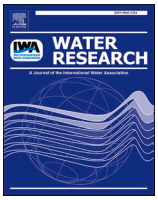
Water Research 274 (2025) 123142

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



Increasing microplastics pollution: An emerging vector for potentially pathogenic bacteria in the environment

Ülkü Nihan Tavşanoğlu [a,b,*], Gülçin Akca [c], Tülay Pekmez [b], Gökben Başaran Kankılıç [d], Tamer Çırak [e], Ali Serhan Çağan [a,f], Selin Özkan Kotiloğlu [g], Hans-Peter Grossart [h,i]

[a] Çankırı Karatekin University, Faculty of Sciences, Biology Department, Çankırı, Türkiye
[b] Çankırı Karatekin University, Health Sciences Institute, Environmental Health Programme, Çankırı, Türkiye
[c] Gazi University, Faculty of Dentistry, Department of Medical Microbiology, Ankara, Türkiye
[d] Kırıkkale University, Faculty of Engineering and Natural Sciences, Department of Biology, Kırıkkale, Türkiye
[e] Aksaray University, Aksaray Technical Sciences Vocational School, Alternative Energy Sources Technology Program, Aksaray, Türkiye
[f] Kastamonu University, Araç Rafet Vergili Vocational School, Wildlife Programme, Kastamonu, Türkiye
[g] Kırşehir Ahi Evran University, Faculty of Arts and Sciences, Molecular Biology Department, Kırşehir, Türkiye
[h] Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Neuglobsow 16775, Germany
[i] Institute of Biochemistry and Biology, Potsdam University, Potsdam 14469, Germany

ARTICLE INFO

Keywords:
Susurluk River Basin
microplastics (MP)
Bacterial biofilm
Harmful bacteria
Türkiye

ABSTRACT

Microplastics (MP), plastic particles <5 mm, are of global concern due to their worldwide distribution and potential repercussions on ecosystems and human well-being. In this study, MP were collected from the urbanized Susurluk basin in Türkiye to evaluate their vector function for bacterial biofilms, both in the wet and dry seasons. Bacterial biofilms were predominantly found on polyethylene (PE), polypropylene (PP), and polystyrene (PS), which constitute the most common MP types in the region. Specific potentially pathogenic bacterial genera, including *Pseudomonas* sp., *Comamonas* sp., *Salmonella* spp., and *Shigella* spp., were prevalent on MP surfaces. Notably, PE and PP harboured numerous genera of potential human and/or animal origin such as *Staphylococcus, Proteus, Escherichia, Enterococcus*, and *Enterobacter*. Water quality played a pivotal role in bacterial biofilm formation on MP. Higher salinity in estuarine areas reduced bacterial abundance on MP, while the more polluted freshwater Nilüfer Stream harboured a higher abundance of total bacteria, particularly of potentially pathogenic strains. Seasonal variations, ambient water conditions, and polymer type are all factors that could influence bacterial colonization on MPs. This catchment-wide evaluation, which includes various habitat types (lentic and lotic systems), the enrichment of cultivable viable bacteria on microplastics (MPs) - a key factor in the spread of pathogens - has significant implications for both environmental and public health. Unlike controlled laboratory experiments or *in-situ* studies with various particles, this study emphasized the dynamic and complex nature of bacterial strains on MPs, which varied depending on seasonal dynamics and antropogenic impacts in open systems. Further research is needed to thoroughly investigate to fully explore the complex interactions among MPs, microbial communities, and their ecological roles, especially in the context of changing environmental factors across entire river catchments.

1. Introduction

The exponential surge in worldwide plastic production, soaring from a mere 1.5 million tons in 1950 to 367 million tons in 2020 (Plastics Europe, 2021), has dramatically increased the amount of microplastics (MP) within the environment, in particular freshwaters (Nava et al., 2023). These diminutive plastic particles are characterized by their size of less than 5 mm, and, in recent studies, have been found to be of paramount importance as indicators of pollutant accumulation and ecological niches for microbial colonization and subsequent biofilm formation (Arias-Andres et al., 2018; Yang et al., 2021). MP enter freshwater ecosystems through multiple pathways, including surface runoff, wastewater treatment plants, fisheries, aquaculture, and atmospheric deposition (Andrady, 2011; Hitchcock, 2020). Consequently,

* Corresponding author.
E-mail addresses: nihan@karatekin.edu.tr, unyazgan@gmail.com (Ü.N. Tavşanoğlu).

https://doi.org/10.1016/j.watres.2025.123142
Received 9 July 2024; Received in revised form 20 December 2024; Accepted 12 January 2025
Available online 13 January 2025

due to the substantial MP influx into the environment, they form an emerging "artificially" ecological niche, known as the "*plastisphere*", fostering the colonization of diverse microorganisms within aquatic ecosystems (Zettler et al., 2013; Kettner et al., 2017; Wang et al., 2020). This colonization is facilitated by the physicochemical and surface properties of MP including large surface area, high porosity, substantial hydrophobicity/hydrophilicity (i.e. wettability) and roughness (Garrett et al., 2008; Bhagwat et al., 2021). Moreover, microbial biofilm community composition can vary with the polymeric composition of different MP types that affect microbial adhesion. Microbial growth, however, is also closely linked to environmental factors such as temperature, salinity, the amount of organic matter, and nutrient availability, which are anticipated to play pivotal roles for microbial substrate colonization (Nguyen et al., 2022; Parida et al., 2022). While it is difficult to assert the superiority of a particular factor over another with certainty, it is essential to recognize that the effectiveness of one environmental factor may depend on the state or condition of the others.

The colonization of MPs by a range of harmful organisms, including pathogenic bacteria capable of adapting and traversing diverse environments, poses a significant and growing threat to aquatic ecosystems (Oberbeckmann et al., 2015; Yang et al., 2020). This colonization facilitates the spread of pathogens, promotes the transfer of harmful chemicals and microorganisms, and exacerbates associated risks, including the development of antibiotic resistance and the release of toxic substances. Consequently, aquatic life and overall ecosystem health are severely impacted (Zettler et al., 2013; Arias-Andres et al., 2018; Miao et al., 2021). Moreover, the transfer of antibiotic resistance genes (ARGs) to aquatic organisms (Arias-Andres et al., 2018) poses significant risks, with potential consequences for human health. These risks emerge through multiple exposure pathways, including the inhalation of airborne MPs (Gasperi et al., 2018), dermal contact (Emenike et al., 2023), and ingestion, particularly via the consumption of contaminated dietary products in freshwater ecosystems (Koelmans et al., 2019).

Several studies have demonstrated the presence of pathogenic bacteria, such as *Vibrio* and *Pseudomonas*, within the microbial communities residing on plastic debris (e.g., Oberbeckmann et al., 2015; Li et al., 2019). Once these potentially pathogenic bacteria establish colonies on plastic debris, their chance to end up in aquatic habitats greatly increases, and thus can be even assumed as invasive species (Zettler et al., 2013).

Freshwater ecosystems harbor distinct microbial communities, each characterized by unique composition and proportions of resident species specific to their regional environment (Thompson et al., 2017). These bacterial species play a crucial role in utilizing and maintaining microbial diversity within ecosystems. Additionally, the process of biofilm formation is inherently dynamic and typically encompasses microbial adhesion, facilitated by the secretion of extracellular polymeric substances (EPS) (Tu et al., 2020) and the proliferation of microbial co-aggregation. Biofilms also represent intricate assemblages of diverse microorganisms, wielding significant influence over the biogeochemical cycles within aquatic ecosystems (Grossart, 2010; Battin et al., 2016).

Furthermore, microbial biofilm formation on MP can lead to alterations in physical and chemical MP properties, rendering them more complex and potentially hazardous. However, compared to marine ecosystems, our understanding of MP biofilm composition, abundance, and public health risks is still limited for freshwater ecosystems (Nava et al., 2023). While a significant proportion of MP research has been conducted through laboratory experiments, there is a pressing need for more empirical data derived from field studies conducted in various freshwater ecosystems, which serve as critical paths for plastic pollution (Mughini-Gras et al., 2021; Yang et al., 2021; Nava et al., 2023).

Currently, the investigation of bacterial diversity in MP biofilms covers molecular technologies such as amplicon sequencing methods (Amaral-Zettler et al., 2020; Forero-López et al., 2022; Nguyen et al., 2023) and shotgun metagenomic sequencing which found a higher predicted diversity compared to 16S rRNA gene sequencing (Logares et al., 2014; Tessler et al., 2017). However, it is important to note that the quantitative polymerase chain (qPCR) approach may lead to an overestimation of viable cell numbers due to the presence of free extracellular DNA (eDNA) (Klein et al., 2012) and DNA originating from deceased cells (Alvarez et al., 2013; Yasunaga et al., 2013; Kruger et al., 2014). Consequently, conventional microbiological methods, grounded in classical cultivation, are still considered as the golden standard for validating the viability and physiology of the isolated strains (Azeredo et al., 2017). Moreover, it is important to determine the vitality of MP biofilm strains and to understand the role of the plastiphere, considered as crucial in plastic biodegradation (Jacquin et al., 2019; Eronen-Rasimus et al., 2022), as an emerging microbial niche, especially of potentially pathogenic bacteria (Yang et al., 2020). Assesing the vitality of isolated strains is pivotal for understanding interspecies interaction and biofilm dynamics. This allowed us to improve our knowledge on occurrence and habitat specificity of cultivable strain types across various freshwater habitats. Notably, these aspects have been relatively understudied in freshwater ecosystems until now (Hoellein et al., 2017; Di Pippo et al., 2020; 2022). While extensive research on biofilm-associated microplastics has been conducted in controlled laboratory experiment or with various particles in-situ studies, our understanding of biofilm formation -encompassing aggregation, growth, and disaggregation- relies heavily on *in vitro* experiments. However, such findings do not always reflect biofilm development *in vivo* or in open systems (Sauer et al., 2022). There is a clear underrepresentation of field studies focusing on biofilm formation on MPs surface in freshwater ecosystems. This gap limits our understanding of how microplastics interact with biofilms in diverse environmental conditions, potentially overlooking significant ecological and public health implications.

