PLASTICS IN AQUATIC SYSTEMS: FROM TRANSPORT AND FATE TO IMPACTS AND MANAGEMENT PERSPECTIVES

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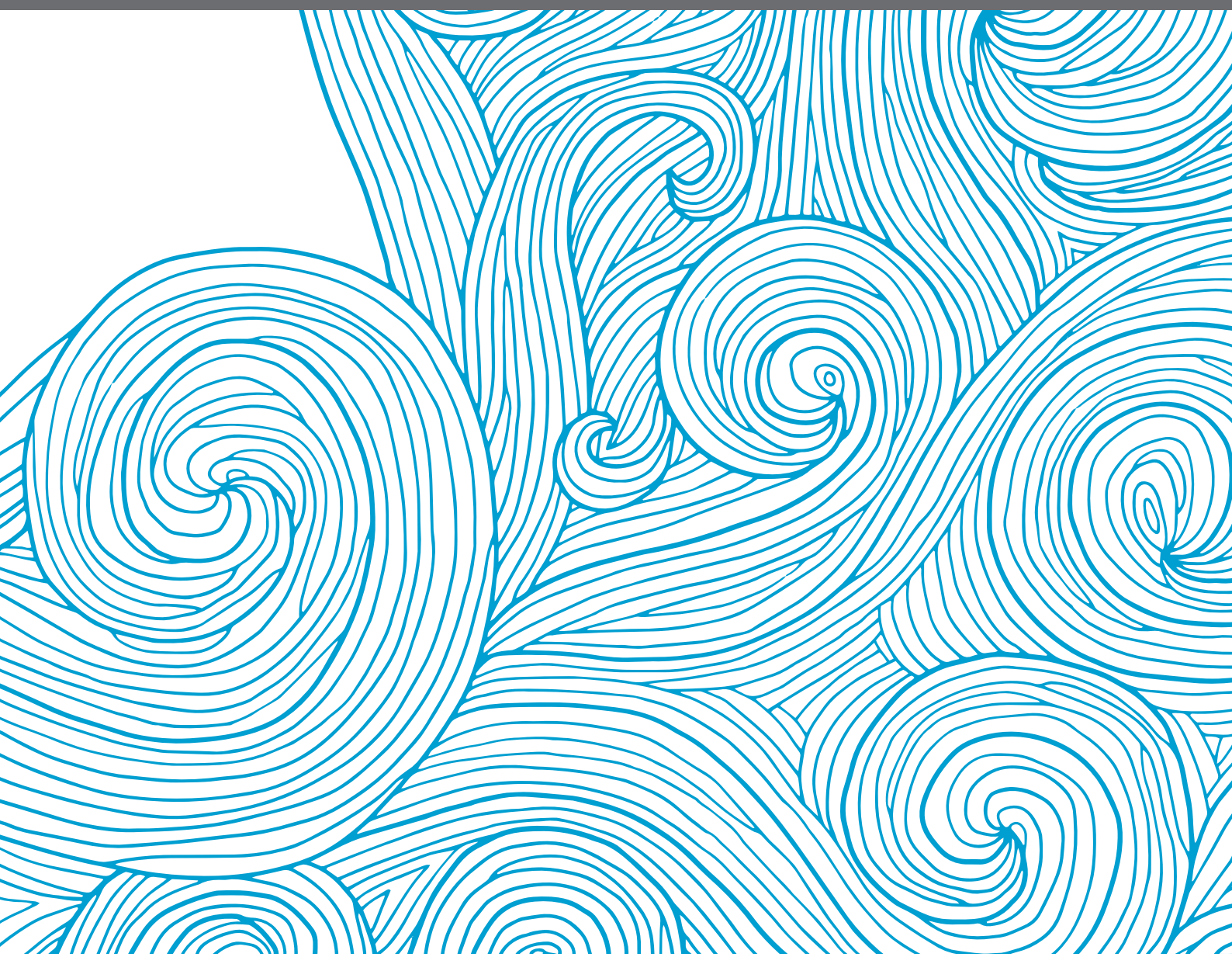

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PLASTICS IN AQUATIC SYSTEMS: FROM TRANSPORT AND FATE TO IMPACTS AND MANAGEMENT PERSPECTIVES

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Editorial: Plastics in aquatic systems: From transport and fate to impacts and management perspectives

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Editorial on the Research Topic
Plastics in aquatic systems: From transport and fate to impacts and management perspectives

Introduction

Plastic is an outstanding material that has become an indispensable part of our daily lives, especially in the current pandemic situation (Noman et al.). However, its ubiquitous application in various fields also leads to significant emissions into the environment, resulting in numerous, mainly negative consequences for biodiverity and ecosystems. This Research Topic takes a closer look at overlooked sources and emissions of plastics into the environment (Folbert et al.), continues with the measurement of environmental concentrations of plastics (Laermanns et al.; Emmerik et al.; Banik et al.; Steele and Miller; Pradit et al.) and discusses the impact of plastics on the environment (Noman et al.; Benson et al.; Mohsen et al.; Merbt et al.; Pradit et al.). We conclude this research topic with a critical assessment of the state of data availability in plastics research (Jenkins et al.). The following sections highlight the most important new findings of this Research Topic.

