Received: 14 April 2023 | Accepted: 27 October 2023

DOI: 10.1111/1462-2920.16536

BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Freshwater plastispheres as a vector for foodborne bacteria and viruses

Ingun Lund Witsø[1] | **Adelle Basson**[1,2] | **Hilde Vinje**[3] | **Ann-Katrin Llarena**[1] | **Carlos Salas Bringas**[4] | **Marina Aspholm**[1] | **Yngvild Wasteson**[1] | **Mette Myrmel**[5]

[1]Faculty of Veterinary Medicine, Food Safety Unit, The Norwegian University of Life Sciences, Ås, Norway

[2]Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[3]Faculty of Chemistry, Biotechnology and Food Science, The Norwegian University of Life Sciences, Ås, Norway

[4]Institute for Marine Operations and Civil Engineering, Norwegian University of Science and Technology, Ålesund, Norway

[5]Faculty of Veterinary Medicine, Virology Unit, The Norwegian University of Life Sciences, Ås, Norway

Correspondence
Ingun Lund Witsø, The Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Food Safety Unit, P.O Box 5003, 1432 Ås, Norway.
Email: ingun.lund.witso@nmbu.no

Funding information
Norges Forskningsråd, Grant/Award Number: 302996

Abstract

There is growing evidence that plastic particles can accumulate microorganisms that are pathogenic to humans or animals. In the current study, the composition of the plastispheres that accumulated on polypropylene (PP), polyvinyl chloride (PVC), and high-density polyethylene (HDPE) pieces submerged in a river in the southeast Norway was characterized by 16S rRNA amplicon sequencing. Seasonal and geographical effects on the bacterial composition of the plastisphere were identified, in addition to the detection of potential foodborne pathogenic bacteria and viruses as part of the plastisphere. The diversity and taxonomic composition of the plastispheres were influenced by the number of weeks in the river, the season, and the location. The bacterial diversity differed significantly in the plastisphere from June and September, with a generally higher diversity in June. Also, the community composition of the plastisphere was significantly influenced by the geographical location, while the type of plastic had less impact. Plastics submerged in river water assembled a variety of microorganisms including potentially pathogenic bacteria and viruses (noro- and adenovirus) detected by qPCR. Cultivation methods detected viable bacteria such as *Escherichia coli* and *Listeria monocytogenes*. The results highlight the need for additional research on the risk of contaminating food with plastic particles colonized with human pathogens through irrigation water.

INTRODUCTION

The constantly increasing plastic production and the subsequent environmental pollution with plastic debris, are currently one of the challenges the planet's ecosystems are facing. Since 2015, approximately 6300 million metric tons of plastic have been produced, of which 79% has been discarded and accumulated in natural environments and landfills (Geyer, 2020; PlasticEurope., 2021). The largest groups of non-fibre plastics produced are polyethylene (PE, 36%) followed by polypropylene (PP, 21%) and polyvinyl chloride (PVC, 12%). PE and PP are predominantly used for plastic packaging while the building and construction sector uses up to 69% of the PVC produced. These mass-produced plastic types do not decompose easily but are slowly fragmented into smaller pieces due to UV radiation and mechanical forces (Geyer et al., 2017).

When present in aquatic environments, plastics accumulate inorganic and organic substances and create plastispheres on their surfaces, a diverse plastic-associated microbial community (Zettler et al., 2013). The adherence of inorganic and organic molecules together with microorganisms results in the formation of an eco-corona (Galloway et al., 2017; Lynch & Dawson, 2014). The surface chemistry of pristine

plastics, which differs from organic surfaces commonly found in the natural aquatic environment, may influence the microbiota's composition and behaviour (Ogonowski et al., 2018; Zettler et al., 2013). Bacteria that reside in plastispheres can benefit from improved nutrient acquisition, protection against desiccation and toxic substances, as well as increased horizontal gene transfer (Rummel et al., 2017). The photochemical and biological degradation of plastics may facilitate the leaching of dissolved organic substances (Romera-Castillo et al., 2018), which has been shown to provide nutrients that promote bacterial growth (Romera-Castillo et al., 2018; Sheridan et al., 2022). However, hazardous chemicals added to synthetic polymers during production, for example, to improve plastic flexibility and heat stability, can also inhibit bacterial growth in plastic leachate (Tetu et al., 2019).

Environmental biofilms can consist of complex microbial communities of algae, bacteria, fungi, viruses, and protozoa embedded in extracellular polymeric substances (EPS) (Burmølle et al., 2014). Several studies have reported that pathogenic variants of microorganisms are found in plastispheres. There are concerns about whether plastics, particularly microplastics, can act as vectors for the introduction of pathogens into food and drinking water chains (Kirstein et al., 2016; Silva et al., 2019; Yang et al., 2020). Viruses, including enteric viruses and respiratory viruses, are ubiquitous and can also settle on naked plastic surfaces. Human entero- and norovirus (NoV) have been found to adhere to naked plastics as well as to biofilms on PE retrieved from treated wastewater (Skraber et al., 2009).

The number of scientific articles describing plastispheres in the ocean is on the rise (Amaral-Zettler et al., 2020; Frere et al., 2018; Moore et al., 2020). However, few studies have specifically addressed the formation of plastispheres in freshwater systems. Hoellein et al. studied bacterial communities on different surfaces in freshwater systems and found more bacteria belonging to the family *Burkholderiaceae* in biofilms on plastic particles compared to other materials from the same environment (Hoellein et al., 2014). Furthermore, by using high throughput amplicon sequencing, McCormick et al., reported enrichment of bacteria belonging to the genera *Arcobacter*, *Campylobacter*, and *Pseudomonas* on plastic compared to among planktonic organisms in river water (McCormick et al., 2016). Notably, *Burkholderia*, *Arcobacter*, and *Campylobacter* include many species that cause intestinal illness in humans. Plastispheres may also contribute to the transmission and spread of antibiotic-resistance genes in marine, freshwater, and soil environments (Zhu et al., 2022).

Plastic from urban and rural sewage can enter aquatic environments (rivers, lakes, and the ocean) through for instance untreated sewage overflow, treated wastewater release, and agricultural use of sludge (Edo et al., 2020; Prata, 2018). Because freshwater sources are frequently used for vegetable irrigation, there is a risk of contamination of fresh produce with plastic particles and their associated microorganisms. Irrigation water droplets have been shown to carry contaminants, including plastic, from the environment to the surface of plants used as food (Allende et al., 2017; Girardin et al., 2005; Jiang et al., 2023; Zhou et al., 2020). Consumers are more concerned about healthy food, sustainability, and nutrition, and there is a general recommendation to increase consumption of fresh produce to improve the sustainability of our food production (Clem & Barthel, 2021; Kamiński et al., 2020). However, food poisoning caused by the ingestion of fresh produce is common, as several large outbreaks caused by both bacteria and viruses have been reported (Alegbeleye et al., 2018). To evaluate the food safety risks associated with plastics we need more knowledge on the accumulation and survival of pathogenic microbes on different types of plastic in freshwater systems.

The current study aims to fill knowledge gaps about the microbiota that assembles on various types of plastic in river water. Pieces of PP, PVC, and HDPE were placed in a river used for irrigation of fresh produce to investigate how plastic type, time in the water and season, and geographical location affected the taxonomic content and diversity of the microorganisms present in the plastisphere. Furthermore, the presence of pathogenic bacteria and viruses was investigated to determine how different types of plastic influenced the microbial hazards of freshwater systems. We combined the use of traditional cultivation methods with modern molecular identification techniques like MALDI-TOF and 16S RRNA amplicon sequencing to get specific and reliable information about the plastic-associated microbial communities.

EXPERIMENTAL PROCEDURES

Sampling area and time of study

Lier is a rural municipality in Viken county in Eastern Norway. The lush and green valley is characterized by nutrient-rich soil provided by marine deposits and a big agricultural industry accounting for 12% of Norway's vegetable cultivation area. Horticulture production is especially prominent, and some outdoor produce includes cabbage, celery, lettuce, and strawberries, in addition to cucumbers, herbs, and lettuce produced in greenhouses (Lier Kommune 2012 (Lier municipality)). The Lier River originates from lakes and streams in a forest area, ends in the narrow Drammen fjord, and is a major source of irrigation water throughout the harvest season from May until October.

Sewage is an important source of pollution in the Lier River. Wastewater management in Lier consists of municipal sewage networks, with treatment plants in

the various districts as well as private, smaller sewage plants. The pollution is related to the transport of wastewater, surface runoff, and drainage water from the sewerage network. When the capacity of the pipes is exceeded, the surplus water is emptied into the Lier River. Sewage also enters the river from a few private septic tanks (Lier Kommune 2012 (Lier municipality)).

The two sampling sites selected for this study, hereafter named Loc1 (59°45′03.3"N 10°17′06.3″ E) and Loc2 (59°47′34.7"N 10°14′17.1″ E) (Figure 1), are both found in dense agricultural areas with vegetable production. Loc2 is located downstream of wastewater treatment plants, while Loc1 is in a more densely populated area with large agricultural activity. The samplings took place in June and September 2021. Physiochemical and biological measurements of the water were done to give background information regarding the water quality of the lier river (Nilsson & Grimsgaard, 2021). Details are provided in the supplementary materials (Table S6).

Biofilm formation on plastic in the Lier River

Biofilm was accumulated on three different plastic types, polypropylene (PP), polyvinyl chloride (PVC), and polyethylene high-density (HDPE) (Astrup AS, Oslo Norway). Each piece of plastic was $6 \times 4 \times 0.6$ cm^3. The pieces were sterilized with hypochlorite and mounted onto custom-made devices (Figure S1) before being inserted into the river. To ensure the device was positioned vertically in the river, an inflatable buoy was attached to one side and a weight to the opposite side. Each device was secured to shore. After harvesting, the plastic pieces were collected in sterile containers filled with river water and transported to the laboratory within 2 h. The pieces were rinsed carefully three times with distilled water to remove loosely attached organic material and immediately analysed for culturable bacterial pathogens, or frozen at −80°C.

Extraction of DNA/RNA from biofilms

Three pieces of each plastic type were regarded as one single sample, and three replicates of each sample were included for each plastic type. DNA was extracted from samples collected after 2 and 4 weeks in the river, in June and September, and from two different locations. For complete sample information see Table S1.