Hence, the objective of this study was to investigate the vector role of predominant MP types within a river basin influenced by antropogenic impacts in Türkiye, focusing on cultivable viable microorganism within the biofilm and their seasonal variations. These variations were analyzed in the context of environmental factors, as the bacterial community vitality is critical for the assemblage. The study provides a snapshot of the ecosystem, highlighting the role of MP pollution in pathogen and ARG facilitation and its broader implications.

2. Material and methods

2.1. Study site

Our current study focused on the investigation of microbial biofilm composition on different MP samples collected from Bursa, Türkiye, which is a city located in the Marmara Region, Northwestern Türkiye. It is the fourth largest city and an important industrial, commercial, and cultural center in the country. The sampling was performed at 10 designated points, and the assessments were structured by categorizing them into four distinct sub-regions: Lake Uluabat, Karadere Stream, Nilüfer Stream, and Estuarine. This categorization was based on similar physico-chemical characteristics of each sampling point (Fig. 1; Table 1). To evaluate the seasonal fluctuations of MP concerning their bacterial biofilm, sampling was carried out during both rainy-wet season (February) and dry season (August) in 2021. Lake Uluabat, a Ramsar site, covers an area of 135–155 km^2 and has a maximum depth of 2.5–3 m. It is an essential source of livelihood for the local population, supporting activities such as fishing, husbandry, agriculture, and tourism. The lake also holds a high biodiversity due to its location on the migratory bird routes and multiple freshwater sources. On the other side, the estuary (Fig. 1), which is 21 km long and 3.5 km wide, discharges into the Marmara Sea and is primarily used for agriculture and fishing activities. Yet, the estuary is connected to the Nilüfer Stream (Fig. 1) and thus exposed to high waste pollution due to several activities in the larger urbanized area of Bursa. The Nilüfer Stream is facing an escalating anthropogenic pollution, a consequence of the increasing

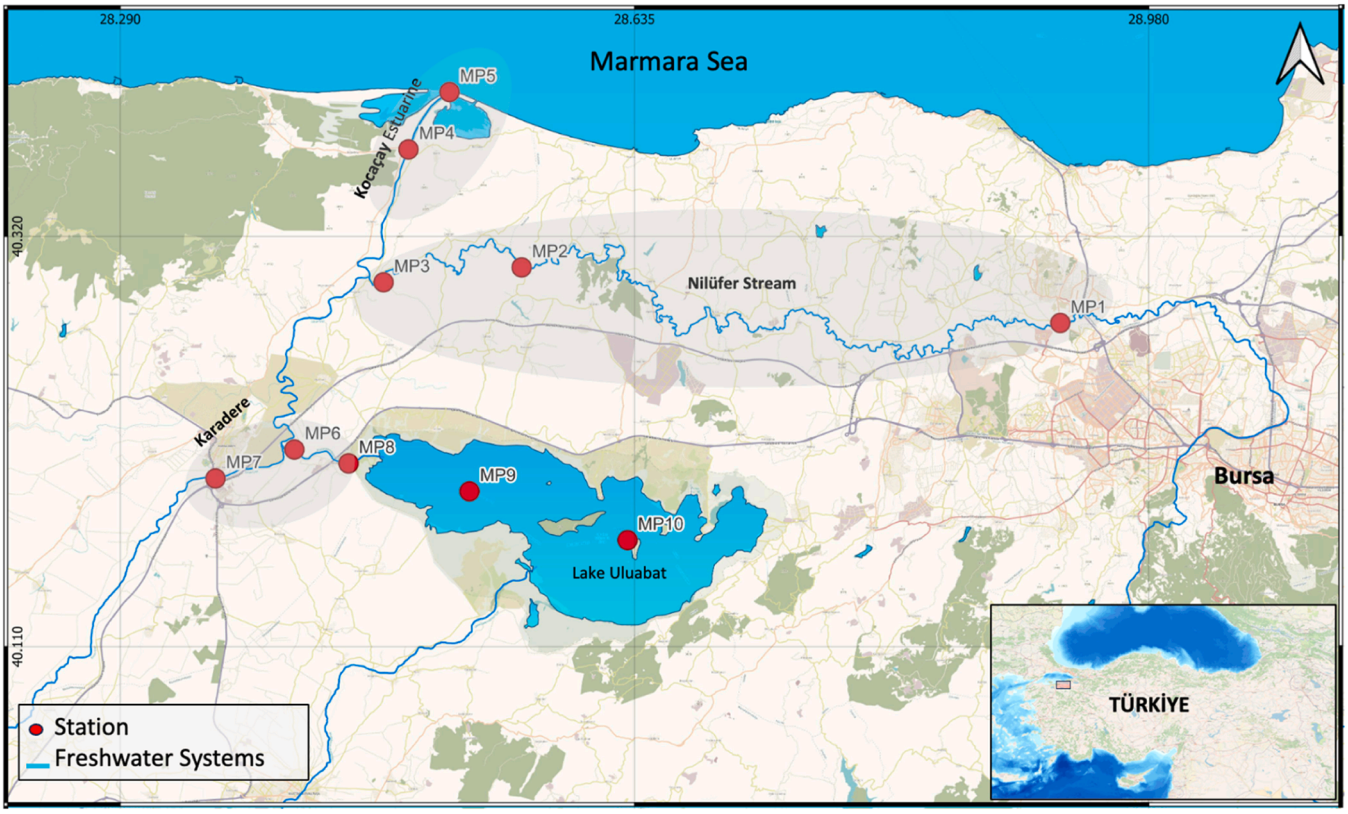


Fig. 1. Sampling locations.

Table 1
General physical and chemical properties of sampling locations (mean ± SE).

	Sites	Temperature (°C)	Salinity (‰)	DO (%)	pH	DIN (μg/L)	PO4 (μg/L)
Dry	Estuary	21.43±0.87	4.36±1.77	38.15±8.72	8.22±0.02	13.05±1.18	1.47±0.18
	Karadere	20.85±0.54	0.36±0.01	41.57±3.62	8.50±0.08	5.82±0.95	0.96±0.09
	Nilüfer	22.58±0.47	1.15±0.01	8.53±0.52	8.30±0.05	37.50±2.38	4.67±0.14
	Uluabat	20.64±0.44	0.36±0.01	86.94±0.72	8.73±0.05	2.30±0.27	0.31±0.02
Wet	Estuary	14.60±2.17	0.32±0.00	74.30±4.79	8.22±0.04	12.10±0.21	0.31±0.08
	Karadere	13.94±1.44	0.32±0.01	50.60±3.51	8.32±0.07	12.72±1.31	0.42±0.07
	Nilüfer	16.51±0.94	0.63±0.03	30.82±6.07	7.88±0.02	13.38±0.27	0.70±0.12
	Uluabat	14.41±1.52	0.29±0.00	84.47±3.66	8.71±0.04	2.58±0.29	0.00±0.00

urbanization and industrialization in the area with an increasing discharge of untreated wastewater directly into the stream. The final sampling location in the Karadere Stream (Fig. 1) is under high domestic and agricultural pollution pressure.

2.2. Sampling

For investigating the bacterial biofilm composition on different MP in the water, samples were collected using manta nets with a mesh size of 300 μm at each station during both wet and dry seasons. All samples were transferred into sterile 250 mL glass jars and kept dark at 4 °C in an icebox during their transportation to the laboratory. Simultaneously, 500 mL-water samples were collected into a sterile glass jar to characterize the ambient bacterial composition in the water column. To investigate the bacterial community composition in the sediment, triplicate sediment samples were collected using a Van Veen grabber. From the pooled sediment sample, 50 mL of homogenized sediment samples were extracted to identify the ambient bacterial community. Additionally, 1 L of sediment samples was collected to investigate MP and their associated bacterial biofilms.

2.3. Microbiological processes of MP

2.3.1. Culture-based identification of MP biofilm bacteria

Bacterial cultures from all samples underwent assessment through conventional microbiological methods. Colony morphologies were employed for counting, selected colonies grown on specific media, which were purified and identified with conventional microbiological techniques. Water samples (500 mL) collected from the stations in sterile glass bottles underwent filtration using sterile 0.22 μm membranes (cat no:18043E, Sartorius AG, Germany). Following filtration of all the water samples through these membranes, the filter membranes were transferred into sterile Falcon tubes containing 1 mL of sterile distilled water. Additionally, each sediment sample (1 gram) was placed into separate Falcon tubes, and 1 mL of sterile distilled water was added to each tube. MP samples were collected from both surface water and sediment at each station. Using sterile tweezers in a fume hood to prevent the risk of contamination, MP were randomly selected and transferred into sterile plastic (PP) Eppendorf® tubes containing 1 mL sterile distilled water. Following a rigorous vortexing process, as described in several studies (e.g. Metcalf et al., 2023; Stevenson et al., 2023), MP in 1.5 mL sterilized distilled water-filled Eppendorf® tubes were subjected to vortexing for

two minutes. Later, the MP were seperated and transferred to another sterile tube for the determination of polymer composition. To ensure the thorough detachment of microorganisms from the MP, a confirmation step was undertaken. MP were subject to cultivation on enriched culture media, subsequently planted and incubated under the same conditions to verify the absence of any microbial growth. Subsequently, all tubes underwent a two-minute vortexing process. Serial dilutions were then prepared from the tubes containing either water, sediment, or microplastics at dilution factors of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. These dilutions were executed in separate Eppendorf® tubes, each containing 1 mL of sterile distilled water (Tavşanoğlu et al., 2020). Fifty μL samples from each tube were separately inoculated into selected culture media (SI-1). All inocula from the samples were cultivated on ten different culture media compositions, comprising both commercially available and chemically defined media, designed to support the growth of specific group of cultivable bacterial species, including enteric and fecal pathogens (SI-1). For isolation and identification of bacterial strains grown on the media, traditional microbiological methods, including Gram staining, were employed. This encompassed the examination of colony pigmentation, morphology, bacterial structure under a light microscope, motility, enzymatic properties, biochemical properties, and other relevant characteristics (Tavşanoğlu et al., 2020; Muñoz-Torres et al., 2023). The purity of the Gram-stained colonies was confirmed using light microscopy. Following the incubation period, the grown colonies were enumerated, and total bacterial counts were determined as colony-forming units (CFU)/mL based on the respective dilution rates. Unidentified cultured strains underwent further analysis utilizing the MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) automated bacterial typing device (VITEK MS v3.0, BioMérieux, Marcy; Etoile France) (Ashfaq et al., 2022).