Overlooked sources of plastics in the environment

Due to the recent pandemic, the use of plastic-made personal protective equipment (PPE) like face masks has increased exponentially. Unfortunately,

careless and mismanaged waste disposal of these useful items has led to a significant increase of plastics introduced into the environment. In their review paper, Noman et al. highlight the poorly studied plastic emission from PPEs into the environment and discuss the possibility of PPEs being disease routes or vectors of pathogens like SARS-CoV-2. Another, less obvious and poorly studied emitters of plastics are sea-based sources such as cruise ships, as found by Folbert et al.. These authors highlight that cruise ship wastewater is highly polluted with personal care items, cosmetics and cleaning products as well as synthetic microfibers from washing machines, with untreated greywater and overboard discharge of biosludge being main introduction pathways of microplastics into marine surroundings. Being the first to look into this source, they also name possible ways for reducing microplastic emissions from cruise ships in the future.

Towards a better understanding of the environmental distribution of plastics

Although long overlooked, rivers are now known to be a main transport pathway and possible sink for plastics. However, the influence of hydrology and river characteristics on local plastic distributions and downstream transport is still poorly understood. Laermanns et al. examine microplastic distribution in water and sediment at the confluence of two rivers in Germany (Elbe and Mulde) to identify the impact of the highly industrialized Mulde river catchment on the microplastic load in the Elbe. Their study indicates that the Mulde contributes to a substantial amount of microplastics in the Elbe River while further identification of possible source areas within the catchment needs further study. Traveling further downstream in the aquatic environment, Banik et al. and Steele and Miller focus on plastics at beaches at Kuakata, Bangladesh and at the California Channel Islands, respectively. Banik et al. study a major tourist beach in Bangladesh and the associated ecological risk of high plastic emissions by tourist activities. At their study site, local tides and currents led to the accumulation of fine sand, and Banik et al. find a correlation between smaller sediment grain sizes and higher microplastic concentrations on the beach. Moving from Bangladesh to the U.S., Steele and Miller report the temporal variation (2016–2020) of plastic pollution on remote beaches of the California Channel Islands and on the adjacent mainland. Steele and Miller find higher accumulation rates for plastic on the remote islands than on the mainland, as well as higher plastic accumulation rates in fall and winter, which might be influenced by tidal height, wind speed and direction, extreme events and anthropogenic sea-based activities. Although fishery-related macroplastic waste made up a high percentage of the found debris, it declined over the course of the 4-year study, possibly due to new regulations leading to lower fishing activities.

Representative monitoring of micro-as well as macroplastics in aquatic environments is still difficult to achieve, although it is essential for guiding policy, developing knowledge, managing operations, and designing and implementing mitigation strategies (Emmerik et al.). These authors develop a “Roadmap” for macroplastic monitoring in the fluvial environment regarding method development, baseline assessment and long-term monitoring that can guide national riverine macroplastic monitoring strategies in the future.

Implications of plastics in the environment

In the aquatic environment, plastics can have a variety of effects on biota, ecosystems and ecosystem services. In their review, Benson et al. compile the implications that micro- and nanoplastics have on food webs and ecotoxicological aspects in freshwater and marine settings. Regarding food web interactions, they highlight the ingestion, exposure routes and bioaccumulation of micro (nano)plastics and the probable ecotoxicological effects on aquatic biota. Additionally, Benson et al. review the adsorption and desorption potential of plastics for persistent organic pollutants, metals and chemical additives. Depending on their use, plastic items can pose additional hazards. The PPE may introduce pathogens such as SARS-CoV-2 into the environment, which could affect the plastisphere and other microbial communities (Noman et al.). Higher abundance of pathogenic bacteria on floating plastics around aquaculture areas compared to that of the surrounding water indicate the impact of anthropogenic activities (Mohsen et al.). Microbial communities (periphyton) form not only on microplastics, but also on rocks and sediments in freshwater environments, and play an essential role in the nutrient cycle (Merbt et al.). Due to the large surface area of the periphyton, it can act as a sink for microplastics (Merbt et al.). Microplastics seem to significantly impact the composition, relative abundances and mechanical properties of prokaryotic and eukaryotic communities of the periphyton, but the underlying mechanisms of these microplastic-biofilm interactions need to be studied in more detail in the future (Merbt et al.). Next to microbial communities, microplastics also change the composition of natural materials. Pradit et al. are the first to observe microplastics attached to the surfaces and pores of pumice stone, an extrusive volcanic rock, on shorelines of Thailand. The lightweight pumice stone has probably been transported to the Gulf of Thailand from the South China Sea, and thus acting as a transport mechanismand a sink for microplastics (Pradit et al.).