The samples were thawed on ice and biofilms from both sides of three plastic pieces were pooled by scraping into a ZR Bashingbead Lysis tube with 0.1- and 0.5-mm beads, containing 750 μL DNA/RNA Shield™ provided with the extraction kit (ZymoBIOMICS DNA/RNA Minprep kit. Nordic BioSites AS, Norway). The samples were homogenized using MP Bio FastPrep-24 (VWR) at 6 m/s for 5×45 s, with 15 s breaks between each cycle while kept on ice. After bead beating, DNA and RNA were extracted separately according to the manufacturer's instructions. Positive and negative controls were included in each round of extraction. The ZymoBIOMICS™ Microbial Community Standard, Catalogue no. D6300 (Zymo Research, California, USA) was used as the positive control. This community is a defined mixture of microbial strains

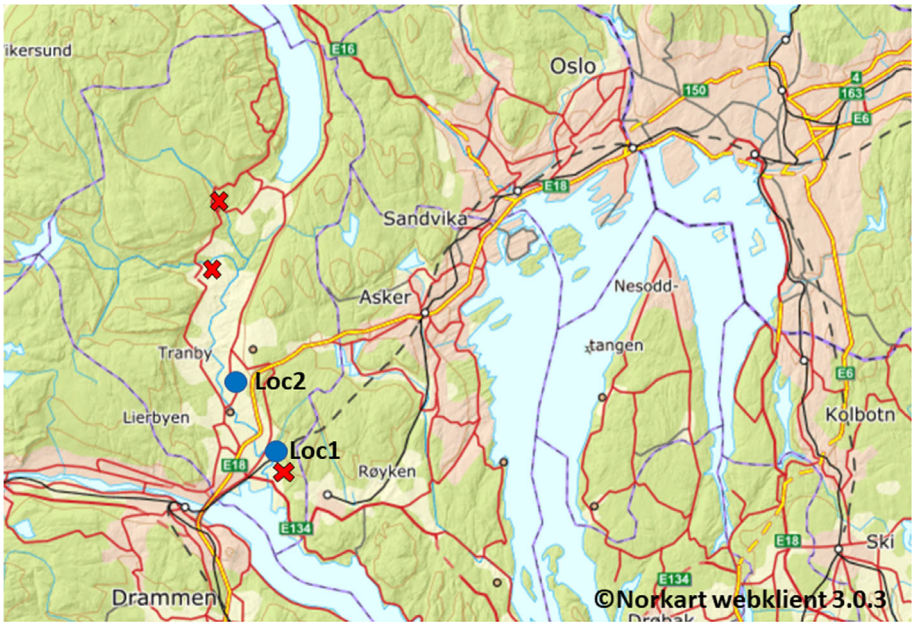

FIGURE 1 Geographic location of the sample sites in Lier River. The two locations (Loc1 and Loc2) are marked as blue dots. The wastewater treatment plants located along the river are marked with red crosses.

created to imitate the composition of a microbiota sample. ZymoBIOMICS™ DNase/RNase-free water was used as a negative control. The concentration and purity of DNA and RNA were measured using Qubit dsDNA BR Assay kit or RNA BR assay kit, respectively, on Fluorometer 2.0 (Invitrogen/Life Technologies, Carlsbad, California, USA) and Nanodrop Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and stored at −80°C until use. The extracted DNA was used for 16S rRNA gene sequencing and detection of specific pathogens by qPCR as described below. Isolated RNA was screened for the viral pathogens adenovirus 40/41 (AdV) and norovirus GI/GII (NoV) with (RT)-qPCR as described below.

16S rRNA amplicon sequencing of bacterial DNA

The bacterial composition of the plastispheres was characterized by amplicon sequencing of the V3-V4 region of the 16S rRNA gene, carried out at Novogene Genome Sequencing Company (Company Limited, Cambridge UK). The amplicon sequencing was performed in two batches. The first batch consisted of samples collected after 2 weeks in the river during June, while the remaining samples were sequenced in the second batch. Briefly, amplicons were generated using the barcoded primers 515F and 806R and the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). Products were size selected by 2% agarose gel electrophoresis. Libraries were prepared by pooling PCR products from each sample followed by end-repair, A-tailing, and ligation with Illumina adapters. Sequences were obtained on the Illumina NovaSeq™ platform in a 2 × 250 bp paired-end run.

Data processing

Data processing was carried out by Novogene Genome Sequencing Company. Briefly, paired-end reads were assigned to samples based on their barcodes, truncated by removing the barcode and primer sequences, and merged using FLASH software version 1.2.11 (http://ccb.jhu.edu/software/FLASH/; Magoc & Salzberg, 2011). Quality filtering was made with the fastp software (version 0.20.0; Chen et al., 2018). Clean tags were compared with the Silva reference database (https://www.arb-silva.de), using Vsearch version 2.15.0. to detect and remove the chimera sequences, resulting in effective tags (Haas et al., 2011; Quast et al., 2012; Rognes et al., 2016). Denoise was performed on the effective tags using the deblur module in the QIIME2 software (version 2020.6). Amplicon Sequence Variants (ASVs) with an abundance of less than five were filtered out to obtain the final ASVs (Bolyen et al., 2019).

The Classify-sklearn module in QIIME2 software was used for species annotation for each ASV. In 2021, Oren and Garrity presented name changes to all bacteria phyla (Oren & Garrity, 2021). As our data were processed against the SSUrRNA database of SILVA138 Database (Quast et al., 2013; Wang et al., 2007) we decided to use phylum names according to the database. The newly proposed names are (old names in brackets): Pseudomonadota (Proteobacteria), Bacillota (Firmicutes), Actinomycetota (Actinobacteria), and Bacteroidota (Bacteroidetes) (Oren & Garrity, 2021).

Diversity analysis and statistics

The absolute abundance of ASVs was normalized based on the minimum number of reads in all samples, in our case sample A218 (21,087 reads). After normalization, the number of reads of all samples was equal to the number of reads of the minimum sample. Subsequent analyses of alpha- and beta-diversity were performed based on the output of the normalized data. The diversity was analysed with 'Plastic', 'Duration', 'Month' and 'Location' as possible associated variables.

The Shannon and Chao1 alpha-diversity indices were calculated in QIIME2 and displayed in plots using the package ggplot2 in RStudio (Version 2023.06.2 + 561) (Bolyen et al., 2019; RStudio, 2020). To investigate the diversity indices two variance (ANOVA) models, with interaction effects, were conducted with the two different alpha measures as response variables and the four possible associated variables as explanatory variables. To simplify the models as much as possible a partial *F*-test was conducted, testing the full models with four-way interaction against reduced models with three-way and two-way interactions, respectively. Consequently, for Chao1 the model included all two-way interactions of the four explanatory variables, and for Shannon, a full model with all interactions up to four ways was conducted. Tukey's post hoc test was performed for pairwise comparisons of the results. The statistical analysis for the alpha diversity was performed in RStudio (version 2023.06.2 + 561). For all statistical analyses, the model assumptions were checked and fulfilled and $p < 0.05$ was considered significant.

Non-Metric Multidimensional Scaling (NMDS) with Bray–Curtis dissimilarity (dissimilarity matrix of ASV relative abundances) and weighted UniFrac distance (abundance-weighted phylogenetic relationship of ASVs) were conducted for visual presentation of the similarity of the bacterial community composition between the samples. All distance matrices were calculated in QIIME2, while the plots were done in RStudio using the *Vegan* and *Phyloseq* packages (Bolyen et al., 2019; McMurdie & Holmes, 2013; Oksanen, 2007). To analyse the influence of the different variables (Plastic, Duration, Month,

and Location) on the bacterial community composition, permutational multivariate analysis of variance (PERMANOVA) with two-way interaction effects were calculated using the Bray–Curtis dissimilarities and weighted UniFrac distance. The decision to keep these models with only the two-way interaction was due to an investigation of the partial R^2 from the adonis function in RStudio. All PERMANOVA analyses were done in RStudio (version 2023.06.2 + 561) using the *Vegan* package with the adonis() function (Oksanen, 2007; RStudio, 2020).

For a more detailed investigation of the taxa in the bacteria diversity, relative abundances were plotted for the 20 most abundant phyla and the 20 most abundant genus using the package ggplot2 in RStudio (Version 2023.06.2 + 561). A visual examination of the plots together with the relative abundance was discussed.

One of the aims of the study was to investigate the difference in relative abundance with a focus on the different types of plastic for pathogenic bacteria. Most of the pathogenic bacteria were under the threshold value and not detectible in all the samples, but for the relative abundance of *Escherichia*/*Shigella* an ANOVA/linear model with up to four-way interactions was conducted with 'Plastic', 'Duration', 'Month' and 'Location' as explanatory variables to test for plastic effects.

Isolation and identification of potential bacterial pathogens from the plastisphere

Bacteria were isolated from biofilm on the plastic pieces immediately after returning to the lab. The surface of one side of the pieces was divided into three similar-sized areas, and each area was swabbed with one dry, sterile swab (Figure S1). Three different agar plates were streaked and incubated; blood agar aerobically at 37°C for 24 h, blood agar anaerobically at 37°C for 48 h, and Luria Broth (LB) agar aerobically at 22°C for 72 h. Single colonies with unique morphologies were purified in two rounds of subculturing under the described conditions (Table S2). The other surface of the plastic piece was divided into five similar-sized areas, swabbed, and streaked onto selective media for *Aeromonas* spp., *Campylobacter* spp., *Escherichia coli*, *Listeria* spp., and *Salmonella* spp. and incubated accordingly (Table S2). Stocks of all the isolates were kept at −80°C until further characterization.

Acquired isolates were subjected to matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). Fresh colonies were prepared on blood or LB agar plates including the calibration strain *E. coli* ATCC® 8739. One colony from each isolate was spotted on a VITEK® MS-DS target slide (bioMérieux, Marcy l'Etoile, France), while *E. coli* ATCC® 8739 was deposited on position xA1, xB1 and xC1, depending on the number of isolates tested. Each sample spot was overlayed with 1 μL of the VITEK® MS- cyano-4-hydroxycinnamic acid (CHCA) matrix solution that dried until crystallization. The target slide was run in the VITEK®MS instrument (bioMérieux, Marcy l'Étoile, France) according to the user manual. Data acquisition and processing were performed with the VITEK®MS Plus/ RUO SARAMIS® database for species identification.