2.3.2. DNA isolation, polymerase chain reaction (PCR) and DNA sequencing

In instances where the MALDI-TOF system failed to reliably characterize the strains, additional PCR and DNA sequencing were applied for the unidentified bacterial isolates. Approximately 2×10^8 bacterial cells of each culture was collected in 1.5 mL Eppendorf tubes containing 1 mL sterile 1X phosphate buffer saline (PBS) and homogenized by vortexing. Then, bacterial cells were pelleted by centrifugation for 10 min at 5000 x *g*. DNA isolation was performed using GeneJet Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions for Gram-positive and Gram-negative bacteria. The whole 16S rRNA gene including V1-V9 regions was PCR-amplified from total DNA using the bacterial primer pair: universal 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Lane, 1991; Janda and Abbott, 2007), using the TEMPase Hot Start DNA Polymerase (Ampliqon, Denmark). PCR was performed in a 50 μL reaction mixture containing 200 μM of dNTPs, 25 mM $MgCl_2$, 20 pmol of both forward and reverse primers, 1 U of Hot Start Taq DNA polymerase, 10X Ammonium buffer, and 100 ng of genomic DNA. PCR cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. Amplicons approximately 1350 bp in size were checked on a 1 % agarose gel, visualized by RedSafe (Intron Biotechnology, Korea) staining, and photographed using Syngene Monitoring System. PCR products were then purified, and bidirectional sequencing was performed using both primers using the Sanger DNA sequencing method by Macrogen (Korea). The obtained sequences were blasted using the NCBI BLAST (https://blast.ncbi.nlm.nih.gov/) 16S ribosomal RNA sequence database (Bacteria and Archaea) and the Megablast program to identify bacteria strains at the genus/species level (GenBank accession numbers: PQ118993 - PQ119375). The highest identity was chosen for genus or species identification (McGinnis and Madden, 2004; Boratyn et al., 2013).

2.4. MP characterization

Folowing the biofilm process outlined below, each MP sample used for biofilm analysis was individually examined using both RAMAN and FTIR spectroscopy to identify the polymer type structures. RAMAN analyses were performed by using a DXR RAMAN Microscope (Thermo Scientific) at ≤7.0 mW laser power with a 633 nm wavelength laser source, and spectra were recorded at a Raman shift range of 3600–150 cm^{-1}. Fourier transform infrared spectrometer (FTIR) analyses were performed by using a Tensor II (Bruker) ATR-FTIR spectrometer. Absorbance spectra were recorded at a wave number range of 400–4000 cm^{-1}. For a comprehensive characterization based on MP type, either RAMAN spectroscopy or ATR-FTIR spectroscopy was applied. Polymer structures were identified by spectra interpretation and self-constructed spectra library matching. To determine bacterial biofilm formation, MP samples were Au/Pd coated by a sputter coater (Leica ACE 600), and the surface properties of MP were evaluated by using a GAIA 3 (Tescan, Czech Republic) Scanning Electron Microscope (SEM) at different magnifications (50 – 10000X).

2.5. Quality assurance/quality control

All types of equipment including forceps, scissors, and Petri dishes were thoroughly cleaned with freshly distilled and MP-free water before and after each use and covered with aluminum foil to prevent any external contamination. To prevent contamination from laboratory conditions, cotton laboratory clothes and nitrile gloves were always worn during sample preparation. During the entire laboratory process, samples were prepared under a sterile bench.

2.6. Data analysis

To determine if there were significant differences in the extent of biofilms between different seasons (dry and wet) and habitats (MP surface, water, sediment), a two-way analysis of variance (ANOVA) was carried out. If there was a significant difference between the different groups, post hoc analysis was performed using Tukey's test. To examine bacterial community composition in relation to polymer type, principal component analysis (PCA) was carried out using the "vegan" package in R. Before the analyses, Hellinger transformation was applied to the species data for normalization. The Shannon diversity index of bacteria at the genus level was computed utilizing the "vegan" R-package. It is commonly used when data exhibit a skewed distribution and contain many zeros. To investigate the variation in bacterial biofilm and environmental variables, ordination analysis was conducted. The bacterial data was transformed using the Hellinger transformation for the ordination analysis (Legendre and Gallagher, 2001). Detrended correspondence analysis indicated that linear response models were suitable for the dataset (gradient length of the first axis: <3 SD) (ter Braak, 1995). Therefore, redundancy analysis (RDA) was employed next. Environmental variables that explaining the maximum variation in the bacterial biofilm data were selected by removing highly collinear and insignificant variables. Variables with high collinearity were identified based on the variance inflation factors (VIFs), and those with VIF value greater than 20 were removed. Subsequently, Monte Carlo permutation tests were used to test the significance of each environmental variable. Variables that did not explain a significant portion of species variance ($P < 0.05$; 999 random permutations) were also removed. All statistical analyses were performed using R (R Core Team, 2022).

3. Results

3.1. Polymer composition

Throughout the sampling seasons, a total of 2052 microplastic (MP) items were analyzed for their polymer composition in Nilüfer Stream,

Lake Uluabat, Karadere River, and the Estuarine region. The most prevalent polymer type was polyethylene (PE), accounting for 50 %, followed by polypropylene (PP) at 27 %, while the remaining 23 % consisted of minor contributions from acrylic, polyurethane, and polyvinyl chloride. Similarly, in the subset of MP samples ($n = 216$) analyzed for biofilm colonization, which included various types such as fibers, films, fragments, and foams (Fig. 2), PE accounted for the highest proportion (57 %), followed by PP and PS as the other predominant polymer types across all sampling locations.

3.2. MP- Associated Biofilm

The observation of cultivable bacterial strains in different environmental compartments revealed seasonal similarities (SI-2). The presence of certain potentially pathogenic bacterial genera such as *Pseudomonas (P. monteili), Comamonas* (*C. testosterone*), *Salmonella*, and *Shigella*, are of significant concern, as they are commonly encountered on MP in both seasons (SI-2). While variations in total bacterial abundance (CFU)/ml were evident among different environmental compartments, the distinctions were primarily notable between the surrounding water and sediment samples contrary to MP samples (Fig. 3, SI-3). The total bacterial colonization of MP surfaces was significantly lower during the dry season than the wet season ($p < 0.005$), even though higher bacterial counts were observed in the surrounding water and sediment during the dry season (Fig. 3).

Consistent with the observed variations in bacterial colonization differences across seasons and sampling subregions, the percentage of individual bacterial strains on the surface of various polymers were high during the wet season at each location (Fig. 4; SI-4). The viable bacterial strains found on MP surfaces during both seasons were predominantly characterized by three dominant phyla: *Proteobacteria, Firmicutes*, and *Bacteroidetes* (Fig. 4; SI-2). Notably, the observed species exhibited a predominance of gram-negative species (SI-4). In assessing the vector role of MP in each subregion, Nilüfer Stream exhibited significantly higher bacterial counts as colony forming unit (CFU)/ml compared to other sampled locations ($p < 0.005$). The presence of polystyrene foam in the stream showed a distinct difference in bacterial biofilms compared to other MP types ($p < 0.005$) (SI-4).

Although the various bacterial MP communities exhibit remarkable similarities at the genus level, a high cultivable vital bacterial diversity was observed in the Estuary during the dry season (Fig. 4a). While in the Karadere stream the cultivable vital bacterial diversity was high during wet season (Fig. 4b). Similarly, the higher bacterial counts noted in the Nilüfer Stream (see SI-3), emphasizing a more pronounced cultivable vital bacterial diversity and biofilm formation in the Nilüfer stream (Fig. 4d). Compared to the other subregions, the cultivable bacterial diversity on MP in Lake Ulubat, a Ramsar Site with high touristic and fishing activities, is relatively low ($H = 1.80$), emphasizing the crucial role of conservational measures in the area (Fig. 4c). The Shannon Diversity Index of cultivable bacterial species was notably high on PECo, PPCo, and PS in the sampling region ($H = 2.12, 2.07, 2.11$, respectively). In contrast, polyamide (PA) and acrylic co-polymer (AcrCo) exhibited a much lower bacterial diversity ($H = 1.36, 1.32$, respectively) (Fig. 4). Nevertheless, *Salmonella* spp. *Shigella* spp. and *Escherichia coli* were consistently found in biofilm communities on MP of various polymer types, such as PE and PP (Fig. 4). The results further highlight that microplastics with varying polymer composition in an environment such as Nilüfer Stream play a significant role as vectors hosting pathogenic bacterial biofilms including *Enterococcus, Shewanella, Citrobacter* (Fig. 4d). The identified taxa displayed variations across seasons, stations, and different types of MP. As a side note, a genus known as *Rhodotorula* spp., belonging to the eukaryotic Basidomycota phylum, was found in a limited number of samples. Remarkably, *Rhodotorula* spp. showcase the unique growth capability on bacterial culture media, underscoring its saprophytic characteristics.