Still a long way to go: Open science in plastic research

As highlighted in this Research Topic, plastic pollution of our environment is not confined by geopolitical boundaries and potentially affects everyone on Earth, and should therefore be studied as comprehensively and collaboratively as possible. An important step in this process is to improve the availability of research data by following the FAIR (Findable, Accessible, Interoperable and Reusable) guidelines. However, the percentage of available data has not increased in the last 5 years in which open science has gained momentum (Jenkins et al.). Analyzing 785 randomly selected studies that were published between 1964 and 2021 on environmental microplastic sampling, Jenkins et al. highlight that only a third of their studied papers contain a data sharing statement. Even of the accessible datasets, less than 20% have descriptions amenable to use in further studies. To increase the accessibility of microplastic research data, Jenkins et al. recommend five strategies: 1) use available standards and practices to describe data; 2) share raw data—or as close to raw as possible; 3) use a trusted digital repository; 4) link datasets to publications; and 5) plan to share data from the onset of a study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Microplastic in Water and Sediments at the Confluence of the Elbe and Mulde Rivers in Germany

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Accumulation of microplastics in aquatic environments is an issue of emerging concern. Initially, research focused on marine systems. However, recent studies also investigate the abundance of microplastics in freshwater environments. Rivers connect terrestrial with marine ecosystems and contribute a considerable share of macro- and microplastics to the oceans. A previous study found a large amount of micro-spheres in Dessau downstream the river mouth of the Mulde. Therefore, the objective of this research was to examine whether the Mulde river with its highly industrialized catchment contributes to the microplastic pollution of the Elbe. Sediment (Van Veen grab sampler) and water samples (filter cascade with the smallest mesh size 50 µm and nets with the smallest mesh size 150 µm) were taken from the Elbe river up- and downstream the confluence with the Mulde. After extensive sample preparation, we examined the samples under a digital microscope and determined polymer types by pyrolysis Gas Chromatography/Mass Spectrometry (pyr-GC-MS). The amount of primary microplastics increased in sediment and water samples just downstream the confluence. Those microplastics originate probably from the Mulde. We measured larger amounts and different shapes of microplastics in filter cascades that have a smaller mesh size compared to the nets.

Keywords: microplastics, rivers, Elbe, Mulde, sediments, pyr-GC-MS

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1 INTRODUCTION

Plastic became a mass product in the second half of the 20th century and has changed almost all areas of our everyday life since then. Littering and uncontrolled disposal of discarded plastic products threaten the environment because plastic is resistant and poorly biodegradable (Thompson et al., 2004; Geyer et al., 2017). Studies on macroplastics and plastic waste are common in the scientific literature. In contrast, microplastics, usually defined as plastic particles smaller than 5 mm (Arthur et al., 2009), has only become a major research topic since the early 2000s (Thompson et al., 2004). Microplastics is an umbrella term and encompasses different categories, e.g., polymer types, shapes (amorphous, fibres, spheres, films, and foams) and origins (primary and secondary microplastic).

Abbreviations: PS, Polystyrene; PP, Polypropylene; PE, Polyethylene; PS-DVB, Polystyrene divinylbenzene; PMMA, Polymethyl methacrylate; PET, Polyethylene terephthalate; PBT, Polybutylene terephthalate.

While primary microplastics are intentionally produced, for example as spheres for industrial purposes, secondary microplastics originate from fragmentation of macroplastic objects by exposure to light, heat, mechanical friction or organisms (Geyer et al., 2017; Kataoka et al., 2019; Meides et al., 2021; Petersen and Hubbart, 2021). So far, researchers studied mostly marine environments, while the contamination of freshwater and terrestrial systems gained far less attention (Dris et al., 2015; Wagner and Lambert, 2018; Scherer et al., 2020). Additionally, the research on microplastics has focused mainly on their abundance and distribution in the environment, while their transport and pathways have remained rather understudied (Horton et al., 2017; Hurley and Nizzetto, 2018; Rochman, 2018; Rillig and Lehmann, 2020). Consequently, we know much less about the sources, transport ways and sinks of microplastics in fluvial environments than in the oceans (Dris et al., 2015; Klein et al., 2015; Wagner and Lambert, 2018).

Rivers transport microplastics to the oceans (Lebreton et al., 2017; Rochman, 2018; Weber and Opp, 2020) and an estimated 80% of marine plastic debris originates from inland sources (Meijer et al., 2021). Several studies have identified urban regions and most notably industrial areas as major sources of microplastic pollution in rivers (Mani et al., 2016; Schmidt et al., 2018; Tibbetts et al., 2018) and therefore as a threat to fluvial and marine environments (Eerkes-Medrano et al., 2015; Blair et al., 2017; Petersen and Hubbart, 2021). The sources of microplastics are manifold, and so are their pathways to rivers. Indeed, microplastics can be transported by surface run-off from agricultural areas, aerial emission from industries or application of sewage sludge, and be released from discharge of waste water treatment plants (WWTP) (Horton and Dixon, 2018; Wagner and Lambert, 2018; Sun et al., 2019; Brandes et al., 2021). Although WWTPs filter more than 90% of microplastics, they still contribute a certain share of microplastic particles to fluvial systems (Murphy et al., 2016; Horton et al., 2017; Kay et al., 2018; Schmidt et al., 2020; Haberstroh et al., 2021; Schell et al., 2021).

Rivers are not only transport systems of microplastics to the oceans, they can also function as sinks themselves (Horton and Dixon, 2018; Frei et al., 2019; Scherer et al., 2020; Waldschläger and Schüttrumpf, 2020). Depending on their density and shape, microplastics tend to float on or close to the water surface or, if denser, can sink (Waldschläger and Schüttrumpf, 2019a). Erosion during flooding events, for example, may re-mobilise the particles (Hurley et al., 2018; Lechthaler et al., 2021). In particular, due to their low density, microplastic particles are re-mobilised more easily than natural sediments (Waldschläger and Schüttrumpf, 2019a). The residence time of microplastic particles in river water affects biofouling, which forms biofilms and changes the particles' surface characteristics and their density (Horton et al., 2017; Horton and Dixon, 2018).