Pathotyping of *E. coli* isolates

To determine whether E. coli can be defined as potential intestinal pathogenic E. coli, a multiplex PCR was run for the presence of virulence genes (Osińska et al., 2022). Genomic DNA was extracted by resuspending colonies from fresh blood agar plates in 500 μL sterile H_2O, heating at 95°C for 10 min, and centrifugation at 3900 × g for 5 min at 4°C. The presence of eight virulence-determinant genes characteristic of different pathogenic *E. coli* was detected using standard PCR. The PCR was run in two multiplex reactions using the Multiplex PCR NZYTaq 2× Master Mix (NZYTech, Lisboa, Portugal), according to the manufacturer's instructions. All reactions were run in a total volume of 20 μL containing 2 μL DNA and 5 pmol of each primer, starting at 50°C for 2 min and 95°C for 5 min. Reaction 1 (*eae*, *bfpA*, and CVD432): 40 cycles of 95°C for 40 s, 58°C for 1 min and 72°C for 2 min, and finally 72°C for 7 min. For reaction 2 (LT, ST, *ipaH*, *stx1*, and *stx2*) the annealing temperature was reduced to 50°C. The PCR products were confirmed by gel electrophorese in 2% agarose and visualized by SYBR safe staining.

qPCR for bacterial pathogens

As 16S rRNA amplicon sequencing cannot be used to determine the pathogenic potential of bacteria at the species level, qPCR was performed to assess the presence of well-known foodborne pathogenic bacterial species/pathotypes. Primers (ThermoFischer) targeting VS1, *tir*, and *hlyA* were used for the detection of *Campylobacter jejuni*, *enteropathogenic E. coli* (EPEC), and *Listeria monocytogenes*, respectively using the AriaMx Real-Time qPCR System (Guilbaud et al., 2005; Haffar & Gilbride, 2010; Stonnet & Guesdon, 1993). Primer sequences are given in Table S3. The reference strains *C. jejuni* CCUG 11284 A, *L. monocytogenes* NCTC 7973, and EPEC OK86 were used as positive controls and to generate the standard curve to determine the efficiency of the respective assays. The qPCRs were run using the FastStart SYBR Green I Master kit (Roche Diagnostics GmbH, Germany), according to the manufacturer's instructions. Each qPCR was run in a total volume of 50 μL and contained 300 nM of each forward and reverse primer, and

250 ng DNA. The qPCR was initiated at 95°C for 10 min prior to 40 cycles starting with 95°C for 15 s. For *C. jejuni*, annealing at 56°C for 30 s, 68°C for 30 s (fluorescence read) and 77°C for 10 s. For EPEC, annealing at 59°C for 30 s, 72°C for 30 s, and 77°C for 10 s (fluorescence read). For *L. monocytogenes*, annealing at 62°C for 1 min (fluorescence read) and 72°C for 10 s. A melting curve analysis was generated by heating to 95°C followed by slow cooling to 60°C and finally heating to 95°C at 0.1°C/s increment.

(RT)-qPCR for viral pathogens

RNA was extracted from the plastispheres as explained previously. One-step TaqMan (RT)-qPCR was used to detect the viral pathogens adenovirus 40/41 (AdV) and norovirus GI/GII (NoV). Primers/probes sequences are given in Table S3. The AriaMx Real-time PCR system (Agilent Technologies, Santa Clara, California, USA) was used for virus detection and analysis of data. Confirmation for the absence of inhibitors was performed with a 10-fold dilution in duplicate during initial screening. All reactions were performed in a total volume of 20 μL. NoV was tested for using the TaqMan™ Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Massachusetts, USA). For NoV GI: 3 μL of RNA, 500 nM QNIF4, 900 nM NVLC1R, and 250 nM probe NVGGI. Cycling conditions were 50°C for 5 min, 95°C for 20 s and 45 cycles of 95°C for 15 s, 55°C for 20 s and 60°C for 1 min. For NoV GII: 3 μL of RNA 500 nM COG2R, 900 nM QNIF2d, and probe 250 nM QNIFS. Cycling conditions were as for NoV GI.

For the detection of AdV, the TaqMan™ Environmental Master Mix 2.0 (Thermo Fisher Scientific) was used with the following setup: 2 μL of DNA, 250 nM each of AdJTVXF, AdJTVXR and the probe 125 nM AdJTVXP (Jothikumar et al., 2005). Cycling conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, 55°C for 15 s and 60°C for 1 min.

RESULTS AND DISCUSSION

Plastisphere bacterial diversity

Biofilm samples from the three different types of plastic were subjected to 16S rRNA marker gene sequencing. After quality filtering and chimera removal, the numbers of effective reads (nochime reads) were 83,000 ± 15,000 (mean ± SD) for two-week-old biofilm and 38,000 ± 2600 for four-week-old biofilm in June. The effective reads from the biofilm collected in September were 41,400 ± 7200 and 37,000 ± 4600 for two- and four-week-old biofilm, respectively. A total of 11,767 ASVs were identified and used for comparative analysis.

Two selected indices of within-sample diversity (alpha-diversity) were used to analyse the effect of the four variables (Plastic, Duration, Month, and Location) on the bacterial diversity in the plastispheres: Shannon index (richness and evenness combined; Figure 2A) and Chao1 index (richness; Figure 2B).

The partial *F*-test, conducted to investigate if a full ANOVA model with all four-way interactions was more appropriate than its reduced versions, showed that the four-way interaction model for Chao1 did not contribute significantly and we could use the simpler model with only two-way interaction terms ($F = 1.174$, $p = 0.332$). Opposite results were shown for the model for Shannon, with all four-way interaction terms contributing significantly and should be included ($F = 6.628$, $p < 0.001$). The two ANOVA models with Shannon and Chao1 as response variables, showed that the main effect of the month had the greatest influence on both alpha-measures (Shannon: *F*-value = 288.448, $p < 0.001$, Chao1: *F*-value = 463.007, $p < 0.001$). All the results, from both ANOVA models, are displayed in Table 1. The most interesting post hoc pairwise Tukey-test results, with respect to the aim of the study, are presented and discussed in detail in Effect of polymer type to Geographic differences in bacterial composition sections for the different variables.

Two different measures of beta-diversity were used in response in the PERMANOVA test to assess the differences in bacterial community structures over the variables: Bray–Curtis dissimilarity distance (relative abundance) and weighted UniFrac distance (relative abundance and phylogeny). The R^2 conducted from the adonis() function showed that the three- and four-way interaction terms had a small explanatory power of the bacteria diversity, for all the terms it was less than 0.07, and hence we decided to retain a less complicated model with only up to two-way interaction. Overall, all variables had a significant effect on the diversity of the bacterial communities for both Bray–Curtis and weighted UniFrac distances, with location (Bray–Curtis: $R^2 = 0.124$, *F*-value = 27.068, $p < 0.001$, weighted UniFrac: $R^2 = 0.186$, *F*-value = 47.946, $p < 0.001$) and month (Bray–Curtis: $R^2 = 0.251$, *F*-value = 54.564, $p < 0.001$. weighted UniFrac: ($R^2 = 0.244$, *F*-value = 62.801, $p < 0.001$) influencing most of the variation (Table 2).

Details of the effect of the variables are presented and discussed in the following sections.

Effect of polymer type

The main effects from the ANOVA test of the impact of the plastic materials on the alpha diversity (Chao1 and Shannon index) for the biofilm communities, showed no significant difference between the three plastic materials (Shannon: *F*-value = 1.346, $p = 0.269$, Chao1:

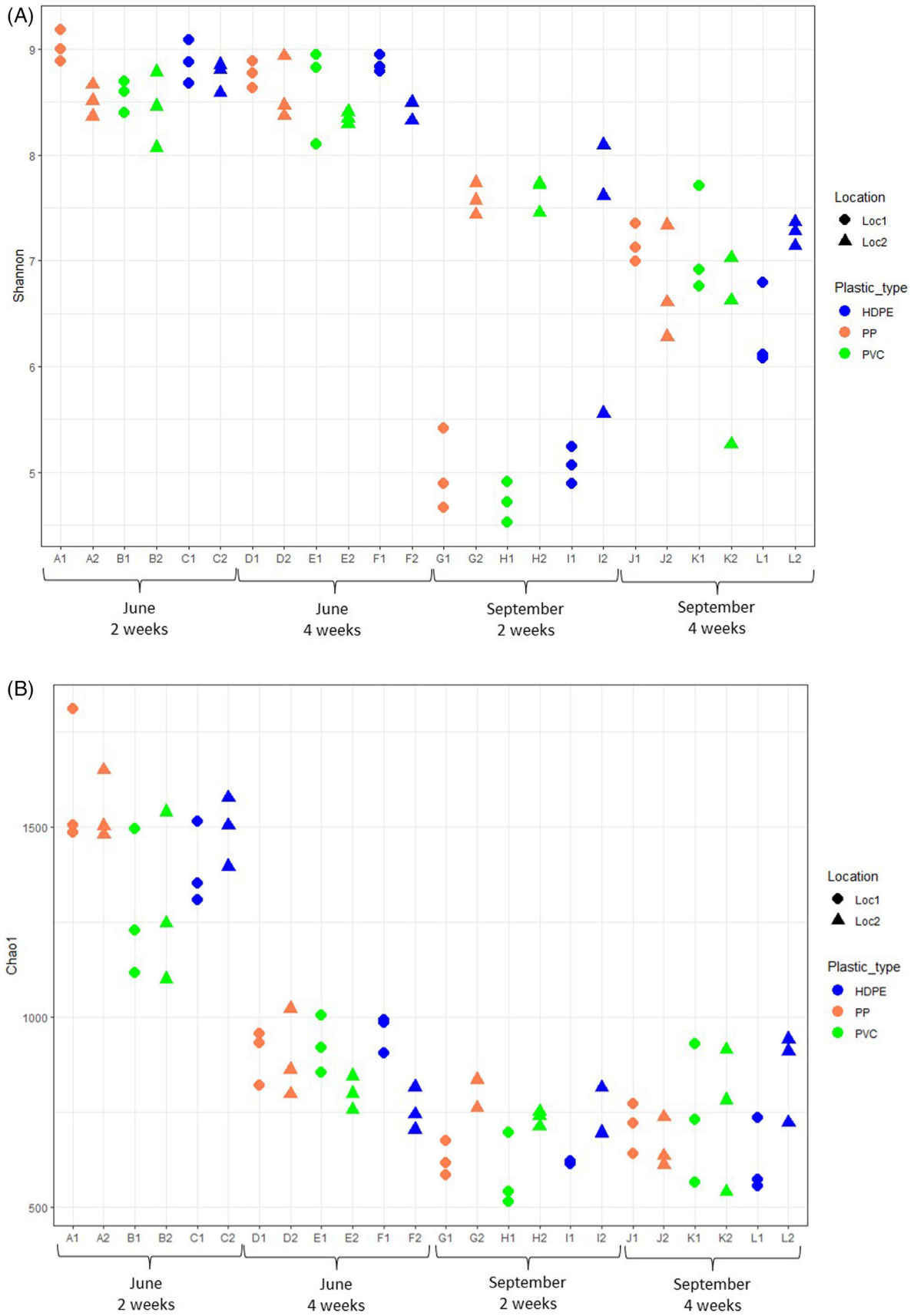

FIGURE 2 Bacterial alpha diversity in the plastispheres on all plastic types (PP, PVC, HDPE) at the two locations during the experiment (2 and 4 weeks of June and September). The x-axis represents the different samples included in the study, while the y-axis denotes the values of the alpha diversity indices. (A) Shannon diversity (richness and evenness). (B) Chao1 index (species richness).

TABLE 1 An investigation of the alpha diversity indices, Shannon and Chao1, was conducted using ANOVA models with interaction effects.

	Chao1		Shannon	
Variables/interaction effects	***F*-value**	**Pr(<*F*)**	***F*-value**	**Pr(<*F*)**
Plastic	3.00	0.054	1.346	0.269
Location	1.247	0.268	21.356	<0.001
Month	280.703	<0.001	463.007	<0.001
Duration	94.977	<0.001	7.337	0.009
Plastic:Location	0.364	0.696	0.425	0.656
Plastic:Month	2.947	0.061	0.498	0.610
Location:Month	8.215	0.005	55.403	<0.001
Plastic:Duration	3.868	0.026	0.006	0.993
Location:Duration	4.217	0.044	44.632	<0.001
Duration:Month	124.88	<0.001	13.825	<0.001
Location:Month:Duration	-	-	41.194	<0.001
Plastic:Location:Duration	-	-	3.157	0.051
Plastic:Location:Duration:Month	-	-	5.196	0.009

Note: The table shows the results from the two-way interaction model for Chao1 and the four-way interaction model for Shannon diversity. The two ANOVA models with Shannon and Chao1 as response variables showed that the effect of month influenced the alpha diversity of the plastispheres the most.