According to our PCA-biplot analysis, which provides insights into the relationship between specific bacterial communities and MP, the bacterial genera *Enterococcus, Diphteroid*, and *Streptococcus* exhibited a high fraction during the dry season. They were positively related to PE, PP, PS, and acrylic copolymer (AcryCo). These bacterial genera and MP types accounted for approximately 54 % of the explained variability in the two-axis PCA plot (Fig. 5). On the other hand, during the wet season,

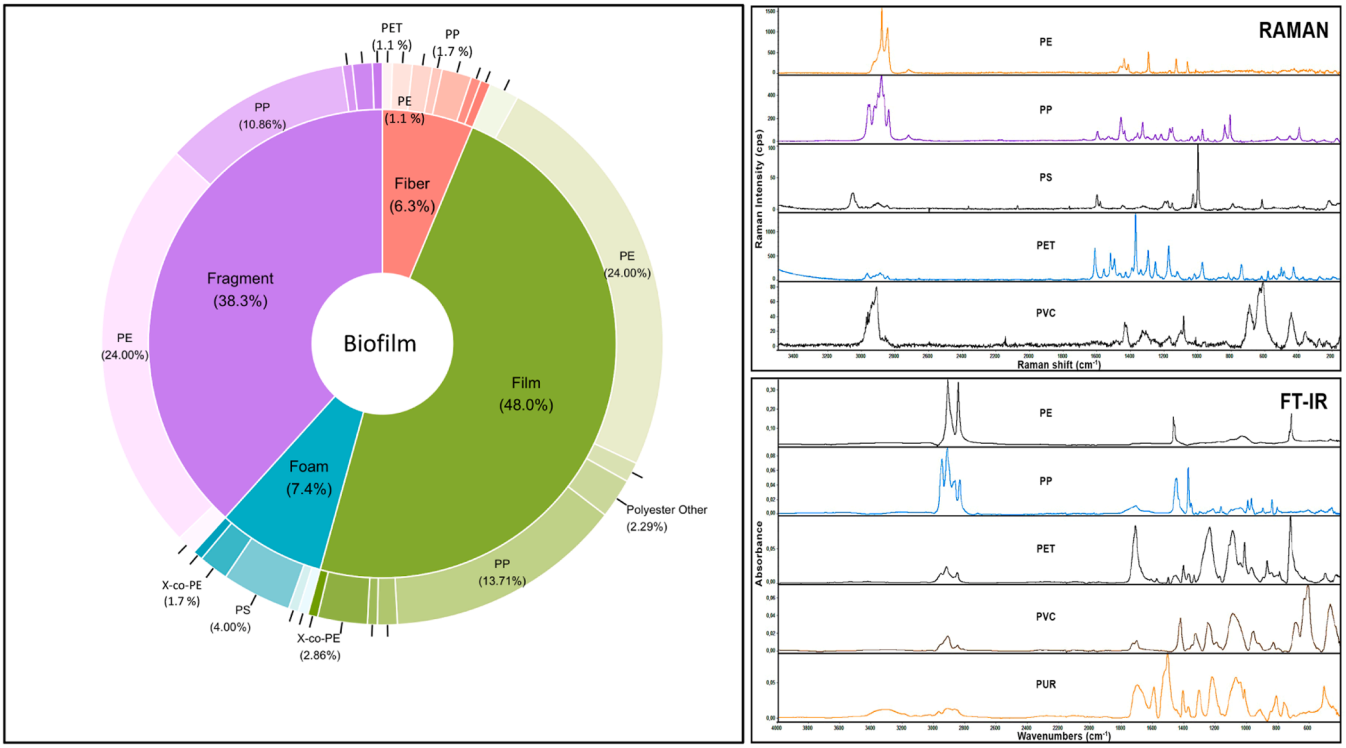


Fig. 2. Polymer composition of different types of microplastics that colonized the bacterial biofilm.

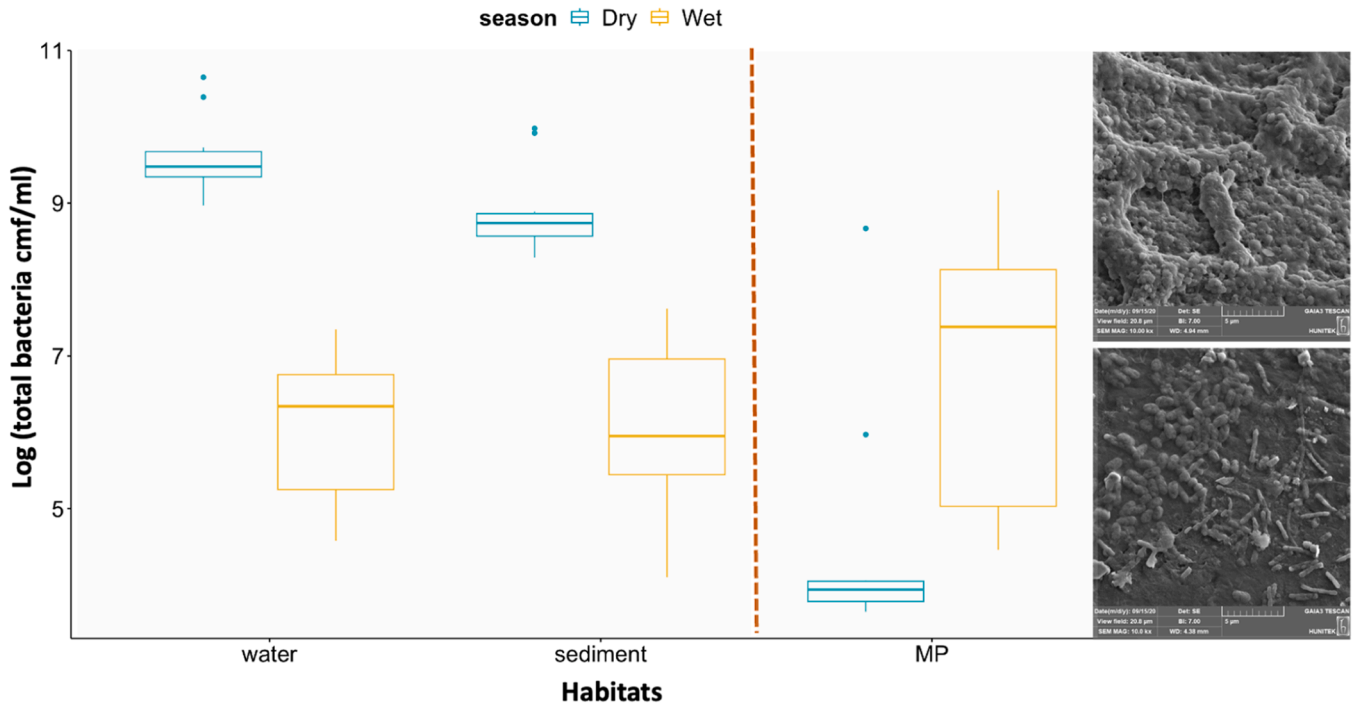


Fig. 3. Box plot of biofilm abundance with the selected SEM images samples.

the bacterial genera *Proteus, Achromobacter, Salmonella*, and *Pseudomonas* constituted a substantial contribution of total biofilm bacteria on PS. This relationship explained 41 % of the variability in the PCA-biplot (Fig. 5). Furthermore, our results revealed bacterial selectivity for specific polymer types, i.e. marker genera of PE and PP were *Staphylococcus, Proteus, Escherichia, Enterococcus*, and *Enterobacter* (Figs 4 and 5).

3.3. MP-Associated bacteria assembly and water quality

The abundance of putative pathogenic bacterial taxa belonging to *Proteus, Enterococccus*, and *Pseudomonas* increased in the highly polluted Nilüfer Stream, characterized by, e.g., high P and N concentrations, low DO% (i.e., PO_4: 2.8 μg/L, DIN: 29.4 μg/L, and DO: 8.5 %). The first axis length of 2.23 indicated that a linear model was appropriate, leading to the performance of RDA. The first RDA axis (RDA1) explained 34 % of the total variance in the species data. After removing variables with high VIFs, we tested the overall significance of the RDA model and the individual axes using permutation tests. Among all environmental variables, temperature ($p = 0.01$), NTU ($p = 0.03$), and PO_4 ($p = 0.04$) were found to be significant parameters affecting bacterial biofilm formation on MP (SI-5 and 6). These significant variables are indicative of low water clarity conditions. Specifically, temperature and NTU were associated with bacteria such as *Enterococcus, Streptococcus, Acinetobacter*. Whereas PO_4 was strongly associated with pathogenic bacteria like *Difteroid, Achromobacter, Proteus*, and *Salmonella*, which possibly indicate anthropogenic sources such as domestic waste discharge into the system (SI-5 and 6). Additionally, the higher salinity observed in the estuary (2.34 ‰) compared to other subregions was associated with a significant reduction in bacterial abundance ($R^2 = 0.87$).

4. Discussion

Microplastics in aquatic environments provide solid surfaces that act as essential habitats for diverse microbial communities. In the present study, we observed that the colonization of different bacterial strains varied depending on seasonal dynamics and anthropogenic impacts. For instance, a distinct difference in bacterial biofilms observed on polystyrene foam compared to other MP types indicates that the hydrophobicity and high buoyancy of PS foam (Battulga et al., 2022) results in a more favorable environment for bacterial biofilm formation in the Nilüfer Stream, which is subjected to high urbanization pressure. During the summer season, biofilms tend to be thicker because of elevated temperatures and reduced dissolved oxygen levels (Chen et al., 2020). While our understanding of hydrodynamic processes influencing microplastic residence time remains limited, recent studies suggest a notable prolongation of residence time within water column during summer (Elegami et al., 2023). Therefore, our results are in line with the notion that the dry season might influence bacterial MP colonization, consistent with previous studies (De Tender et al., 2015; Oberbeckmann et al., 2016; Zhang et al., 2021). The substantial bacterial MP colonization observed during the wet season in our study indicates an increased inflow from the catchment and a MP redistribution between riparian and aquatic environments. These processes create favorable niches for bacterial growth. The observed viable bacterial strains, namely *Proteobacteria, Firmicutes*, and *Bacteroidetes*, align with previous studies in aquatic environments (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2016; Curren and Leong, 2019). This prevalence of Gram-negative bacteria on positively charged MP surfaces may be attributed to their superior adhesion capacity facilitated by electrostatic interactions, particularly in comparison to MP with negatively charged surfaces (Gottenbos et al., 2001). The slight positive charge of MP surfaces contributed to their enhanced stability in nature (Larue et al., 2021). Moreover, the prevalence of these strains might be linked to the observed variety in MP polymer types specific to the distinct subregions (Fig. 4). Differences in bacterial biofilms on different MP types might be due to the release of different chemoattractants, e.g. nitrogen (Junaid et al., 2022). The presence of distinct bacterial taxa on MP, such as *Enterobacter* spp. and *E. coli*, can be attributed to sources and factors related to extensive industrialization, urbanization, and the discharge of human and animal wastes into the water body from the upstream catchment area. The high MP abundance stemming from a dense human population likely facilitates the prevalence of these bacterial coliform species (Zhang et al., 2015a; 2015b). Additionally, wastewater contamination from various anthropogenic sources further contributes to the deterioration of the freshwater resources in these regions. Anthropogenic activities including land use are crucial in shaping the