Consequently, the occurrence and distribution of microplastics in fluvial systems is rather complex and depends on the distribution of sources and pollutants (Kay et al., 2018; Haberstroh et al., 2021) as well as on fluvial dynamics, such as seasonality (Mani and Burkhardt-Holm, 2020) and flood events (Hurley et al., 2018). In this context, recent studies suggest that smaller and medium-sized rivers are of crucial importance for the microplastic distribution in the whole catchment. They point out that the abundance of microplastics in smaller rivers might be far higher than in larger rivers due to the proximity of point sources (Heß et al., 2018; Constant et al., 2020). Therefore, tributaries are more and more seen as relevant contributors of microplastics to larger rivers (Mani et al., 2016; Scherer et al., 2020).

Our study focuses on the confluence of the Mulde (major tributary) and the Elbe rivers (Junge, 2020). In a previous study, Scherer et al. (2020) showed that the site of Dessau along the Elbe was highly contaminated with microbeads. By sampling both, sediment and water of the Elbe, upstream and downstream of the confluence, we intend to 1) clarify if the Mulde contributes to a substantial share of microplastic to the Elbe and 2) analyse the spatial distribution of microplastic contamination of the area on a local scale. Furthermore, we 3) quantify and characterize the microplastic particles by optical microscopy and pyrolysis Gas Chromatography/Mass Spectrometry (pyr-GC-MS).

2 MATERIALS AND METHODS

2.1 Research Area and Sampling

The Elbe river is one of the largest rivers in Central Europe. It originates in the Giant Mountains in the Czech Republic and debouches after 1094 km course close to Cuxhaven into the North Sea in Germany. Its catchment covers an area of approximately 148,000 km^2 with roughly 24.5 million inhabitants. The Elbe can be separated in the Upper Elbe (Czech Republic and German Elbe Sandstone Mountains until Castle Hirschstein), the Middle Elbe (from Castle Hirschstein until the barrage at Geesthacht close to Hamburg) and the tide influenced Lower Elbe from Geesthacht to the open North Sea at Cuxhaven-Kugelbake (Scherer et al., 2020).

The Mulde river is the fourth main tributary of the Elbe, after Vltava, Saale and Havel, and covers a catchment of approximately 7,400 km^2 (Schneider and Reincke, 2006) (**Figure 1**). Intense and long-term anthropogenic activities of ore mining, smelting and metalworking industries led to continuous inputs of trace metals to the river, that are detectable in both, water and sediments of the Mulde (Junge, 2020). Additionally, plastic-processing industries in the surroundings of the cities of Bitterfeld and Dessau could potentially contribute microplastics to the river (Scherer et al., 2020).

In January 2020 samples were taken in the Elbe nearby the city of Dessau-Roßlau upstream and downstream of the confluence with the Mulde river, close to both riversides (**Figure 1**). Seven water samples were retrieved with two different methods. For smaller particles, a filter cascade with mesh sizes of 100 and 50 µm was used for four samples (fractionated pressure filtration) (Klein et al., 2018; Stock et al., 2019). The filter cascade was connected with a pump which was placed in a depth of 30 cm below the water surface filtering 530–680 L per sample. For larger particles, an Apstein plankton net (opening: 0.022 m^2,

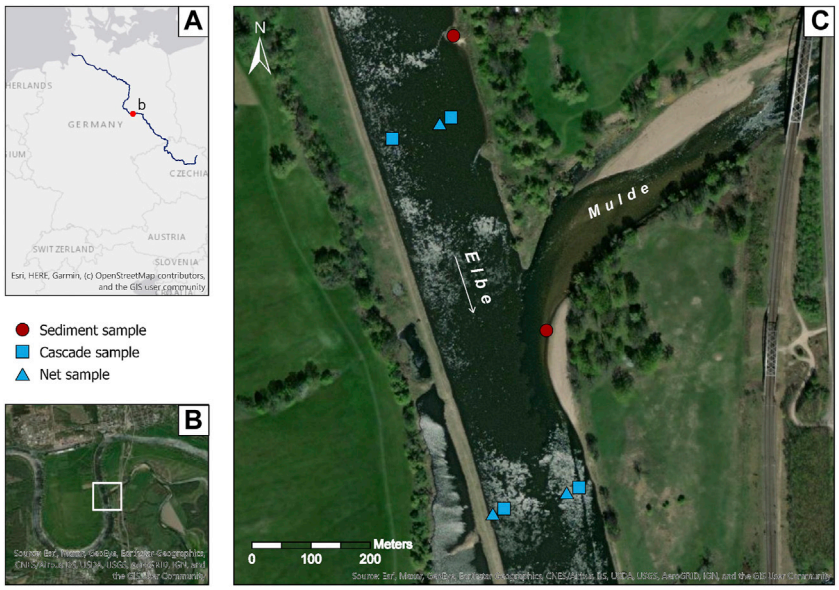

FIGURE 1 | (A,B) Location of the Elbe–Mulde confluence in Dessau-Roßlau (Germany). **(C)** One sediment sample and three water samples were taken upstream of the confluence, and four water samples and one sediment sample were taken downstream.

diameter 17 cm, length 110 cm) with two connected nets of 150 and 300 µm mesh size was used for three samples. For measuring the water volume, a flow meter was fixed on the plankton net (40 and 53 m^3 per sample) (Klein et al., 2018; Stock et al., 2019). Samples were stored in glass jars until further processing.