TABLE 2 A PERMANOVA with two-way interaction effects using Bray–Curtis and weighted UniFrac distance was calculated to analyse the influence of the different variables on the beta diversity of the bacterial community composition.

	Bray–Curtis			Weighted UniFrac		
Variable/interaction effects	***R*-square**	***F*-value**	**Pr(>*F*)**	***R*-square**	***F*-value**	**Pr(>*F*)**
Plastic	0.029	3.195	0.001	0.030	3.859	0.001
Location	0.124	27.068	0.001	0.186	47.946	0.001
Duration	0.075	16.460	0.001	0.068	17.695	0.001
Month	0.251	54.564	0.001	0.244	62.801	0.001
Plastic:Location	0.017	1.846	0.022	0.009	1.267	0.24
Plastic: Duration	0.016	1.780	0.034	0.020	2.603	0.009
Location:Duration	0.043	9.419	0.001	0.031	8.004	0.001
Plastic:Month	0.018	2.708	0.003	0.014	1.919	0.04
Location:Month	0.092	19.995	0.001	0.110	28.546	0.001
Duration:Month	0.067	14.643	0.001	0.062	15.976	0.001

Note: All variables had a significant effect on the beta diversity of the bacterial communities, with location and month influencing most of the variation.

F-value = 3.083, $p = 0.055$) as displayed in Figure 2 and Table 1. The post hoc Tukey test revealed a significantly higher diversity richness in the plastispheres collected from PP compared to those collected from PVC (diff = −284.824, 95% CI: [−526.176; −43.472], $p = 0.006$), after the two-week-old plastispheres collected in June (Figure 2B). This difference, however, was not observed during the other sampling times, indicating that the plastic materials had a non-significant influence on the bacterial richness. On the other hand, considering the beta diversity of the bacterial communities, the plastic materials significantly influenced the plastisphere diversity for both Bray–Curtis and weighted UniFrac (Figure 3, Table 2). A few studies demonstrate that the structure and composition of the bacterial communities are not influenced by differences in plastic materials (Di Pippo et al., 2020; Zhang et al., 2021). The impact of plastic materials on the bacterial community, however, might differ between pristine and weathered plastic (Erni-Cassola et al., 2020; Zadjelovic et al., 2023).

According to previous studies, other factors, such as season, incubation duration, and location, have a greater effect on the composition of the associated microbiota than the plastic surface itself (Oberbeckmann & Labrenz, 2020; Pinto et al., 2019; Zhang et al., 2021). Although all these studies were conducted in a marine environment, the results coincide with the results of this study.

The physiochemical properties of plastic surfaces, such as roughness, hydrophobicity, topography, and

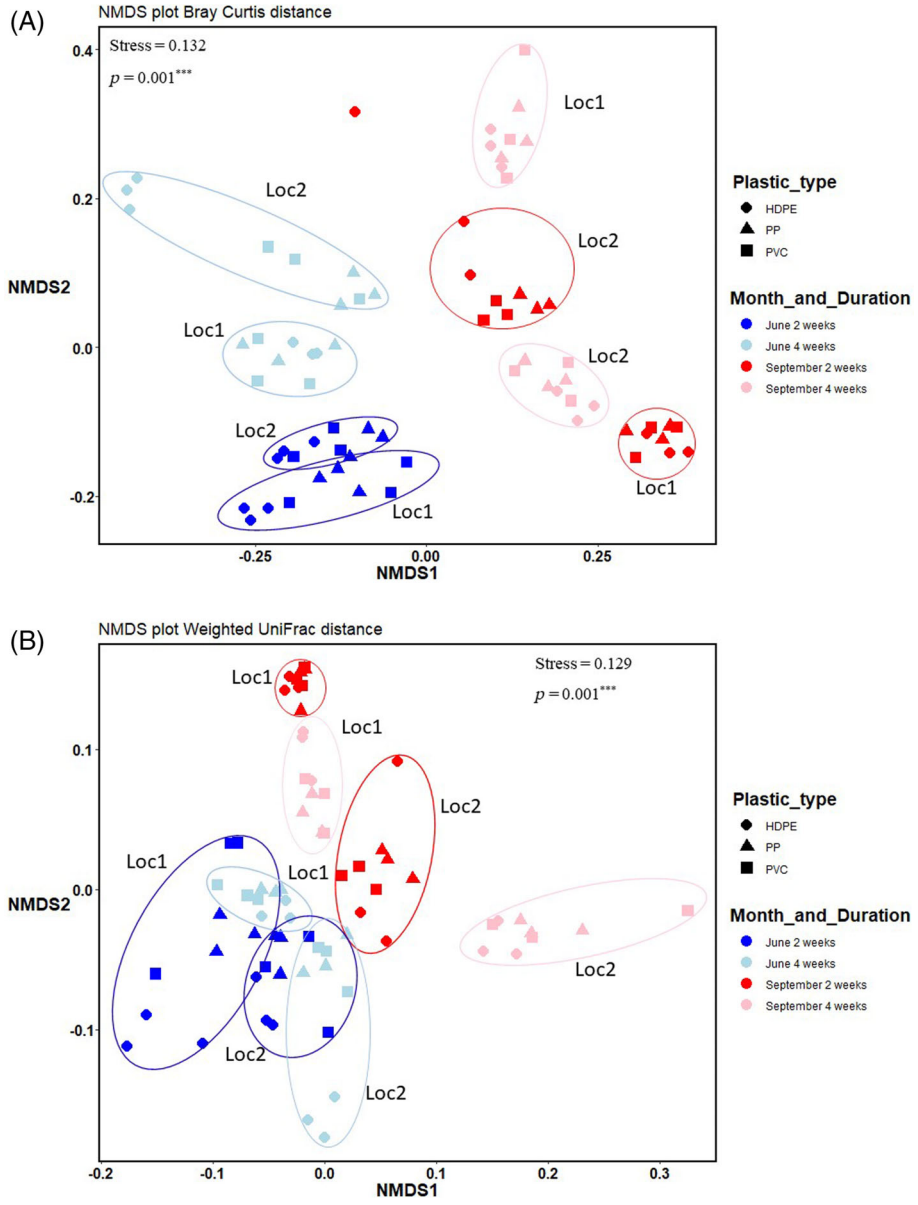

FIGURE 3 Bacterial beta-diversity between the communities on the surface of plastic materials after being in the river at different periods and locations. NMDS ordination plot representation of (A) Bray–Curtis and (B) Weighted UniFrac distances of all samples. Each point represents one sample, and each colour represents the variables at which the plastispheres were harvested (duration in the rives and month), while the symbols represent the plastic materials. Stress values indicate the NMDS goodness-of-fit.

electrostatic interaction are shown to influence the initial attachment of microorganisms and biofilm formation (Rummel et al., 2017; Verran & Boyd, 2001; Whitehead & Verran, 2006). As the biofilm matures, the secondary colonizers are influenced more by the internal biofilm processes rather than the surface-related properties of the plastic materials. Additionally, organic, and inorganic components in the water create a conditioning layer on the plastic's surface that could affect the colonizing bacteria. A study by Rochman et al. showed that HDPE and PP sorbed higher concentrations of organic pollutants from the environment, specifically polychlorinated biphenyls, and polycyclic aromatic hydrocarbons, compared to PVC which may affect the composition of the plastispheres on the different plastic materials (Rochman et al., 2013). There are also several studies showing that the surface topography and roughness of the polymer do not affect the initial attachment of microorganisms (Hook et al., 2012; Nauendorf et al., 2016). However, because the surface topology and hydrophobicity of the three plastic materials used in the present study were relatively similar (Figure S3), they probably did not have a major impact on bacterial colonization, which is corroborated by our results.

Effect of duration in the river and month on plastisphere communities

The results from the ANOVA model showed a significant interaction effect of duration and month on the alpha diversity indices of Chao1 (F-value = 124.880, $p < 0.001$) and Shannon (F-value = 13.825, $p < 0.001$; Table 1). The Tukey post hoc test for the interaction effects of Duration: Month showed that the Shannon diversity was significantly higher for plastispheres collected at 2 and 4 weeks in June compared to 2 and 4 weeks in September (2 weeks: diff = −2.515, 95% CI: [−2.890; −2.140], $p < 0.001$, 4 weeks: diff = −1.774, 95% CI: [−2.149; −1.399], $p < 0.001$). These results are also illustrated in Figure 2A. The post hoc tests showed that the bacterial diversities of the plastispheres remained stable throughout the sampling period in June (diff = −0.100, 95% CI: [−0.475;0.274], $p = 0.891$), while the plastisphere diversity collected in September varied considerably, from low after 2 weeks in the river, to higher after 4 weeks (diff = 0.640, 95% CI: [−0.265; −1.015], $p < 0.001$), also seen in Figure 2A. The richness (Chao1) of the plastisphere formed after 2 weeks in June was higher compared after 4 weeks (diff = −561.365, 95% CI: [661.793; 460.937], $p < 0.001$), as well as after 2 weeks (diff = −749.426, 95% CI: [−849.854; −648.999], $p < 0.001$) and 4 weeks (diff = −711.073, 95% CI: [811.501; 610.645], $p < 0.001$) in September, as shown in Figure 2B. Notably, the samples collected after 2 weeks in June were sequenced in a separate batch and resulted in twice as many effective tags. Especially the Chao1 richness estimator is significantly affected by experimental procedures in separate rounds of sequencing, thus caution should be taken when comparing results from different sequencing batches even after normalization of the data (He et al., 2013).

The NMDS analysis based on Bray–Curtis distance, suggested that the plastispheres from June clustered separately from September. Also, the samples collected after 2 weeks clustered separately from those collected after 4 weeks, which was true for both June and September (Figure 3A). This indicates that plastispheres collected after 2 and 4 weeks, and at various months had a distinct bacterial composition.

The weighted Unifrac distance, which considers the phylogenetic relationship of the organisms, indicated a minor overlap between June samples harvested after 2 and 4 weeks, whereas September samples clustered according to time spent in the river (Figure 3B). This was supported by the PERMANOVA analysis which showed a significant interaction effect of duration and month on the bacterial communities shown in Table 2 (Bray–Curtis: F-value = 14.643, $p < 0.001$, weighted UniFrac: F-value = 15.976, $p < 0.001$).