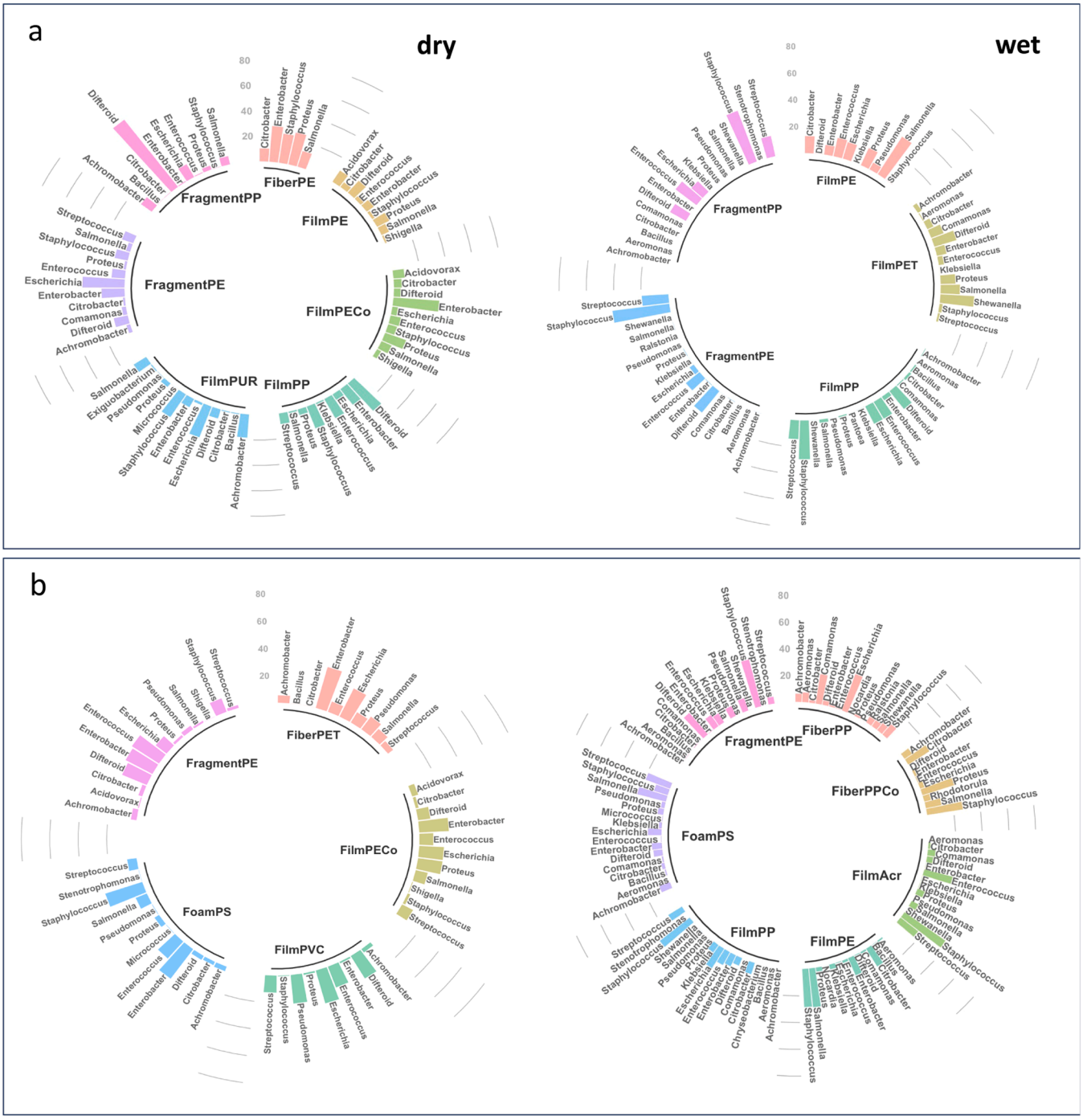


Fig. 4. Sunburst plot of Biofilm community in different polymer type during dry and wet seasons. a) Estuarine, b) Karadere River, c) Lake Uluabat, d) Nilüfer Stream. Abbreviations; PS: Polysytrene, PP: Polypropylene, PE: Polyethylene, PPCo: Polypropylene Copolymer, PECo: Polyethylene Copolymer, PA: Polyamid, ABS: Acrylonitrile Butadiene Styrene, Acr: Acrylic, PET: Polyethylene Terephthalate.

bacterial community associated with MP (Junaid et al., 2022; Moyal et al., 2023). Therefore, close associations between human activities, MP pollution, and the presence of specific bacterial taxa on various MP underscore the need for a vigilant monitoring and sustainable management practices to mitigate potential public health concerns.

4.1. *Presence of potential pathogens on MP*

The presence of certain potentially pathogenic bacterial genera such as *Pseudomonas (P. monteili), Comamonas (C. testosterone), Salmonella*, and *Shigella*, are of significant concerns, as they are commonly encountered on MP in both seasons (SI-3). Our results are consistent with findings in freshwaters from China, USA and other countries, i.e., the presence of *Pseudomonas, Acinetobacter, Bacillus, Escherichia, Klebsiella*, and *Aeromonas* (Junaid et al., 2022). These bacteria are common potential human pathogens and are associated with various infections in the gastrointestinal and urogenital tract via bacteremia and other routes. Moreover, exposure to bacterial biofilms on MP have triggered changes in the human gut microbiota and affect the human immune system (Hirt and Body-Malape, 2020). Thus, presence of these bacteria is of

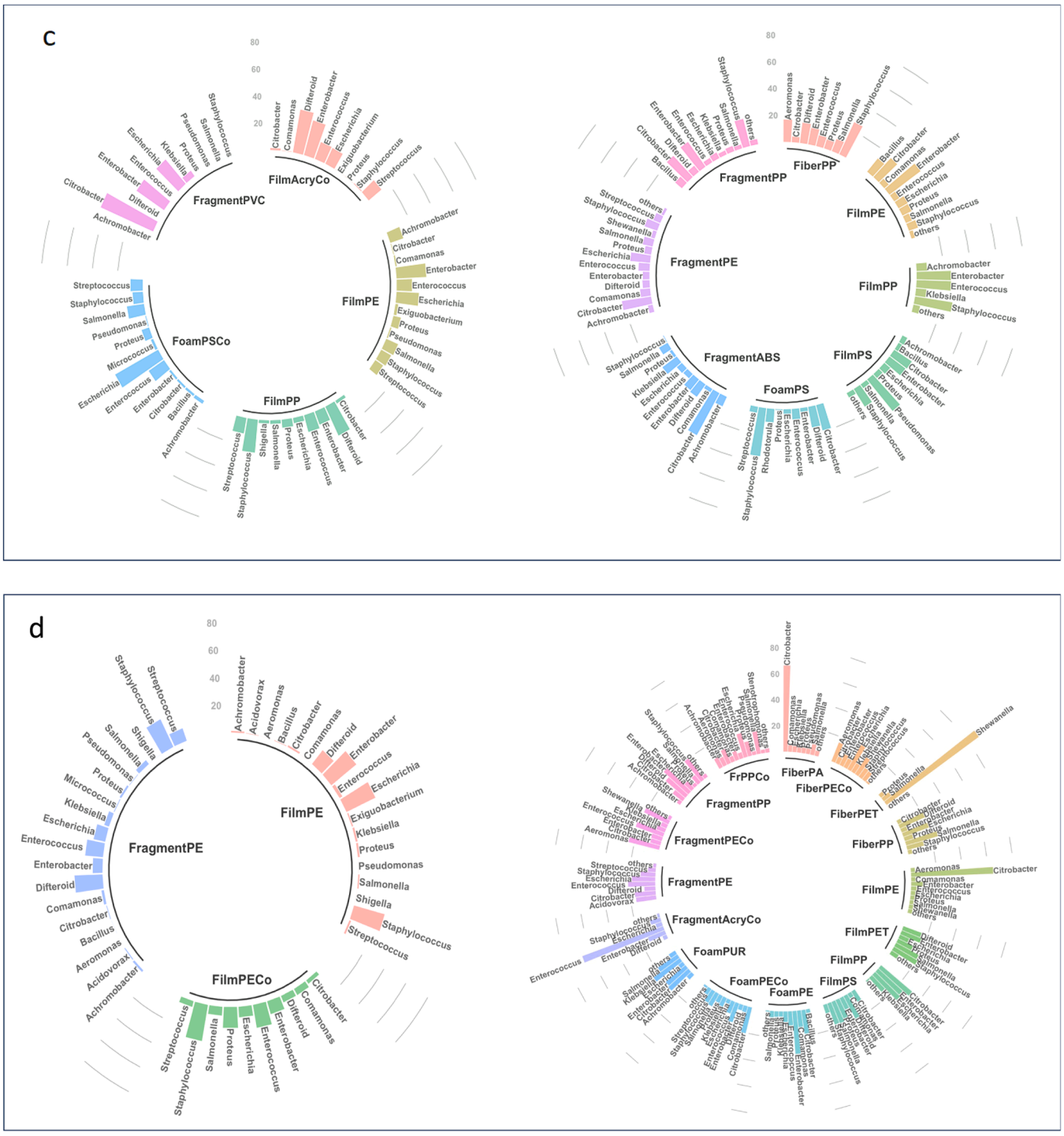


Fig. 4. (*continued*).

particular concern due to their potential harm to human health. Their occurrence on MP highlights a high potential to impact environmental and human health as MP also enhance the environmental spread of these pathogenic bacteria by long-distance transport.