Additionally, two sediment samples were taken at the left shoreline of the Elbe, from the river banks upstream and downstream of the Mulde confluence with a Van Veen grab sampler (1.2 kg of sediment upstream and 1.23 kg of sediment downstream, respectively) (**Figure 1**). Sediments were transferred into glass jars until further processing.

2.2 Sample Preparation

In the laboratory, the sediment samples were dried in an oven at 40°C for 5 days. All subsequent sample preparation steps in the laboratory were done under a laminar flow box to avoid contamination. The samples were dry-sieved on a vibrating shaker using a five-sieves cascade (Microtrac Retsch GmbH, Haan, Germany) with the mesh sizes of 1,000, 500, 100, 50 and 20 µm (Enders et al., 2019). Subsequently, they were placed on a transversal cross-shaker and treated with 35% hydrogen peroxide (H_2O_2) to dissolve the organic matter (Mai et al., 2018). After all visible reaction has ceased, the samples were transferred to glass separation funnels. A saturated potassium formate [K(HCOO)] solution was added for a density separation to remove the inorganic sediment material (Mai et al., 2018; Enders et al., 2019; Stock et al., 2019). Finally, the supernatant containing the microplastics was vacuum-filtered onto glass microfibre filters (Fisherbrand MF 100 by Fisher Scientific, Waltham, Massachusetts, United States) (Pagter et al., 2018).

The water samples were vacuum-filtered with stainless-steel sieves with mesh sizes of 50 and 100 µm for the samples of the filter cascade, and mesh sizes of 150, 300 and 500 µm for the samples of the nets. Similarly to the sediment samples, the organic matter was destroyed by adding hydrogen peroxide (H_2O_2) and the samples were filtered on glass microfibre filters.

2.3 Identification of Microplastics in Water and Sediment Samples

2.3.1 Visual Identification

Each filter was photographed under a digital microscope (VHX-2000 by Keyence Corporation, Osaka, Japan) equipped with 200 × magnifying lenses. Presumable microplastic particles were counted and their shape determined (Hidalgo-Ruz et al., 2012). The number of particles was related to the dry weight of the sediment samples (number of particles per kg dry sediment) or the volume of the water samples (number of particles per m^3 of water), respectively (Mai et al., 2018). Then, 121 particles were picked, photographed and their size measured in the same digital microscope. Kindly note that the 100 µm water sample downstream on the left side was pyrolysed before pictures could be taken.

2.3.2 Pyr-GC-MS Analysis

The photographed single particles were analysed to identify the polymer type of frequently occurring particles (e.g., spheres, fibres etc.). The remaining particles were extracted *via* pressurized liquid extraction similar to sample preparation published by Dierkes et al. (2019). Instead of the described

TABLE 1 | Parameters of the pressurized liquid extraction for the pyr-GC-MS analysis.

Parameter	Pre-extraction	Microplastic extraction
Extraction solvent	Methanol	Tetrahydrofuran
Top volume (ml)	5	18
Bottom volume (ml)	5	7
Rinse volume (ml)	5	0
Extraction temperature (°C)	100	170
Cycle time (min)	10	15
Cycles	2	3

TABLE 2 | Indicator compounds and selected indicator ions in the pyr-GC-MS analysis (m/z, mass/charge; t_R, retention time).

Polymer	Pyrolysis product	Indicator ion (m/z)	t_R (min)
Polypropylene	2,4-Dimethyl-hept-1-ene	126	4.59
		70	4.59
Polyethylene	1,14-Octadeca-diene	81	11.69
	1-Pentadecene	97	11.72
Polystyrene	Styrene	104	5.21
		91	5.21
Poly(4-fluorstyrene)	4-flourstyrene	122	5.29

equipment (ASE-350, Dionex, Sunnyvale, CA, United States) an alternative extraction system was used (EDGE, CEM, Matthews, NC, United States) with parameters listed in **Table 1**.

Briefly, filters were transferred to aluminium-coated cups (Q-cups; CEM, Matthews, NC, United States) covered with calcined (600°C, 2.5 h) sea sand and automatically extracted with methanol (MeOH, LC-MS grade; Merck, Darmstadt, Germany) to reduce disturbing organic matrix effects. Subsequently, microplastic particles were extracted with tetrahydrofuran (THF, HPLC grade, unstabilised; Sigma-Aldrich, Schnelldorf, Germany). While MeOH-extracts were discarded, THF-extracts were collected in 60 ml-vials previously filled with 200 mg calcined silica gel. Fluorinated polystyrene [poly(4-fluorstyrene), PFS; PolymerSource, Montreal, Canada) was used as internal standard (10 ml of 1 mg ml^{-1} in dichloromethane (DCM, picograde; Sigma-Aldrich, Schnelldorf, Germany)] (Lauschke et al., 2021) (**Table 1**; **Supplementary Table S1**). Calcined silica gel was used to capture precipitating synthetic polymers as THF was subsequently evaporated for Pyr-GC-MS analysis. Adhered microplastics were manually rinsed off vial walls with DCM for at least three times. Then, silica gel was manually homogenised in an agate mortar and aliquots of 20 mg were weighted into 80 µl pyrolysis cups (Eco-Cup LF, Frontier Laboratories, Saikon, Japan) and pyrolysed at 600°C. Pyr-GC-MS analysis was conducted as described by Dierkes et al. (2019), except that a DB-5ms capillary separation column (Agilent, Santa Clara, CA, United States) was used. For the single particles, scan mode was used (qualitative analysis), while for the remaining mass-based quantification selected ion monitoring (SIM) mode was applied. Therefore, pyrolysis products and indicator ions, respectively, were monitored as shown in **Table 2**. Kindly note that the utilised pyr-GC-MS method is currently limited to determine mass concentrations of the three polymers PE, PP and PS.