Biofilms in streams are dynamic structures that are influenced by a variety of physical and chemical parameters in the surrounding water (Battin et al., 2016; Zhang et al., 2021). The observed variations between plastic-associated biofilms collected in June and September are probably due to environmental changes in the aquatic ecosystem. For example, during the June sampling, the water temperature was rising, whereas in September, it was stable (June; average temp. 10.6°C ± 6.13, September; average temp. 15.11°C ± 0.33; Table S6). Furthermore, the flow cytometry analysis revealed that the total bacterial count in the river water was higher in September compared to June which could also influence the diversity of the plastispheres (Table S6, Figure S2).

Geographic differences in bacterial composition

The two ANOVA models with two-way interaction showed a significant interaction effect between location and month (Shannon: F-value = 55.403, $p < 0.001$, Chao1: F-value = 8.215, $p = 0.005$; Table 1). As illustrated in Figure 2, and shown by the Tukey post hoc test results, the Shannon diversity decreased significantly for both locations from June to September (Shannon: Loc1: diff = −2.887, 95% CI: [−3.262; −2.511], $p < 0.001$. Loc2: diff = −1.403, 95% CI: [−1.778; −1.027, $p < 0.001$). The same was observed for Chao1 richness (Loc1: diff = −526.476, 95% CI: [−626.903; −426.048], $p < 0.001$. Loc2: diff: −372.659. 95% CI: [−473.087; −272.231], $p < 0.001$). Further, the results from the Tukey post hoc test also showed that Loc1 and Loc2 had no difference in within diversity in June (Shannon: diff: −0.281, 95% CI: [−0.656; 0.093], $p = 0.204$. Chao1: diff = −46.942, 95% CI: [147.370; 53.485], $p = 0.606$), while Loc1 had significant less within diversity compared to Loc2 in September for both diversity measures (Shannon: diff = 1.202, 95% CI: [0.827; 1.577], $p < 0.001$, Chao1: diff = 106.873, 95% CI: [6.446; 207.301], $p = 0.032$), also supported by Figure 2.

The ANOVA models also showed a significant interaction effect of location and duration (Shannon: F-value = 44.632, $p < 0.001$. Chao1: F-value = 4.217, $p = 0.044$; Table 1), with a significant difference in the Shannon diversity at Loc1 between two and 4 weeks (Tukey post hoc: diff = 0.936, 95% CI: [0.560; 1.311], $p < 0.001$). However, this interaction effect was not found significant between 2 and 4 weeks at Loc2.

For the richness of the plastispheres, the post hoc Tukey test showed significant difference between 2 and 4 weeks at Loc1 (diff = −206.406, 95% CI: [−306.384; 105.978], $p < 0.001$) and between 2 and 4 weeks at Loc2 (diff = 316.605, 95% CI: [417.033; 216.177], $p < 0.001$) (Figure 2B).

Regarding the beta diversity, the location influenced the bacterial composition in the plastisphere biofilms

(Bray–Curtis: F-value = 27.068, $p < 0.001$, weighted UniFrac: F-value = 47.946, $p < 0.001$) as shown in Table 2 and illustrated in Figure 3. In contrast to the plastisphere samples collected in June, both Bray–Curtis- and weighted UniFrac distance matrices indicated that those collected in September clustered according to both location and duration of incubation in the river, also shown with a significant interaction effect of location and duration from the PERMANOVA (Bray–Curtis: F-value = 9.419, $p < 0.001$, Weighted UniFrac: F-value = 8.004, $p < 0.001$; Figure 3, Table 2).

Contributions from organic matter and the release of high concentrations of nutrients (e.g., nitrogen and phosphorus) from agriculture and other human activities influence biofilm formation (Harrison et al., 2018). Previous reports have shown that the water quality in the Lier River is affected by extensive agricultural activities in the surrounding areas, especially in the southern part of the river (Lier Kommune 2019 (Lier municipality)). The difference in the bacterial communities at the two locations might be explained by increased runoff and contamination from the area's agriculture, industry, and wildlife. The area surrounding Loc2 is regarded as critical in terms of sewage overflow from the drainage network particularly during heavy rains (VIVA, 2018), and such events will influence the bacterial communities at this site. However, the detection of PMMoV in Loc1 also shows that the river at this point was exposed to human faecal contamination (Table S6). Our findings suggest that the location of sampling, rather than the surface composition, is a more important determinant of biofilm composition, which is consistent with previous research (Curren & Leong, 2019; Oberbeckmann et al., 2014). However, less is known about how organic and inorganic substances attaching to the plastic surface affect the composition of the plastisphere present in freshwater systems.

Plastisphere community composition

Phylum level

The results from the beta-diversity PERMANOVA tests indicated that the bacterial composition of plastisphere biofilms varies depending on the number of weeks in the river, the month, and the location, together with plastic types (Figure 3). These results are visually supported by Figure 4 showing the compositions of the top 20 most abundant phyla. Although there are differences between the sample groups, there are also some similarities. Proteobacteria (Pseudomonadota), Cyanobacteria, and Firmicutes (Bacillota) were generally the dominating phyla in all plastisphere samples (Figure 4A). These phyla have also previously been reported as predominant in plastisphere communities and are identified as primary colonizers during biofilm development in aquatic environments (Curren & Leong, 2019; Gong et al., 2019; Li et al., 2021; Oberbeckmann et al., 2014). The existence of a 'core'-plastisphere community- defined as a group of shared, stable, and consistent members of microbial communities from similar habitats- has recently been proposed (Amaral-Zettler et al., 2020; Di Pippo et al., 2022; Zhang et al., 2022). Proteobacteria and Cyanobacteria have been suggested as such members (Marques et al., 2023; Tu et al., 2020), and the present study supports this as these phyla were found in high abundance in all plastic communities analysed (Figure 4A). Proteobacteria accounted for more than 50% of the bacterial abundance in the plastisphere and have been identified as prevalent in freshwater biofilms in several other studies (Battin et al., 2016; Hoellein et al., 2014; McNamara & Leff, 2004; Rickard et al., 2003).

Figure 4A illustrates the stability of the bacterial communities in the plastisphere during June, with apparently minor variations in the abundance of the most dominant phyla (Proteobacteria, Cyanobacteria, Verrucomicrobiota, Firmicutes, Actinobacteriota (Actinomycetota) and Bacteroidetes (Bacteroidota)) after 2 and 4 weeks in the river (Figure 4A).

In September, the abundance of Proteobacteria in the plastispheres reached over 70% (Figure 4A, Table S4). As visualized in Figure 4A and presented in Table S4, the abundance of Cyanobacteria in the plastisphere was around 2% at Loc1, while it reached 18% in the two-week-old biofilms from Loc2. The abundance of Bacteroidota was between 5.57% and 6.51% at Loc1 and reached 10.34% at Loc2. While Actinobacteriota had an abundance between 1.6% and 3% at Loc1 and up to 7.38% at Loc2.

In the four-week-old plastisphere from Loc2, the abundance of Proteobacteria and Cyanobacteria was dominating (both >40%), while the relative abundance of the other phyla remained unchanged (Figure 4A, Table S4). Cyanobacteria frequently dominate microbial communities when the levels of nutrients and other organic compounds are high (Huisman et al., 2018). Bacteria belonging to this phylum can produce toxins affecting other bacteria, giving them a possible growth advantage (Volk & Furkert, 2006). The greater abundance of this phylum in samples collected in late September may thus be attributable to a competitive advantage under prevailing conditions in the river.

Genus level

Details about the most abundant genera are visualized in Figure 4B and presented in Table S5. *Sphaerotilus*, a member of the phylum Proteobacteria, was consistently among the most abundant genera across all plastisphere samples. *Sphaerotilus*, *Rhodoferax*, *Chloroplast*, *Pseudorhodobacter*, and *Lutolibacter*

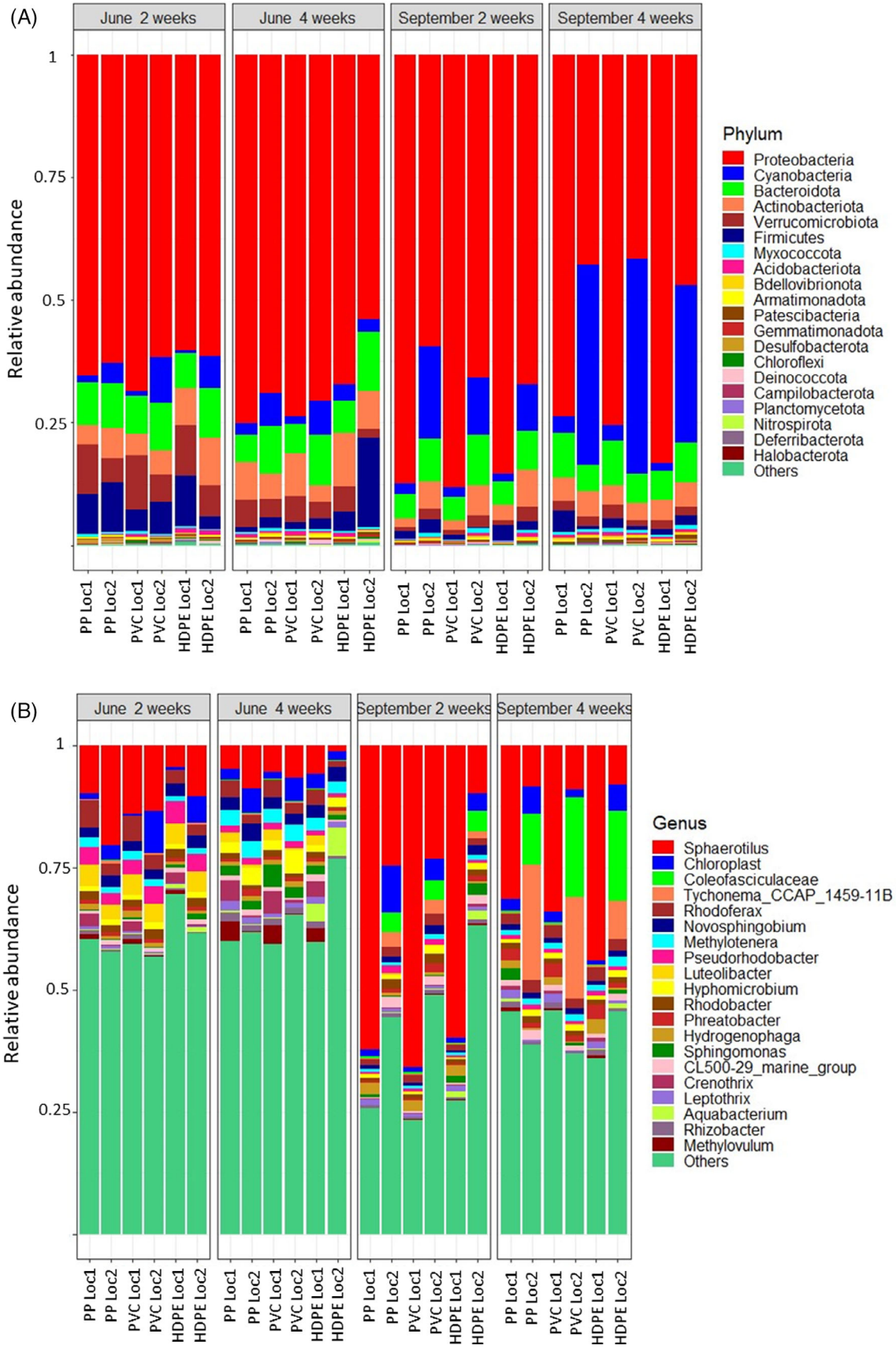

FIGURE 4 Relative abundance of the 20 most abundant (A) phyla and (B) genera in the plastisphere communities. Each colour represents one phylum or genus, and the length of the patch represents the relative abundance of the phylum/genus. A group of three replicates constitutes each bar.

were among the most abundant genera in the plastispheres collected in June, and their relative abundance was similar regardless of location and weeks in the river (Figure 4B, Table S5). The taxonomic profile is influenced by a high occurrence of low abundant species, indicated by the high abundance of 'others', also reflected in higher richness and diversity (Chao1 and Shannon) in the June samples compared to the September samples (Figure 2).

