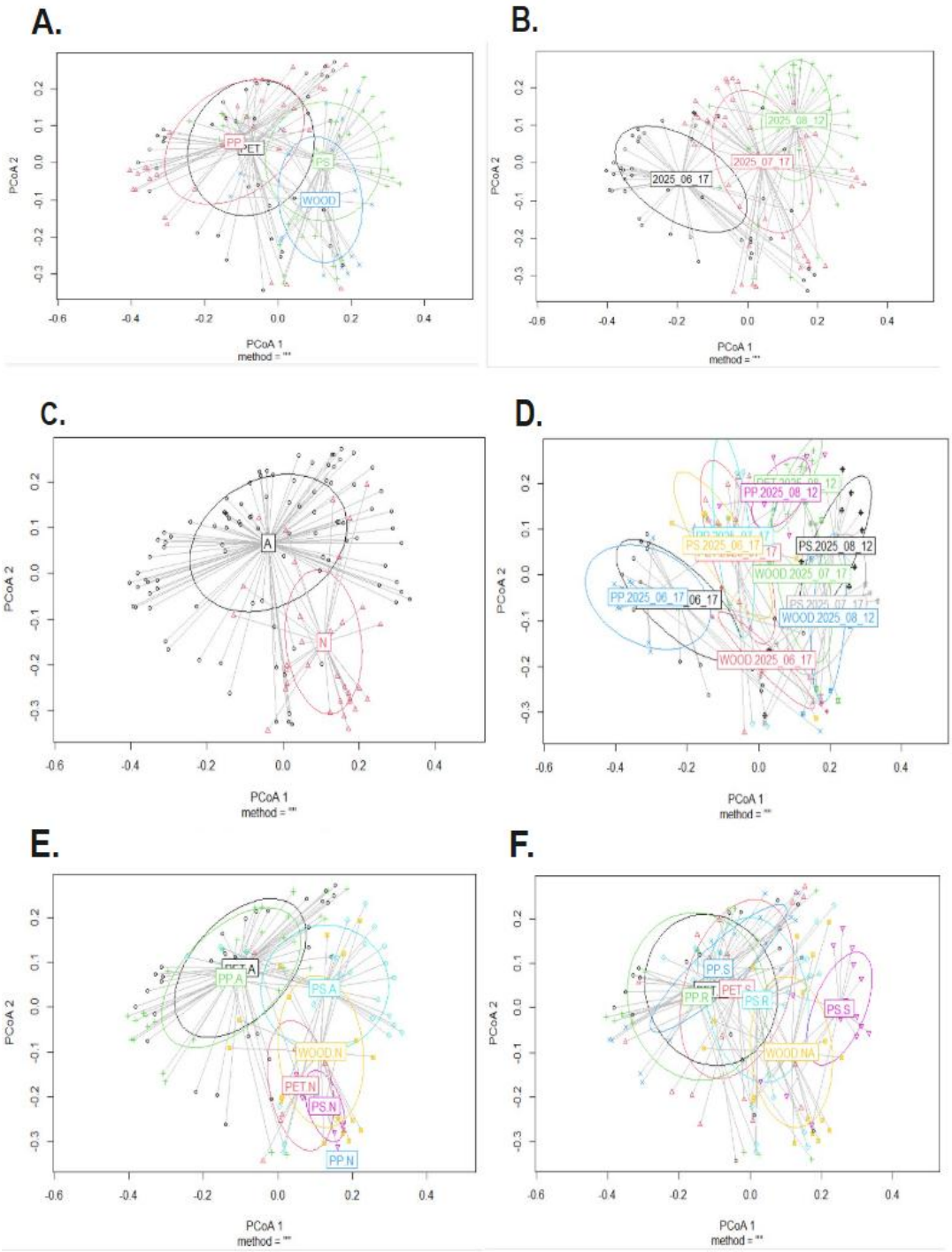
Supplementary figure 2. Alpha diversity regression analyses at the Family level.