2.3.3 Validation and Quality Control

To estimate the recovery rates of microplastic particles during the sample preparation, artificial quartz sand-silt mixture (approx. 1,500 g with a ratio of 60% sand and 40% silt) were spiked with PS, PET, LDPE, PP and PVC (30 particles of 200–2,000 µm each). This artificial validation sample was treated equally to the sediment samples. The total recovery rate for the whole extraction process equals 71.3%.

In addition, two blank samples were used to assess the influence of airborne contamination in the laboratory (Klein et al., 2018; Mai et al., 2018). In one of the blank samples, we detected two, in the other six fibres. Furthermore, blank filters were put next to the microscope during visual identification to quantify contamination during the analysis (Scherer et al., 2020). Here, one fibre was found on the filter. Therefore, the airborne contamination during sample preparation and visual analysis can be considered as low and no correction of the concentration of microplastics was done.

3 RESULTS

3.1 Visual Identification

In total, 1,782 presumable microplastics were counted and categorized under the microscope, 393 in sediment samples and 1,389 in water samples. Within the sediment samples, spheres occurred most frequently with a notable difference of 35 presumable microplastic particles kg^{-1} upstream and 208 presumable microplastic particles kg^{-1} downstream of the Mulde confluence (**Figure 2**; **Supplementary Table S1**). In contrast, the numbers of fibres, films, and fragments remained rather low in both locations (27 presumable microplastic particles kg^{-1} or less). Most spheres were in the size fraction between 500 and 1,000 µm and between 50 and 100 µm.

Due to the different sampling methods of the water samples, either with the Apstein plankton net or the filter cascade, comparisons need to be considered carefully. Indeed, the particle sizes differ between the sampling techniques because of different mesh sizes of the cascade and the net, respectively. We found substantially more fibres in the cascade than in the plankton net (188 versus 15 presumable microplastic particles m^{-3} **Figure 3** and **Supplementary Figure S1**). While the number of fibres, especially in the cascade samples, remained large in all samples, the occurrence of spheres seems to be related to the sampling sites. Upstream of the confluence, we found a few spheres only. In contrast, the number of larger spheres in the net increased downstream and especially on the left riverside that is adjacent to the Mulde.

3.2 Pyr-GC-MS Analysis

Altogether, 121 single presumable microplastic particles were picked, 87 from water samples (20 upstream and 67 downstream) and 34 particles from sediment samples (18 upstream and 16 downstream). More than half of these 121 isolated presumable microplastic particles were spheres, from which almost all (62 out of 68) were identified as PS or PS-DVB, while a certain number of

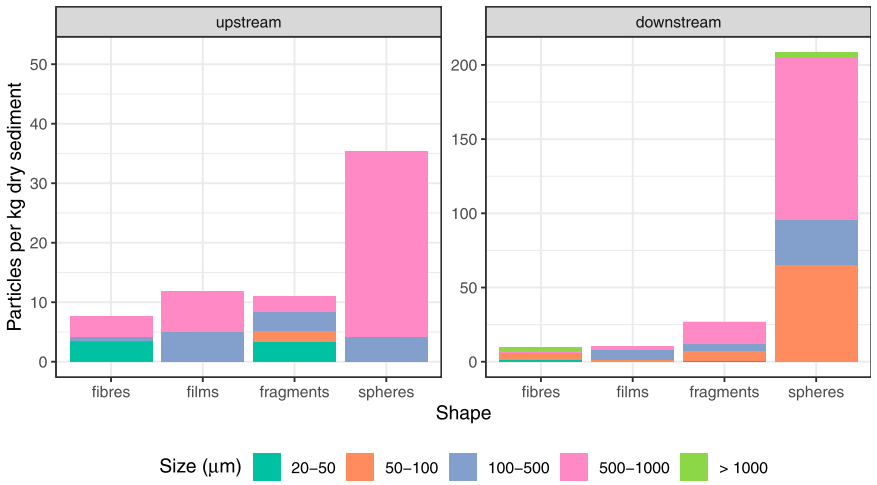

FIGURE 2 | Number of presumable microplastics in sediment samples by shape. Note different scales in the ratio 1:4 on the *y* axis.