In September, the bacterial genus composition from the plastisphere samples was significantly influenced by the location and time in the river, as indicated by alpha and beta diversity analyses as described in Plastisphere bacterial diversity section (Figures 2 and 3, Tables 1 and 2). At Loc1 the plastispheres from September remained stable with minor variations in the most abundant genera; *Sphaerothilus*, *Chloroplast*, *Hydrogenophaga*, and *Rhoderoferax*, with *Sphaerotilus* as the most dominating genus with an abundance of more than 60% in the two-week-old plastispheres (Figure 4B, Table S5). The most dominating genera in the two-week-old plastispheres from Loc2 were *Sphaerothilus*, *Chloroplast*, *Coleofasciculaceae*, and *Tychonema_CCAP_1459-11B*. In contrast, in the four-week-old plastispheres from Loc2, the abundance of *Sphaerothilus* was less than 10%, while *Coleofasciculaceae* and *Tychonema_CCAP_1459-11B* accounted for roughly 40% of the abundance in the bacterial communities (Figure 4B, Table S5).

Sphaerotilus is a common environmental bacterial genus often found in sewage and activated sludge and is known to cause problems such as clogged pipes and bulking of activated sludge in wastewater treatment (Pellegrin et al., 1999). The presence of *Sphaerotilus* in freshwater systems indicates severe organic pollution caused by leakage of sewage and waste from agriculture (Stoessel, 1989). As mentioned above, possible sources of contamination in the Lier River are septic tanks and surrounding agriculture (Lier kommune 2017 (Lier municipality); VIVA, 2018), thus the high abundance of *Sphaerotilus* in the biofilms indicates heavy contamination of the water. Although community variation due to geographical and seasonal influence was observed, the bacterial communities, in general, were dominated by genera associated with freshwater environments and plastispheres, supporting the idea of a core plastisphere microbiome (Di Pippo et al., 2022).

Detection of pathogens

From the 16S amplicon sequencing data, most ASVs from the plastisphere samples were affiliated to Proteobacteria which is known to include a range of potential foodborne pathogens such as *E. coli*, *Salmonella* sp., and *Campylobacter*. The phylum Firmicutes, which includes the pathogenic species *Bacillus* sp. and *Listeria* sp. also, had a high relative abundance, particularly in plastispheres collected in June. At the genus level, the plastisphere samples contained bacteria belonging to the genera *Escherichia*/*Shigella*, *Listeria*, *Aeromonas*, *Bacillus*, and *Enterobacter*. In the samples collected in June, *Escherichia*/*Shigella* were among the most abundant with a relative abundance of approximately 0.5% (Table 3). The ANOVA model showed a significant effect of both main effects of plastic (F-value = 39.551, $p < 0.001$), month (F-value = 57.225, $p < 0.001$), location (F-value = 40.952, $p < 0.001$) and duration (F-value = 16.343, $p < 0.001$) on the relative abundance of *E. coli* /*Shigella*. Statistical analysis of the rest of the pathogenic genera detected by 16S amplicon data was not performed, due to relative abundance below the threshold value and high variability across samples.

As 16S amplicon sequencing does not discriminate between pathogenic and benign bacterial species, we combined the use of traditional cultivation methods, with microbial identification techniques like MALDI-TOF and qPCR to get more specific and reliable information about the pathogenic potential of the species inhabiting the plastic-associated biofilms.

Bacterial isolates from plastisphere samples were identified by MALDI TOF as *L. monocytogenes*, *E. coli*, *Providencia rettgeri*, *Citrobacter freundii*, *Mammaliicoccus vitulinus*, *Klebsiella pneumoniae*, and *Enterobacter* spp. The presence of *L. monocytogenes* and enteropathogenic *E. coli* (EPEC) was also confirmed by qPCR (Table 4), while the well-known waterborne pathogen *Campylobacter* was not detected in any of the samples. All these detected species are either environmental isolates or commensals in the digestive tracts of humans or animals. Further characterization of the *E. coli* isolates using PCR, showed that all but one of the six *E. coli* isolates carried virulence genes associated with intestinal pathogenicity. Usually, *E. coli* are commensal bacteria colonizing the gastrointestinal tracts of humans and animals. However, some strains

TABLE 3 The abundance (%) of genera known to include foodborne pathogens.

	June		September	
Taxonomy	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**
Escherichia-Shigella	0.257	0.598	0.086	0.064
Aeromonas	0.041	0.055	0.239	0.065
Listeria	0.131	0.167	0.112	0.017
Bacillus sp.	0.180	0.187	0.126	0.024
Enterobacter	0.006	0.003	0.000	0.009
Serratia	0.003	0.005	0.000	0.003
Yersinia	0.001	0.000	0.000	0.000

Note: Plastisphere samples representing different seasons and time spent in the river were characterized by 16S amplicon sequencing.

TABLE 4 Pathogenic viruses and bacteria detected by (RT)-qPCR.

	June		September		
Pathogen	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**	**Sum**
Adenovirus	0/6	0/6	0/6	1/6	1/24
Norovirus GI	0/6	0/6	0/6	0/6	0/24
Norovirus GII	0/6	0/6	0/6	3/6	3/24
EPEC	6/6	2/6	2/6	1/6	11/24
C. jejuni	0/6	0/6	0/6	0/6	0/24
L. monocytogenes	4/5	6/6	0/6	0/6	10/23

Note: A total of 24 samples were run, with six samples within the different months and time spent in the river. The results are shown as the number of positives/number of total samples analysed.

carry specific virulence factors that cause severe disease that have been responsible for severe foodborne outbreaks. The panel of virulence genes in the multiplex PCR used in this study is limited, and it is not possible to unravel the whole virulence profile of the isolates without whole genome sequencing. Nevertheless, this unusual combination of virulence genes characteristic of three different pathogroups indicates that these isolates are representatives of one *E. coli* clone that could be characterized as a hybrid pathogen (Santos et al., 2020).

As for water quality, *E. coli* was found in samples collected from the river water (Table S6; Nilsson & Grimsgaard, 2021). The levels of *E. coli* found in the water samples collected in June exceeded what is acceptable for irrigation water (100 MPN/100 mL) (Table S6; Eckner et al., 2014; Nilsson & Grimsgaard, 2021). This indicates that different pathogens with a faecal-oral transmission route may occur in the river water. Several large foodborne outbreaks have been linked to fresh produce such as green leaf lettuce and sprouts (EFSA, 2011; Panel et al., 2020). The source of contamination is usually wastewater, manure from production animals, and, domestic animals, and the finding of *E. coli* in a water sample usually indicates recent faecal contamination.

The presence of pathogenic bacteria species, including the ones detected in this study is also previously found on plastic debris, mainly in marine environments (Silva et al., 2019; Tavelli et al., 2022; Tu et al., 2020; Yang et al., 2020). On the contrary, a recent study by Song et al. suggested a low likelihood of plastic-mediated dissemination of *E. coli* and found no *E. coli* on HDPE particles incubated in freshwater and marine environments (Song et al., 2020). Only a limited number of studies have focused on the presence of pathogenic bacteria in freshwater. A recent study by Di Pippo et al. discovered, using PCR-based techniques, that plastic particles from freshwater environments were colonized by biofilm-forming opportunistic pathogens, suggesting that the plastispheres are suitable vectors for pathogenic bacteria in a freshwater ecosystem (Di Pippo et al., 2022). Nevertheless, more research is needed to assess the likelihood of pathogens that colonize plastic surfaces, to survive, transfer to a host, and eventually cause disease (Beloe et al., 2022). The most used molecular approach to provide information on the complexity of microbial communities in plastispheres is analyses based on taxonomic data from 16S amplicon sequencing (McCormick et al., 2016), followed by assessments of microbial pathogenicity by qPCR (Di Pippo et al., 2022). However, these techniques provide limited information regarding microbiome complexity and specific taxa harbouring virulence determinants (Laudadio et al., 2018; Zadjelovic et al., 2023). Given the limited number of studies on freshwater plastispheres, comprehensive metagenomic analysis of the plastispheres is recommended to gain a more complete description and understanding of these microbial communities (Beloe et al., 2022; Wu et al., 2019).

Regarding pathogenic viruses, NoV GII (genogroup II) was detected in September in all three 4-week samples from Loc1, while Norovirus NoV GI was not detected in any samples (Table 4). These results corroborate the PMMoV results indicating more human faecal contamination in Loc1 (Table S6). Adenovirus was only detected in one sample collected in September. There is a range of viruses that can infect humans through the faecal-oral route, however, when it comes to foodborne outbreaks of diseases NoVs are the agents that are most registered. According to WHO estimates based on data from 135 countries, NoVs contributed to the largest number of foodborne diseases (WHO, 2015). In the US and UK, estimates are that NoVs caused 58% (years 2000–2008) and 16% (year 2018) of foodborne diseases, respectively (Holland & Mahmoudzadeh, 2020; Scallan et al., 2011). Norovirus is also the leading agent of acute gastroenteritis worldwide and is found in high numbers in raw and treated sewage (Bosch et al., 2018). Enteric adenovirus (AdV) is mostly connected with gastroenteritis in children but can be found in sewage at levels comparable to NoV (Myrmel et al., 2015). The relatively few

biofilm samples being positive for the two groups of viruses in the present study is likely due to sampling during the summer period with lower levels of NoV in the communities (Myrmel et al., 2015) and to COVID-19 restrictions that reduced transmission of virus between humans (Kraay et al., 2021). However, the results show that NoV and AdV can be part of the plastisphere. Whether this influences the transmission or the pathogenic potential of the viruses is unknown.

One of the most important sources of pollution in the Lier River is sewage, primarily from small, private treatment installations in the rural area. The pollution is also related to the common transportation of wastewater, surface water, and drainage water in the municipal sewage pipelines. The challenge with these common pipes is overflow, as the capacity is often exceeded during heavy rainfall, and the surplus water is emptied into the Lier River (Lier kommune 2017 (Lier municipality)), increasing the risk of contamination with pathogens (Tryland et al., 2014). The rainfall in the sampling area was higher in June compared to September (Nilsson & Grimsgaard, 2021). Also, urban activities and wildlife are associated with pathogenic contamination of water (Jung et al., 2014; Selvakumar & Borst, 2006). There was also increased water turbidity during June which suggests an increased risk for contamination with viruses and bacteria (Jung et al., 2014).