MP serve as effective carriers for microorganisms, facilitating their survival and long-distance transport (Curren and Leong, 2019; Yang et al., 2020). Consequently, bacterial biofilms on MP act as reservoirs also for pathogenic microorganisms (Keswani et al., 2016; Kesy et al., 2019). Certain members of the biofilm consortia on MP can be opportunistic human pathogens, such as *Pseudomonas monteilii* and *Pseudomonas mendocina* (Wu et al., 2019). Additionally, complex microbial consortia present in MP biofilms may facilitate horizontal gene transfer, e.g. of antibiotics resistant genes, between bacterial species (Arias-Andres et al., 2018; Hirt and Body-Malapel, 2020). These findings suggest that specific bacterial genera have distinct associations with specific MP types depending on season and presumably other environmental factors. Furthermore, our results revealed bacterial selectivity for specific polymer types, i.e. marker bacterial genera of PE and PP were *Staphylococcus, Proteus, Escherichia, Enterococcus*, and *Enterobacter* (Figs 4 and 5). Similarly, water quality, particularly salinity, was an important environmental factor for MP biofilm formation (Li et al., 2019; Miao et al., 2021). These findings corroborate earlier results,

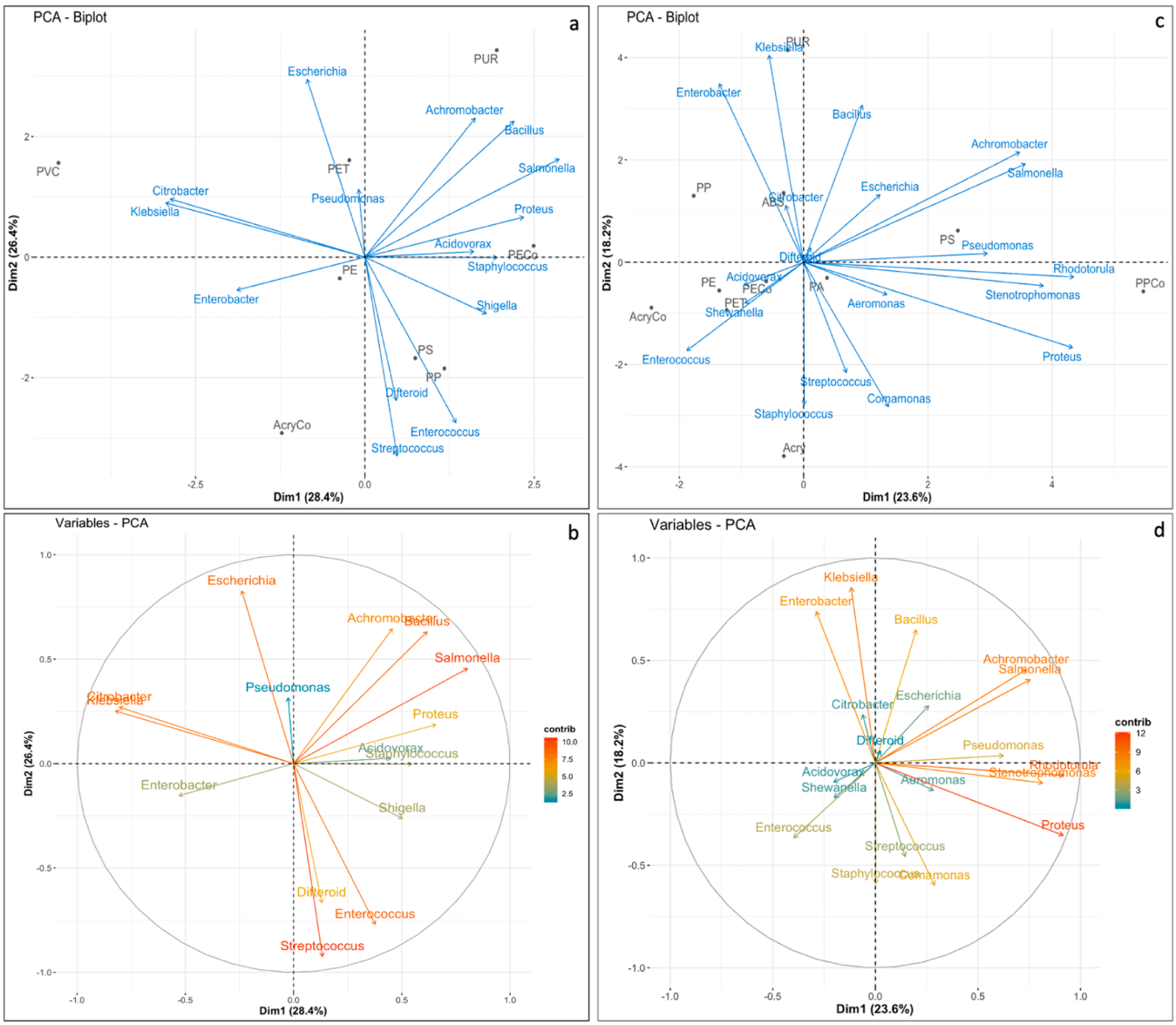


Fig. 5. PCA of the microbial assemblage using different polymer types; a-b) Dry season, c-d) Wet season.

indicating a more pronounced MP biofilm at increased nutrient levels (Miao et al., 2020) in the surrounding water. The correlation between environmental conditions and bacterial biofilm on MP are in line with these results.

Multiple factors, encompassing physical MP characteristics and environmental conditions, exert a significant influence on bacterial interactions on MP, leading to challenges in assessing their hydrophobicity (Min et al., 2020; Foster et al., 2020; He et al., 2022). Due to methodological limitations of our study, we were unable to conduct contact angle measurements on the collected MP samples, thus, impeding a precise determination of their hydrophobicity. Previously, the "Mathers Hydrophobicity Parameter (MHP)" model has demonstrated a good compatibility with water contact angle measurements, providing a reliable method for determining the hydrophobicity of polymers (Foster et al., 2020). Hydrophobicity changed with PAN-<Nylon6<PVC<PS<PE<PP according to the MHP model supporting previous findings (Min et al., 2020). It is worth noting that bacteria tend to adhere more rapidly to hydrophobic materials than hydrophilic ones (He et al., 2022). The comparison of bacterial colonization across different plastic types and bacterial species revealed variability among the examined species and plastics. However, eroded plastics and LDPE generally exhibited a higher bacterial abundance compared to HDPE and PS (Hossain et al., 2019). While, PS and PP display more tightly clustered groups, PE communities show a high degree of heterogeneity in their distribution (Frere et al., 2018). The study examined the adhesion of biofilms on MP under simulated water conditions. The MP—PVC, PA, and HDPE—differed in surface morphology and functional group composition. Each MP type exhibited varying capacities for biofilm formation and hosted distinct bacterial species compositions. However, all biofilms contained potentially pathogenic bacteria. After 28 days of incubation, the biofilm biomass increased in the order of PVC > HDPE > PA (He et al. 2023). In our study, the highest total bacterial colonization was observed on PS, PE, and PP, aligning well with their hydrophobicity. Considering the risk assesment in the region, no specific polymer risk index calculations have been conducted for biofilm-associated microplastics. However, the high abundance of PE and PP suggests a potential risk, with assigned risk scores of 1 for PE and 11 for PP, as reported by Lithner et al. (2011) (SI-7). Notably, the presence of polyurethane (PUR), which has the highest hazard score, particularly in the Nilüfer Stream and Estuarine area where the stream meets the Marmara Sea, highlights this locations as a high-risk environment in terms of hazard score (Fig. 4). Complex bacterial interactions within MP biofilms

emphasize the need for more comprehensive research to better understand health risks associated with MP in various aquatic environments.

Once a bacterial biofilm is formed on MP, substrate properties may lose their effectiveness for further microbial colonization (Kettner et al., 2019). Consequently, factors related to colonization time, such as dynamic equilibrium and community succession, though not studied in our current study, are also recognized as influential general determinants (Yu et al., 2023). Furthermore, MP undergo oxidation, e.g. when exposed to UV-radiation, and mechanical aging when exposed to marine and freshwater systems, leading to alteration in MP surface chemistry and roughness (Loeb and Neihof, 1975; Garrett et al., 2008; Rummel et al., 2017). Considering all these factors, MP appears to provide an effective micro-niche for microbial colonization, especially for potential pathogenic bacteria, due to their inherent surface hydrophobicity and roughness characteristics (He et al., 2022). However, further studies are needed to better understand the ecological implications and potential interactions between bacterial genera and MP in freshwater ecosystems.

5. Conclusion

MP biofilms not only provide new micro-habitats for colonization of diverse bacterial taxa including potentially pathogenic taxa, but also promote their growth and reproduction in aquatic environments. For survival and long-distance transport, MP are suitable bacterial carriers. Thereby, different MP types with specific microbial biofilms act as reservoirs for distinct microorganisms such as many pathogens. Our results, presenting a snapshot of environmental conditions, indicate that MP transport is intensified by increased inflow from the catchment during the wet season, with high human activities further amplifying this impact. This process enhances bacterial colonization on MPs and subsequently influences community structure. Many bacteria including pathogens can survive and form stable populations in biofilms. Thus, information on bacterial composition and survival on MP is essential for developing protocols and strategies for toxin and pathogen detection to prevent or reduce the build-up of bacterial pathogen reservoirs in water reclamation systems. Different plastic types may create distinct ecological niches for unique bacterial colonizers. Furthermore, culture-based methods provide some of the most established, accessible and cost-effective microbiological protocols, which could be extremely useful in helping to address key questions in plastisphere research. Our findings suggest that MP surface charge plays a significant role in bacterial adhesion with positively charged MP being more favorable substrates for Gram-negative bacteria. Moreover, pathogenic bacteria exhibited higher abundances on PE and PECo, the main MP polymers in the region. Consequently, it is evident that understanding of MP uptake mechanisms and the consequences of MP propagation through the food web are essential to develop effective management strategies for mitigating MP pollution and related health risks in aquatic ecosystems.