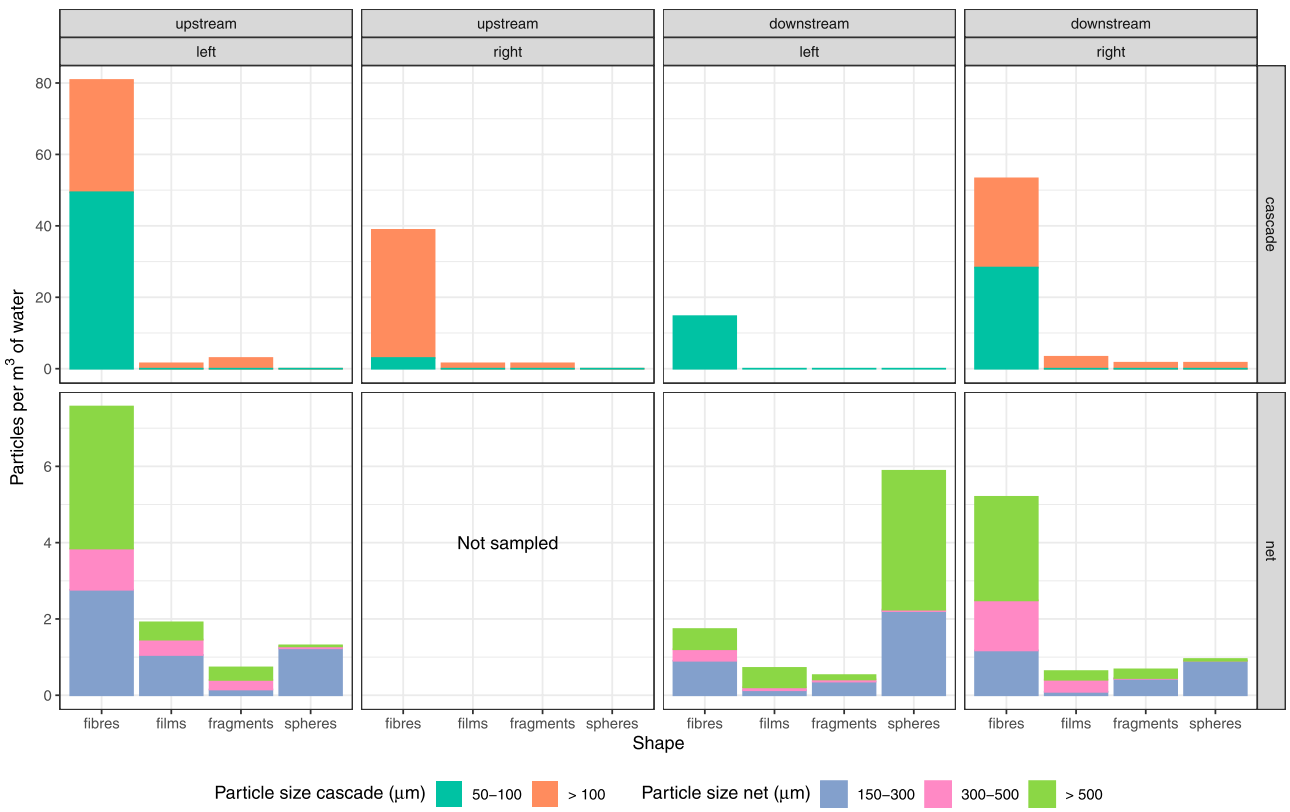

FIGURE 3 | Number of microplastics in water samples by shape. Note different scales on the *y* axes for cascade and net samples.

fragments consisted of PE, PP or PS. 36 particles could not be identified (**Supplementary Table S1**).

In the remaining sediment sample upstream of the confluence, we found PE almost exclusively (**Figure 4**). Especially in the coarser fractions (500–1000 µm and < 1000 µm), larger concentrations of 0.45 and 0.21 mg kg^{-1} PE were detected. In the sediment sample downstream, we measured ca. 0.8 mg kg^{-1} PE with high shares in the fraction of 500–1000 µm, and more than 1 mg kg^{-1} PS with

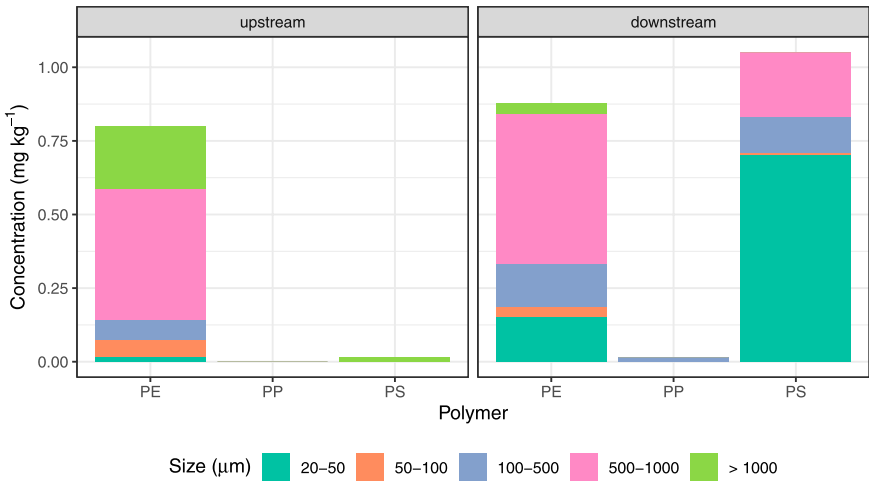

FIGURE 4 | Mass concentration of microplastics in sediment samples by polymer.