CONCLUSION

This study shows that microbes colonize and accumulate on different types of plastic materials in a freshwater environment. The season, time spent in the river, and geographical location, as opposed to the plastic materials, determine the composition of the resulting plastisphere. Analyses of the taxonomical characteristics of the plastisphere indicated a bacterial community composition previously recognized as a 'core' microbiome of the plastisphere.

Since irrigation water can contain substantial amounts of plastics, integration in plastispheres might boost the survivability of microbes in a variety of conditions, potentially facilitating the spread of pathogenic microorganisms to fresh produce. The identification of potentially harmful bacteria and viruses in plastispheres in natural river water and the lack of research on the risk of pathogen transmission induced by the dissemination of plastic in freshwater ecosystems highlight the importance of this study.

AUTHOR CONTRIBUTIONS

Ingun Lund Witsø: Conceptualization (equal); data curation (equal); formal analysis (lead); investigation (equal); methodology (equal); resources (lead); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Adelle Basson:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (lead); writing – original draft (supporting); writing – review and editing (equal). **Hilde Vinje:** Formal analysis (equal); visualization (supporting); writing – review and editing (supporting). **Ann-Katrin Llarena:** Conceptualization (equal); investigation (supporting); methodology (supporting); resources (equal); writing – review and editing (equal). **Carlos Salas Bringas:** Conceptualization (supporting); funding acquisition (equal); methodology (supporting); resources (equal); writing – review and editing (equal). **Marina Aspholm:** Conceptualization (equal); funding acquisition (equal); methodology (supporting); writing – review and editing (equal). **Yngvild Wasteson:** Conceptualization (equal); funding acquisition (equal); project administration (lead); resources (equal); writing – review and editing (equal). **Mette Myrmel:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (equal); investigation (supporting); methodology (supporting); project administration (lead); writing – original draft (supporting); writing – review and editing (equal).

ACKNOWLEDGEMENTS

This work is supported by The Norwegian Research Council grant (PLASTPATH project number 302996). The following are acknowledged for their contributions to the study: Kaja Berge Grimsgaard and Vilde Irene Nilsson (Faculty of Veterinary Medicine, NMBU) for providing background information regarding the water quality of Lier River. Razak Seidu (Department of Ocean Operations and Civil Engineering, Norwegian University of Science and Technology) for organizing the analyses of the hydrophobicity of the plastic pieces. Letemichael Negash Welekidan, Adriana Dorota Osinska, Mamata Khatri, and Alejandro Jimènez Melèndez for laboratory work. Unni Lise Jonsmoen (Faculty of Veterinary Medicine, NMBU) and Jonas Segervald (Department of Physics, Umeå University) for surface roughness measurements.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The full set of sequence reads generated by the 16S rRNA amplicon sequencing is available in the Dryad data repository: https://doi.org/10.5061/dryad.9zw3r22mx.

ORCID

Ingun Lund Witsø https://orcid.org/0000-0003-2041-4887
Hilde Vinje https://orcid.org/0000-0003-0318-0214
Ann-Katrin Llarena https://orcid.org/0000-0001-5278-7253

Marina Aspholm https://orcid.org/0000-0003-3175-6022
Yngvild Wasteson https://orcid.org/0000-0001-6527-8407
Mette Myrmel https://orcid.org/0000-0002-2794-4023

REFERENCES

Alegbeleye, O.O., Singleton, I. & Sant'Ana, A.S. (2018) Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. *Food Microbiology*, 73, 177–208.

Allende, A., Castro-Ibáñez, I., Lindqvist, R., Gil, M.I., Uyttendaele, M. & Jacxsens, L. (2017) Quantitative contamination assessment of *Escherichia coli* in baby spinach primary production in Spain: effects of weather conditions and agricultural practices. *International Journal of Food Microbiology*, 257, 238–246.

Amaral-Zettler, L.A., Zettler, E.R. & Mincer, T.J. (2020) Ecology of the plastisphere. *Nature Reviews Microbiology*, 18(3), 139–151.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M. & Packmann, A.I. (2016) The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, 14(4), 251–263.

Beloe, C.J., Browne, M.A. & Johnston, E.L. (2022) Plastic debris As a vector for bacterial disease: an interdisciplinary systematic review. *Environmental Science & Technology*, 56(5), 2950–2958.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A. et al. (2019) Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857.

Bosch, A., Gkogka, E., Le Guyader, F.S., Loisy-Hamon, F., Lee, A., van Lieshout, L. et al. (2018) Foodborne viruses: detection, risk assessment, and control options in food processing. *International Journal of Food Microbiology*, 285, 110–128.

Burmølle, M., Ren, D., Bjarnsholt, T. & Sørensen, S.J. (2014) Interactions in multispecies biofilms: do they actually matter? *Trends in Microbiology*, 22(2), 84–91.

Chen, S., Zhou, Y., Chen, Y. & Gu, J. (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17), i884–i890.

Clem, J. & Barthel, B. (2021) A Look at Plant-Based Diets. *Missouri Medicine*, 118(3), 233–238.

Curren, E. & Leong, S.C.Y. (2019) Profiles of bacterial assemblages from microplastics of, profiles of bacterial assemblages from microplastics of tropical coastal environments tropical coastal environments. *The Science of the Total Environment*, 655, 313–320.

Di Pippo, F., Crognale, S., Levantesi, C., Vitanza, L., Sighicelli, M., Pietrelli, L. et al. (2022) Plastisphere in lake waters: microbial diversity, biofilm structure, and potential implications for freshwater ecosystems. *Environmental Pollution*, 310, 119876.

Di Pippo, F., Venezia, C., Sighicelli, M., Pietrelli, L., Di Vito, S., Nuglio, S. et al. (2020) Microplastic-associated biofilms in lentic Italian ecosystems. *Water Research*, 187, 116429.

Eckner, K., Lunestad, B., Robertson, L. & Grahek-Ogden, D. (2014) Kvalitetskrav for vann til jordvanning. Norwegian Scientific Committee for Food and Environment (VKM).

Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F. & Rosal, R. (2020) Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. *Environmental Pollution*, 259, 113837.

EFSA. (2011) Shiga toxin-producing *E. Coli* (STEC) O104: H4 2011 outbreaks in Europe: taking stock. *EFSA Journal*, 9(10), 2390.

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.

Frere, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H. et al. (2018) Microplastic bacterial communities in the bay of Brest: influence of polymer type and size. *Environmental Pollution*, 242 (Pt A, 614–625.

Galloway, T.S., Cole, M. & Lewis, C. (2017) Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology & Evolution*, 1(5), 116.

Geyer, R. (2020) Production, use, and fate of synthetic polymers. In: *Plastic waste and recycling*. 13–32: Elsevier.

Geyer, R., Jamback, J.R. & Law, K.L. (2017) Production, use, and fate of all plastics ever made. *Science Advances*, 3(7), 1–5.

Girardin, H., Morris, C.E., Albagnac, C., Dreux, N., Glaux, C. & Nguyen-The, C. (2005) Behaviour of the pathogen surrogates *Listeria innocua* and *Clostridium sporogenes* during production of parsley in fields fertilized with contaminated amendments. *FEMS Microbiology Ecology*, 54(2), 287–295.

Gong, M., Yang, G., Zhuang, L. & Zeng, E.Y. (2019) Microbial biofilm formation and community structure on low-density polyethylene microparticles in lake water microcosms. *Environmental Pollution*, 252, 94–102.

Guilbaud, M., de Coppet, P., Bourion, F., Rachman, C., Prévost, H. & Dousset, X. (2005) Quantitative detection of *Listeria monocytogenes* in biofilms by real-time PCR. *Applied and Environmental Microbiology*, 71(4), 2190–2194.

Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G. et al. (2011) Chimeric 16S rRNA sequence formation and detection in sanger and 454-pyrosequenced PCR amplicons. *Genome Research*, 21(3), 494–504.

Haffar, M. & Gilbride, K.A. (2010) The utility and application of real-time PCR and FISH in the detection of single-copy gene targets in *Escherichia coli* O157:H7 and *Salmonella Typhimurium*. *Canadian Journal Microbiology*, 56(3), 254–262.

Harrison, J. P., Hoellein, T. J., Sapp, M., Tagg, A. S., Ju-Nam, Y. & Ojeda, J. J. (2018). Microplastic-associated biofilms: a comparison of freshwater and marine environments. *Freshwater Microplastics*, 58, 181-201: Springer.

He, Y., Zhou, B.-J., Deng, G.-H., Jiang, X.-T., Zhang, H. & Zhou, H.-W. (2013) Comparison of microbial diversity determined with the same variable tag sequence extracted from two different PCR amplicons. *BMC Microbiology*, 13(1), 208.

Hoellein, T., Rojas, M., Pink, A., Gasior, J. & Kelly, J. (2014) Anthropogenic litter in urban freshwater ecosystems: distribution and microbial interactions. *PLoS One*, 9(6), e98485.

Holland, D. & Mahmoudzadeh, N. (2020) Foodborne disease estimates for the United Kingdom in 2018. *Food Standards Agency*. United Kingdom, London, 27.

Hook, A.L., Chang, C.-Y., Yang, J., Luckett, J., Cockayne, A., Atkinson, S. et al. (2012) Combinatorial discovery of polymers resistant to bacterial attachment. *Nature Biotechnology*, 30(9), 868–875.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H. & Visser, P.M. (2018) Cyanobacterial blooms. *Nature Reviews Microbiology*, 16(8), 471–483.

Jiang, J.J., Hanun, J.N., Chen, K.Y., Hassan, F., Liu, K.T., Hung, Y.H. et al. (2023) Current levels and composition profiles of microplastics in irrigation water. *Environmental Pollution*, 318, 120858.

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. & Erdman, D.D. (2005) Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Applied and Environmental Microbiology*, 71(6), 3131–3136.

Jung, A.-V., Le Cann, P., Roig, B., Thomas, O., Baurès, E. & Thomas, M.-F. (2014) Microbial contamination detection in water resources: interest of current optical methods, trends and needs in the context of climate change. *International Journal of Environmental Research and Public Health*, 11(4), 4292–4310.

Kamiński, M., Skonieczna-Żydecka, K., Nowak, J.K. & Stachowska, E. (2020) Global and local diet popularity rankings, their secular trends, and seasonal variation in Google trends data. *Nutrition*, 79-80, 110759.

Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M. et al. (2016) Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. *Marine Environmental Research*, 120, 1–8.

Kraay, A.N.M., Han, P., Kambhampati, A.K., Wikswo, M.E., Mirza, S.A. & Lopman, B.A. (2021) Impact of nonpharmaceutical interventions for severe acute respiratory syndrome coronavirus 2 on norovirus outbreaks: an analysis of outbreaks reported by 9 US states. *The Journal of Infectious Diseases*, 224(1), 9–13.