CRediT authorship contribution statement

Ülkü Nihan Tavşanoğlu: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Gülçin Akca:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Tülay Pekmez:** Investigation, Formal analysis. **Gökben Başaran Kankılıç:** Investigation, Formal analysis. **Tamer Çırak:** Investigation, Formal analysis. **Ali Serhan Çağan:** Investigation. **Selin Özkan Kotiloğlu:** Formal analysis. **Hans-Peter Grossart:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (Tübitak-project no: 119Y031). We thank to Belda Erkmen, Gizem Bezirci and Kerem Gökdağ for technical assistance in the field, Fatma Feisal Almas, Merve Seyfe and Melike Seyfe for the laboratory assistance. HPG was funded by the European Union's Horizon 2020 Research and Innovation programme, under the Grant Agreement number 965367 (PlasticsFatE).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.123142.

Data availability

Data will be made available on request.

References

Alvarez, G., Gonzalez, M., Isabal, S., et al., 2013. Method to quantify live and dead cells in multi-species oral biofilm by real-time PCR with propidium monoazide. AMB Express. 3, 1. https://doi.org/10.1186/2191-0855-3-1.

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. Front. Ecol. Environ. 13, 541–546. https://doi.org/10.1890/150017.

Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151. https://doi.org/10.1038/s41579-019-0308-0.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Arias-Andres, M., Klümper, U., Rajas-Jimenez, K., Grossart, H.-P., 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. Environ. Pollut. 237, 253–261. https://doi.org/10.1016/j.envpol.2018.02.05.

Ashfaq, M.Y., Da'na, D.A., Ghouti, M.A., 2022. Application of MALDI-TOF MS for identificaton of environmental bacteria: a review. J. Environ. Manage 305, 114359. https://doi.org/10.1016/j.jenvman.2021.114359.

Azeredo, J., Azeveredo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A.R., Desvaux, M., et al., 2017. Critical review on biofilm methods. Crit. Rev. Mcirobiol. 43, 313–351. https://doi.org/10.1080/1040841X.2016.1208146.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14, 251–263. https://doi.org/10.1038/nrmicro.2016.15.

Battulga, B., Kawahigashi, M., Oyuntsetseg, B., 2022. Characterization of biofilms formed on polystyrene microplastics (PS-MPs) on the shore of the Tuul River, Mongolia. Environ. Res. 212, 113329. https://doi.org/10.1016/j.envres.2022.113329.

Bhagwat, G., O'Connor, W., Grainge, I., Palanisami, T., 2021. Understanding the fundamental basis for biofilm formation on plastic surfaces: role of conditioning films. Front. Microbiol. 12, 687118. https://doi.org/10.3389/fmicb.2021.687118.

Boratyn, G.M., Camacho, C., Cooper, P.S., Coulouris, G., Fong, A., Ma, N., Madden, T.L., et al., 2013. BLAST: a more efficient report with usability improvements. Nucl. Acids Res. 41, W29–W33. https://doi.org/10.1093/nar/gkt282.

Chen, Z., Zhao, W., Xing, R., Xie, S., Yang, X., Cui, P., et al., 2020. Enhanced in situ biodegradation of microplastics in sewage sludge using hyperthermophilic composting technology. J. Hazard. Mater. 384, 121271. https://doi.org/10.1016/j.jhazmat.2019.121271.

Curren, E., Leong, S.C.Y., 2019. Profiles of bacterial assemblages from microplastics of tropical coastal environments. Sci. Total Environ. 655, 313–320. https://doi.org/10.1016/j.scitotenv.2018.11.250.

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. Environ. Sci. Technol. 49, 9629–9638. https://doi.org/10.1021/acs.est.5b01093.

Di Pippo, F., Venezia, C., Sighicelli, M., Pietrelli, L., Di Vito, S., Nuglio, S., Rossetti, S., 2020. Microplastic-associated biofilms in lentic Italian ecosystems. Water Res. 187, 116429. https://doi.org/10.1016/j.watres.2020.116429.

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. Environ. Pollut. 310, 119876. https://doi.org/10.1016/j.envpol.2022.119876.

Elegami, H., Frei, S., Boos, J.-P., Trommer, G., Gilfedder, B.S., 2023. Quantifying microplastic residence times in lakes using mesocosm experiments and transport modelling. Water Res. 229, 119463. https://doi.org/10.1016/j.watres.2022.119463.

Emenike, E.C., Okorie, C.J., Ojeyemi, T., Egbemhenghe, A., Iwuozor, K.O., Saliu, O.D., Okoro, H.K., Adeniyi, A.G., 2023. From ocens to dinner plates: the impact of microplastics on human health. Heliyon 9, e20440. https://doi.org/10.1016/j.heliyon.2023.e20440.

Eronen-Rasimus, E.L., Näkki, P.P., Kaartokallio, H.P., 2022. Degradation rates and bacterial community compositions vary among commonly used bioplastic materials in a brackish marine environment. Environ. Sci. Technol. 56, 15760–15769. https://doi.org/10.1021/acs.est.2c06280.

Forero-López, A.D., Brugnoni, L.I., Abasto, B., Rimondino, G.N., Lassalle, V.L., Ardusso, M.G., et al., 2022. Plastisphere on microplastics: in situ assays in an estuarine environment. J. Hazard. Mater. 440, 129737. https://doi.org/10.1016/j.jhazmat.2022.129737.

Foster, J.C., Akar, I., Grocott, M.C., Pearce, A.K., Mathers, R.T., O'Reilly, R.K., 2020. 100th anniversary of macromolecular science viewpoint: the role of hydrophobicity in polymer phenomena. ACS Macro Lett. 9, 1700–1707. https://doi.org/10.1021/acsmacrolett.0c00645.

Frere, 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. Environ. Pollut. 242, 614–625. https://doi.org/10.1016/j.envpol.2018.07.023.

Garrett, T.R., Bhakoo, M., Zhang, Z., 2008. Bacterial adhesion and biofilms on surfaces. Prog. Nat. Sci. 18, 1049–1056. https://doi.org/10.1016/pnsc.2008.04.001.

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: are we breathing it in? Curr. Opin. Environ. Sci. Health 1, 1–5. https://doi.org/10.1016/j.coesh.2017.10.002.

Gottenbos, B., Grijpma, D.W., van der Mei, H.C., Feijen, J., Busscher, H.J., 2001. Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. J. Antimicrob. Chemother 48, 7–13. https://doi.org/10.1093/jac/48.1.7.

Grossart, H.P., 2010. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. Environ. Microbiol. Rep. 2, 706–714. https://doi.org/10.1111/j.1758-2229.2010.00179.x.

He, S., Jia, M., Xiang, Y., Song, B., Xiong, W., Cao, J., Peng, H., Yang, Y., Wang, W., Yang, Z., Zeng, G., 2022. Biofilm on microplastics in aqueous environment: physicochemical properties and environmental implications. J. Hazard. Mater. 424, 127286. https://doi.org/10.1016/j.jhazmat.2021.127286.

He, S., Tong, J., Xiong, W., Xiang, Y., Peng, H., Wang, W., Yang, Y., Ye, Y., Hu, M., Yang, Z., et al., 2023. Microplastics influence the fate of antibiotics in freshwater environments: biofilm formation and its effect on adsorption behavior. J. Hazard. Mater. 442, 130078. https://doi.org/10.1016/j.jhazmat.2022.130078.

Hirt, N., Body-Malapel, M., 2020. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. Part. Fibre Toxicol. 17, 57. https://doi.org/10.1186/s12989-020-00387-7.

Hitchcock, J.N., 2020. Storm events as key moments of microplastic contamination in aquatic ecosystems. Sci. Total Environ. 734, 139436. https://doi.org/10.1016/j.scitotenv.2020.139436.

Hoellein, T.J., McCormick, A.R., Hittie, J., London, M.G., Scott, J.W., Kelly, J.J., 2017. Longitudinal patterns of micro-plastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. Freshw. Sci. 36, 491–507. https://doi.org/10.1086/693012.

Hossain, M.R., Jiang, M., Wei, Q., Leff, L.G., 2019. Microplastic surface properties affect bacterial colonization in freshwater. J. Basic Microbiol. 59, 54–61. https://doi.org/10.1002/jobm.201800174.

Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., et al., 2019. Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the "Plastisphere". Front. Microbiol. 10, 865. https://doi.org/10.3389/fmicb.2019.00865.

Janda, J.M., Abbott, S.L., 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin. Microbiol. 45, 2761–2764. https://doi.org/10.1128/JCM.01228-07.

Junaid, M., Siddiqui, J.A., Sadaf, M., Liu, S., Wang, J., 2022. Enrichment and dissemination of bacterial pathogens by microplastics in the aquatic environment. Sci. Total Environ. 830, 154720. https://doi.org/10.1016/j.scitotenv.2022.154720.

Keswani, A., Oliver, D.M., Gutierrez, T., Quilliam, R.S., 2016. Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. Mar. Environ. Res. 118, 10–19. https://doi.org/10.1016/j.marenvres.2016.04.006.

Kesy, K., Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2019. Spatial environmental heterogeneity determines young biofilm assemblages on microplastics in Baltic Sea mesocosms. Front. Microbiol. 10, 1665. https://doi.org/10.3389/fmicb.2019.01665.

Kettner, M.T., Rojas-Jimenez, K., Oberbeckmann, S., Labrenz, M., Grossart, H.-P., 2017. Microplastics alter composition of fungal communities in aquatic ecosystems. Environ. Microbiol. 19, 4447–4459. https://doi.org/10.1111/1462-2920.13891.

Kettner, M.T., Oberbeckmann, S., Labrenz, M., Grossart, H.-P., 2019. The eukaryotic life on microplastics in brakish ecosystems. Front. Microbiol. 10, 538. https://doi.org/10.3389/fmicb.2019.00538.

Klein, M.I., Scott-Anne, K.M., Gregoire, S., et al., 2012. Molecular approaches for viable bacterial population and transcriptional analyzes in a rodent model of dental caries. Mol. Oral Microbiol. 27, 350–361. https://doi.org/10.1111/j.2041-1014.2012.00647.x.

Koelmans, A.A., Mohamed, Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res. 155, 410–422. https://doi.org/10.1016/j.watres.2019.02.054.