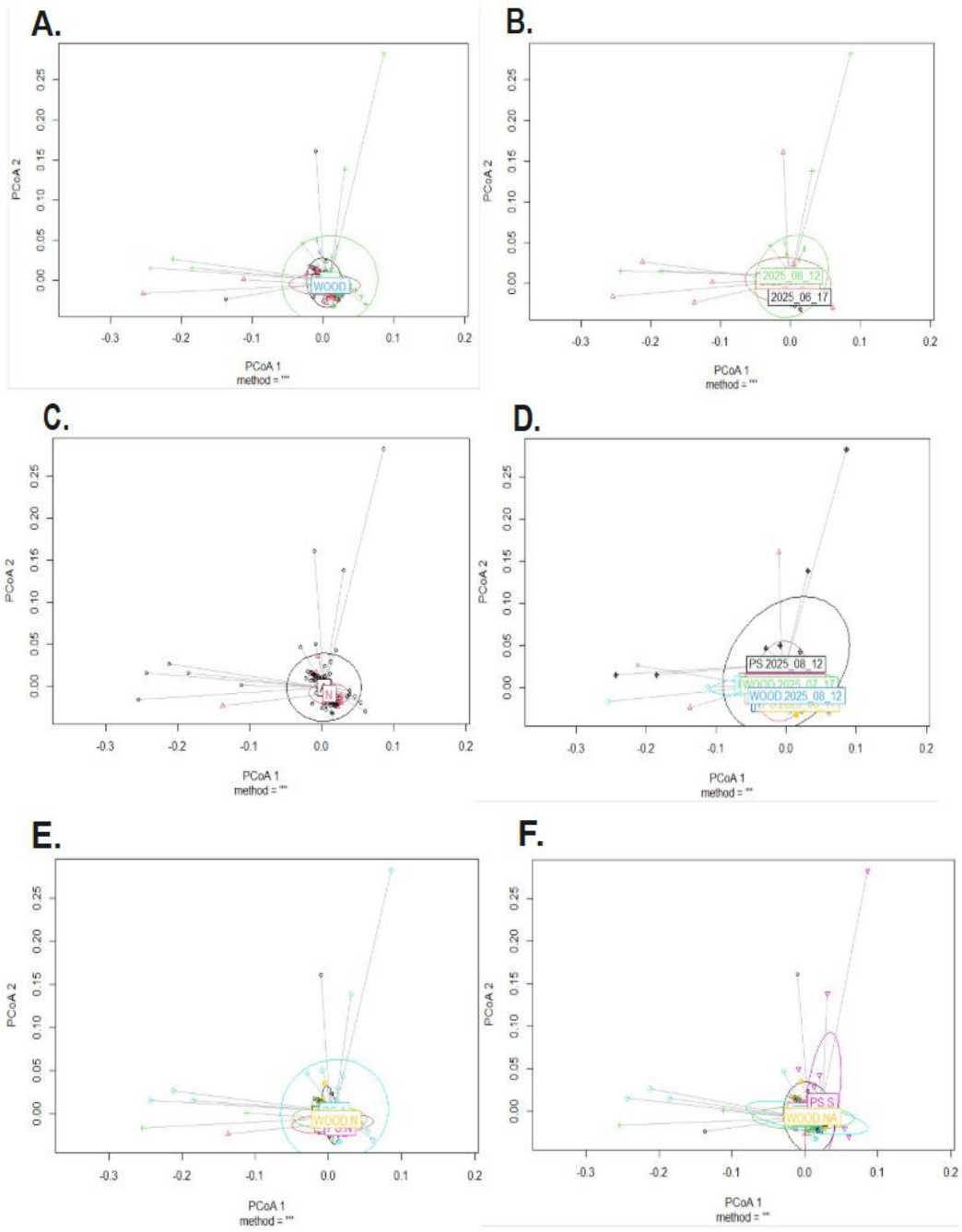
Supplementary figure 3. Heatmap shows the microbial communities composition at the Genus level. Artificially deployed samples were found to be different from pre-existing plastic samples irrespective of substrate type and sampling months.



Supplementary figure 4. Jaccard ordination plots at ASV level for different variables including **A.** substrate, **B.** collection date, **C.** artificials/naturals, **D.** substrate+collection date, **E.** substrate+artificials/naturals and **F.** substrate+singles/rafts.



Supplementary figure 5. UniFrac ordination plots at ASV level for different variables including **A.** substrate, **B.** collection date, **C.** artificials/naturals, **D.** substrate+collection date, **E.** substrate+artificials/naturals and **F.** substrate+singles/rafts.



Supplementary figure 6. Weighted UniFrac at ASV level ordination plots for different variables including **A.** substrate, **B.** collection date, **C.** artificials/naturals, **D.** substrate+collection date, **E.** substrate+artificials/naturals and **F.** substrate+singles/rafts.