Laudadio, I., Fulci, V., Palone, F., Stronati, L., Cucchiara, S. & Carissimi, C. (2018) Quantitative assessment of shotgun metagenomics and 16S rDNA amplicon sequencing in the study of human gut microbiome. *OMICS: A Journal of Integrative Biology*, 22(4), 248–254.

Li, C., Wang, L., Ji, S., Chang, M., Wang, L., Gan, Y. et al. (2021) The ecology of the plastisphere: microbial composition, function, assembly, and network in the freshwater and seawater ecosystems. *Water Research*, 202, 117428.

Lier kommune. (2012) Lier kommune Landbruksplan. https://www.lier.kommune.no/globalassets/10.-politikk-og-samfunn/samfunn/planer/landbruk-og-miljo/landbruksplan-lier-kommune-2012.pdf (red.).

Lier kommune. (2017) Tilsynet for små avløpsanlegg i Drammensregionen.

Lier kommune. (2019) Temaplan overvann, Lier Kommune.

Lynch, I. & Dawson, K.A. (2014) In: Lead, J.R. & Valsami-Jones, E. (Eds.) *Nanoscience and the environment*, Frontiers of Nanoscience, ch. 4, 127–156. Amsterdam, Netherlands: Elsevier Science.

Magoc, T. & Salzberg, S.L. (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963.

Marques, J., Ares, A., Costa, J., Marques, M.P.M., de Carvalho, L.A. E.B. & Bessa, F. (2023) Plastisphere assemblages differ from the surrounding bacterial communities in transitional coastal environments. *Science of the Total Environment*, 869, 161703.

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), e01556.

McMurdie, P.J. & Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8(4), e61217.

McNamara, C.J. & Leff, L.G. (2004) Bacterial community composition in biofilms on leaves in a northeastern Ohio stream. *Journal of the North American Benthological Society*, 23(4), 677–685.

Moore, R.E., Millar, B.C. & Moore, J.E. (2020) Antimicrobial resistance (AMR) and marine plastics: can food packaging litter act as a dispersal mechanism for AMR in oceanic environments? *Marine Pollution Bulletin*, 150, 110702.

Myrmel, M., Lange, H. & Rimstad, E. (2015) A 1-year quantitative survey of Noro-, adeno-, human Boca-, and hepatitis E viruses in raw and secondarily treated sewage from two plants in Norway. *Food and Environmental Virology*, 7(3), 213–223.

Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M. et al. (2016) Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. *Marine Pollution Bulletin*, 103(1), 168–178.

Nilsson, V.I. & Grimsgaard, K.B. (2021) Vannkvaliteten i Lierelva relatert til jordvanning. Brage: Faculty of Veterinary Sciences, Norwegian University of Life Sciences. Unpublished thesis.

Oberbeckmann, S. & Labrenz, M. (2020) Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. *Annual Review of Marine Science*, 12, 209–232.

Oberbeckmann, S., Loeder, M.G.J., Gerdts, G. & Osborn, A.M. (2014) Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in northern European waters. *FEMS Microbiology Ecology*, 90(2), 478–492.

Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I. et al. (2018) Evidence for selective bacterial community structuring on microplastics. *Environmental Microbiology*, 20(8), 2796–2808.

Oksanen, J. (2007) Vegan: community ecology package. R package ersion 1.8–5. http://www.cran.r-project.org

Oren, A. & Garrity, G.M. (2021) Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 71(10), 005056.

Osińska, A., Korzeniewska, E., Korzeniowska-Kowal, A., Wzorek, A., Harnisz, M., Jachimowicz, P. et al. (2022) The challenges in the identification of *Escherichia coli* from environmental samples and their genetic characterization. *Environmental Science and Pollution Research*, 30, 11572–11583.

Panel, E.B., Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M. et al. (2020) Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA Journal*, 18(1), e05967.

Pellegrin, V., Juretschko, S., Wagner, M. & Cottenceau, G. (1999) Morphological and biochemical properties of a *Sphaerotilus* sp. isolated from paper mill slimes. *Applied and Environmental Microbiology*, 65(1), 156–162.

Pinto, M., Langer, T.M., Hüffer, T., Hofmann, T. & Herndl, G.J. (2019) The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. *PLoS One*, 14(6), e0217165.

PlasticEurope. (2021) Plastics—The facts: an analysis of European latest plastics production, demand and waste data. *Plastics*, 1, 40.

Prata, J.C. (2018) Microplastics in wastewater: state of the knowledge on sources, fate and solutions. *Marine Pollution Bulletin*, 129(1), 262–265.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590–D596.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41 (Database issue, D590–D596.

Rickard, A.H., McBain, A.J., Ledder, R.G., Handley, P.S. & Gilbert, P. (2003) Coaggregation between freshwater bacteria within biofilm and planktonic communities. *FEMS Microbiology Letters*, 220(1), 133–140.

Rochman, C.M., Hoh, E., Hentschel, B.T. & Kaye, S. (2013) Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. *Environmental Science & Technology*, 47(3), 1646–1654.

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584.

Romera-Castillo, C., Pinto, M., Langer, T.M., Álvarez-Salgado, X.A. & Herndl, G.J. (2018) Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. *Nature Communications*, 9(1), 1430.

RStudio, T. (2020) RStudio: integrated development for R. Rstudio Team, PBC, Boston, MA. http://www.rstudio.com

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. *Environmental Science & Technology Letters*, 4(7), 258–267.

Santos, A.C.D.M., Santos, F.F., Silva, R.M. & Gomes, T.A.T. (2020) Diversity of hybrid-and hetero-pathogenic *Escherichia coli* and

their potential implication in more severe diseases. *Frontiers in Cellular and Infection Microbiology*, 10, 339.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L. et al. (2011) Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, 17(1), 7–15.
Selvakumar, A. & Borst, M. (2006) Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health*, 4(1), 109–124.
Sheridan, E.A., Fonvielle, J.A., Cottingham, S., Zhang, Y., Dittmar, T., Aldridge, D.C. et al. (2022) Plastic pollution fosters more microbial growth in lakes than natural organic matter. *Nature Communications*, 13(1), 4175.
Silva, M.M., Maldonado, G.C., Castro, R.O., de Sá Felizardo, J., Cardoso, R.P., Anjos, R.M.D. et al. (2019) Dispersal of potentially pathogenic bacteria by plastic debris in Guanabara Bay, RJ, Brazil. *Marine Pollution Bulletin*, 141, 561–568.
Skraber, S., Ogorzaly, L., Helmi, K., Maul, A., Hoffmann, L., Cauchie, H.M. et al. (2009) Occurrence and persistence of enteroviruses, noroviruses and F-specific RNA phages in natural wastewater biofilms. *Water Research*, 43(19), 4780–4789.
Song, J., Jongmans-Hochschulz, E., Mauder, N., Imirzalioglu, C., Wichels, A. & Gerdts, G. (2020) The travelling particles: investigating microplastics as possible transport vectors for multidrug resistant *E. coli* in the Weser estuary (Germany). *Science of the Total Environment*, 720, 137603.
Stoessel, F. (1989) On the ecology of ciliates in riverwaters: The evaluation of water quality via ciliates and filamentous bacteria. *Aquatic Sciences*, 51(3), 235–248.
Stonnet, V. & Guesdon, J.L. (1993) *Campylobacter jejuni*: specific oligonucleotides and DNA probes for use in polymerase chain reaction-based diagnosis. *FEMS Immunology and Medical Microbiology*, 7(4), 337–344.
Tavelli, R., Callens, M., Grootaert, C., Abdallah, M.F. & Rajkovic, A. (2022) Foodborne pathogens in the plastisphere: can microplastics in the food chain threaten microbial food safety? *Trends in Food Science & Technology*, 129, 1–10.
Tetu, S.G., Sarker, I., Schrameyer, V., Pickford, R., Elbourne, L.D.H., Moore, L.R. et al. (2019) Plastic leachates impair growth and oxygen production in *Prochlorococcus*, the ocean's most abundant photosynthetic bacteria. *Communications Biology*, 2(1), 184.
Tryland, I., Myrmel, M., Østensvik, Ø., Wennberg, A.C. & Robertson, L.J. (2014) Impact of rainfall on the hygienic quality of blue mussels and water in urban areas in the inner Oslofjord, Norway. *Marine Pollution Bulletin*, 85(1), 42–49.
Tu, C., Chen, T., Zhou, Q., Liu, Y., Wei, J., Waniek, J.J. et al. (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, 139237.
Verran, J. & Boyd, R.D. (2001) The relationship between substratum surface roughness and microbiological and organic soiling: a review. *Biofouling*, 17(1), 59–71.
VIVA. (2018) Saneringsplan Vann og Avløp, Vestviken interkommunale vei-, vann- og avløp.
Volk, R.-B. & Furkert, F.H. (2006) Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. *Microbiological Research*, 161(2), 180–186.
Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73(16), 5261–5267.
Whitehead, K.A. & Verran, J. (2006) The effect of surface topography on the retention of microorganisms. *Food and Bioproducts Processing*, 84(4), 253–259.
WHO. (2015) WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015. *World Health Organization*.
Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M. & Wang, Y. (2019) Selective enrichment of bacterial pathogens by microplastic biofilm. *Water Research*, 165, 114979.
Yang, K., Chen, Q.-L., Chen, M.-L., Li, H.-Z., Liao, H., Pu, Q. et al. (2020) Temporal dynamics of antibiotic Resistome in the Plastisphere during microbial colonization. *Environmental Science & Technology*, 54(18), 11322–11332.
Zadjelovic, V., Wright, R., Borsetto, C., Quartey, J., Cairns, T., Langille, M. et al. (2023) *Microbial hitchhikers harbouring antimicrobial-resistance genes in the riverine plastisphere*: Research Square.
Zettler, E.R., Mincer, T.J. & Amaral-Zettler, L.A. (2013) Life in the "plastisphere": microbial communities on plastic marine debris. *Environmental Science & Technology*, 47(13), 7137–7146.
Zhang, B., Yang, X., Liu, L., Chen, L., Teng, J., Zhu, X. et al. (2021) Spatial and seasonal variations in biofilm formation on microplastics in coastal waters. *Science of the Total Environment*, 770, 145303.
Zhang, W., Jiang, C., Chen, L., Bhagwat, G., Thava, P. & Yang, Y. (2022) Spatial turnover of core and occasional bacterial taxa in the plastisphere from a plateau river, China. *Science of The Total Environment*, 838, 156179.
Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S. et al. (2020) Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, East China: multiple sources other than plastic mulching film. *Journal of Hazardous Materials*, 388, 121814.
Zhu, D., Ma, J., Li, G., Rillig, M.C. & Zhu, Y.-G. (2022) Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens. *The ISME Journal*, 16(2), 521–532.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Witsø, I.L., Basson, A., Vinje, H., Llarena, A.-K., Bringas, C.S., Aspholm, M. et al. (2023) Freshwater plastispheres as a vector for foodborne bacteria and viruses. *Environmental Microbiology*, 25(12), 2864–2881. Available from: https://doi.org/10.1111/1462-2920.16536