Kruger, N.J., Buhler, C., Iwobi, A.N., et al., 2014. "Limits of control"– crucial parameters for a reliable quantification of viable campylobacter by real-time PCR. PLoS ONE 9, e88108. https://doi.org/10.1371/journal.pone.0088108.

Lane, D.J., 1991. in: Nucleic Acid Techniques in Bacterial Systematics. E Stackebrandt and M Goodfellow (eds) Wiley, London, pp 115.

Larue, C., Sarret, G., Castillo-Michel, H., Pradas, Del Real, A.E., 2021. A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms. Small 17, e2005834. https://doi.org/10.1002/smll.202005834.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280. https://doi.org/10.1007/s004420100716.

Li, W., Zhang, Y., Wu, N., Zhao, Z., Xu, W., Ma, Y., Niu, Z., 2019. Colonization characteristics of bacterial communities on plastic debris influenced by environmental factors and polymer types in the Haihe Estuary of Bohai Bay, China. Environ. Sci. Technol. 53, 10763–10773. https://doi.org/10.1021/acs.est.9b03659.

Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409 (18), 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038.

Loeb, G.I., Neihof, R.A., 1975. Marine conditioning films. Applied Chemistry at Protein Interfaces. American Chemical Society, Washington, DC, pp. 319–335. https://doi.org/10.1021/ba-1975-0145.ch016, 145.

Logares, R., Sunagawa, S., Salazar, G., et al., 2014. Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. Environ. Microbiol. 16, 2659–2671. https://doi.org/10.1111/1462-2920.12250.

McGinnis, S., Madden, T.L., 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucl. Acids Res. 32, W20–W25. https://doi.org/10.1093/nar/gkh435.

Metcalf, R., White, H.L., Ormsby, M.J., Oliver, D.M., Quilliam, R.S., 2023. From wastewater discharge to the beach: survival of human pathogens bound to microplastics during transfer through the freshwater-marine continuum. Environ. Pollut. 319, 120955. https://doi.org/10.1016/j.envpol.2022.120955.

Miao, L., Wang, C., Adyel, T.M., Wu, J., Liu, Z., You, G., Meng, M., Qu, H., Huang, L., Yu, Y., Hou, J., 2020. Microbial carbon metabolic functions of biofilms on plastic debris influenced by the substrate types and environmental factors. Environ. Int. 143, 106007. https://doi.org/10.1016/j.envint.2020.106007.

Miao, L., Yu, Y., Adyel, T.M., Wang, C., Liu, Z., Liu, S., Huang, L., You, G., Meng, M., Qu, H., Hou, J., 2021. Distinct microbial metabolic activities of biofilms colonizing microplastics in three freshwater ecosystems. J. Hazard. Mater. 403, 123577. https://doi.org/10.1016/j.jhazmat.2020.123577.

Min, K., Cuiffi, J.D., Mathers, R.T., 2020. Ranking environmental degradation trends of plastic marine debris based on physical properties and molecular structure. Nat. Comm. 11, 727. https://doi.org/10.1038/s41467-020-14538-z.

Moyal, J., Dave, P.H., Wu, M., Karimpour, S., Brar, S.K., Zhong, H., Kwong, R.W.M., 2023. Impacts of biofilm formation on the physicochemical properties and toxicity of microplastics: a concise review. Rev. Environ. Contam. Toxicol. 261, 8. https://doi.org/10.1007/s44169-023-00035-z.

Muñoz-Torres, P., Márquez, S.L., Sepúlveda-Chavera, G., Cárdenas-Ninasivincha, S., Arismendi-Macuer, M., et al., 2023. Isolation and identification of bacteria from three geothermal sites of the Atacama Desert and their plant-beneficial characteristics. Microorganisms 11, 2635. https://doi.org/10.3390/microorganisms11112635.

Mughini-Gras, L., van der Plaats, R.Q.J., van der Wielen, P.W.J.J., Bauerlein, P.S., de Roda Husman, A.M., 2021. Riverine microplastic and microbial community compositions: a field study in the Netherlands. Water.. Res. 192, 116852. https://doi.org/10.1016/j.watres.2021.116852.

Nava, V., Chandra, S., Aherne, J., Alfonso, M.B., Antao-Geraldes, A.M., et al., 2023. Plastic debris in lakes and reservoirs. Nature 619, 317–322. https://doi.org/10.1038/s41586-023-06168-4.

Nguyen, H.T., Choi, W., Kim, E.J., Cho, K., 2022. Microbial community niches on microplastics and prioritized environmental factors under various urban riverine conditions. Sci. Total Environ. 849, 157781. https://doi.org/10.1016/j.scitotenv.2022.157781.

Nguyen, N.H.A., Marlita, M., El-Temsah, Y.S., Hrabak, P., Riha, J., Sevcu, A., 2023. Early stage biofilm formation on bio-based micro-plastics in a freshwater reservoir. Sci. Total Environ. 858, 159569. https://doi.org/10.1016/j.scitotenv.2022.159569.

Oberbeckmann, S., Löder, M.G., Labrenz, M., 2015. Marine microplsatic-associated biofilms- a review. Environ. Chem. 12, 551–562. https://doi.org/10.1071/EN15069.

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, e0159289. https://doi.org/10.1371/journal.pone.0159289.

Parida, P.K., Behera, B.K., Dehury, B., Rout, A.K., Sarkar, D.J., Rai, A., Kumar Das, B., Mohapatra, T., 2022. Community structure and function of microbiomes in polluted stretches of river Yamuna in New Delhi, India, using shotgun metagenomics. Environ. Sci. Pollut. Res. 29, 71311–71325. https://doi.org/10.1007/s11356-022-20766-1.

Plastics Europe, 2021. Plastics – The Facts 2021. An Analysis of European Plastics Production, Demand and Waste Data. Plastics Europe Association of Plastic Manufacturers, Brussels. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/.

R Core Team, 2022. R: A Language and Environment Fot Statistical Computing. R foundation for statistical computing, Vienna, Austria. URL. https://www.R-profect.org/.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. 4, 258–267. https://doi.org/10.1021/acs.estlett.7b00164.

Sauer, K., Stoodley, P., Goeres, D.M., Stoodley, L.H., Burmolle, M., Stewart, P.S., Bjarnsholt, T., 2022. The biofilm life cycle: expanding the conceptual model of

biofilm formation. Nat. Rev. Microbiol. 608–620. https://doi.org/10.1038/s41579-022-00767-0.

Stevenson, E.M., Buckling, A., Cole, M., Lindeque, P.K., Murray, A.K., 2023. Culturing the plastisphere: comparing methods to isolate culturable bacteria colonising microplastics. Front. Microbiol. 14, 1259287. https://doi.org/10.3389/fmicb.2023.1259287.

Tavşanoğlu, U.N., Başaran Kankılıç, G., Akca, G., Çırak, T., Erdoğan, Ş., 2020. Microplastics in a dam lake in Turkey: type: mesh size effect, and bacterial communities. Environ. Sci. Pollut. Res. 27, 45688–45698. https://doi.org/10.1007/s11356-020-10424-9.

ter Braak, C.F.K., 1995. Ordination. In: Jongman, R.H.G., ter Braak, C.J.F., van Tongeren, O.F.R. (Eds.), Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, pp. 91–173.

Tessler, M., Neumann, J.S., Afshinnekoo, E., Pineda, M., Hersch, R., et al., 2017. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. Sci. Rep. 7, 6589. https://doi.org/10.1038/s41598-017-06665-3.

Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., et al., 2017. A communal cataloque reveals Earth's multiscale microbial diversity. Nature 551, 457–463.

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. Sci. Total Environ. 734, 139237. https://doi.org/10.1016/j.scitotenv.2020.139237.

Wang, J., Qin, X., Guo, J., Jia, W., Wang, Q., Zhang, M., Huang, Y., 2020. Evidence of selective enrichment of bacterial assemblages and antibiotic resistant genes by microplastics in urban rivers. Water Res. 183, 116113. https://doi.org/10.1016/j.watres.2020.116113.

Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., Wang, Y., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res. 165, 114979. https://doi.org/10.1016/j.watres.2019.114979.

Yang, Y., Liu, W., Zhang, Z., Grossart, H.-P., Gadd, G.M., 2020. Microplastics provide a new niches in aquatic environments. Appl. Microbiol. Biotechnol. 104, 6501–6511. https://doi.org/10.1007/s00253-020-10704-x.

Yang, G., Gong, M., Mai, L., Zhuang, L., Zeng, E.Y., 2021. Diversity and structure of microbial biofilms on microplastics in riverine waters of the Pearl River Delta, China. Chemosphere 272, 129870. https://doi.org/10.1016/j.chemosphere.2021.129870.

Yasunaga, A., Yoshida, A., Morikawa, K., et al., 2013. Monitoring the prevalence of viable and dead cariogenic bacteria in oral specimens and in vitro biofilms by qPCR combined with propidium monoazide. BMC Microbiol. 13, 157. https://doi.org/10.1186/1471-2180-13-157.

Yu, Y., Miao, L., Adyel, T.M., Waldschläger, K., Wu, J., Hou, J., 2023. Aquatic plastisphere: interactions between plastics and biofilms. Environ. Pollut. 322, 121196. https://doi.org/10.1016/j.envpol.2023.121196.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "plastisphere": microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/es401288x.

Zhang, K., Gong, W., Lv, J., Xiong, X., Wu, C., 2015a. Accumulation of floating microplastics behind the Three Gorges Dam. Environ. Pollut. 204, 117–123. https://doi.org/10.1016/j.envpol.2015.04.023.

Zhang, W., Ma, X., Zhang, Z., Wang, Y., Wang, J., Wang, J., Ma, D., 2015b. Persistent organic pollutants carried on plastic resin pellets from two beaches in China. Mar. Pollut. Bull. 99, 28–34. https://doi.org/10.1016/j.marpolbul.2015.08.002.

Zhang, B., Yang, X., Liu, L., Chen, L., Teng, J., Zhu, X., Zhao, J., Wang, Q., 2021. Spatial and seasonal variations in biofilm formation on microplastics in coastal waters. Sci. Total Environ. 770, 145303. https://doi.org/10.1016/j.scitotenv.2021.145303.