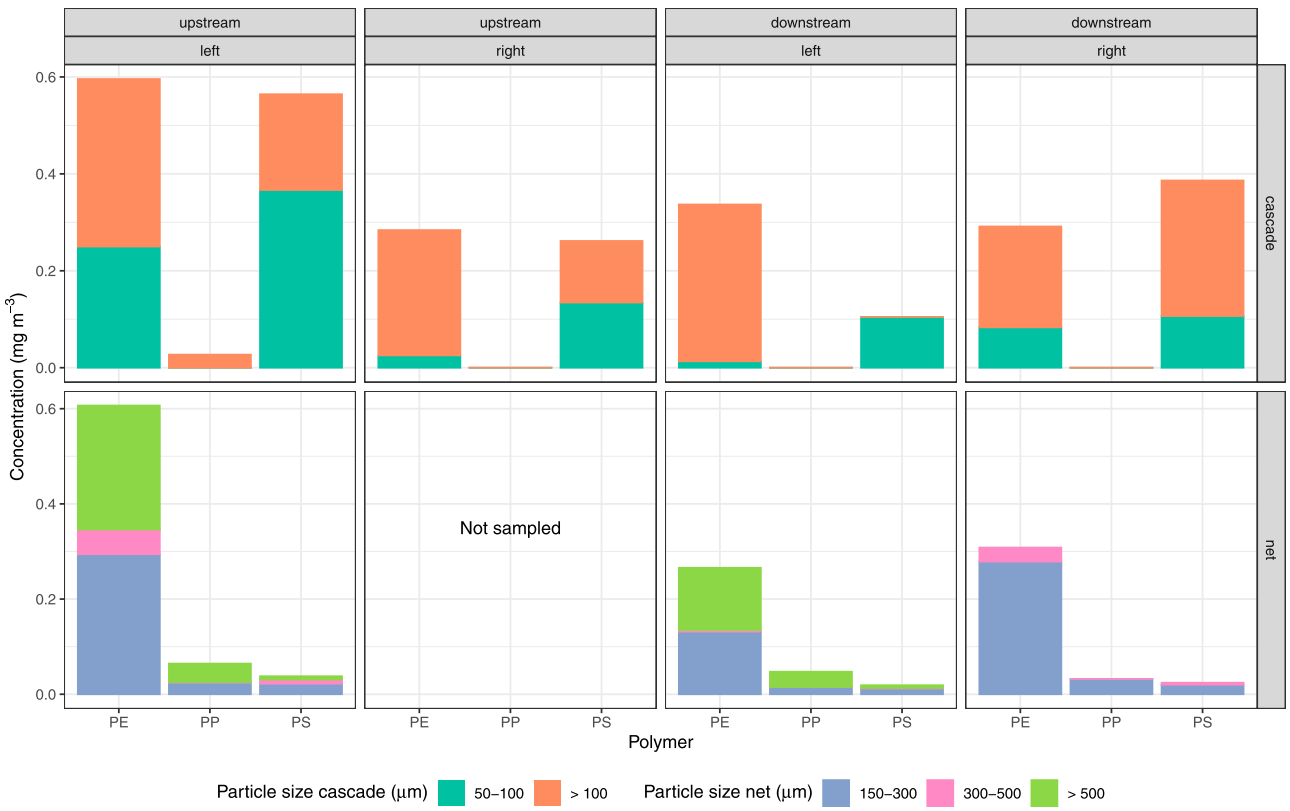

FIGURE 5 | Mass concentration of microplastics in water samples by polymer.

especially high shares in the finest fraction of 20–50 µm. Hardly any PP was detected.

The concentrations of microplastics (all polymers taken together) in the water samples varied between 0.33 mg m^{-3} (downstream, left side, net sample) and 1.19 mg m^{-3} (upstream, left side, cascade sample). PE and PS were roughly equally common in the cascade samples, with maxima of 0.60 mg m^{-3} and 0.56 mg m^{-3}, respectively (upstream, left side, all fractions summed up) while the net samples

indicated higher shares of PE. The concentration of PP remained below 0.05 mg m^{-3} (**Figure 5**).

4 DISCUSSION

4.1 Microplastic Abundance in Water and Sediments

The sediment and water samples which we took upstream of the Elbe–Mulde confluence show that–regardless of the sampling site and type–the Elbe carries a certain load of microplastics. They are mainly composed of fibres; however, the water also contains small amounts of spheres, fragments, and films. Whether these values truly reflect the average microplastic load remains open, since our samples provide a snapshot at the moment of sampling only. Indeed, the number of microplastics in the river water can vary over the seasons and with flood events (Constant et al., 2020; Mani and Burkhardt-Holm, 2020; Napper et al., 2021). This also holds true for the sediment samples that were taken at the riverside. There, the occurrence of microplastics depends mostly on deposition and re-mobilisation, driven by the water level, flooding, flow dynamics and exposure to the atmosphere during low discharge (Hurley et al., 2018; Constant et al., 2020; Mani and Burkhardt-Holm, 2020).

The remarkable amount of (PS) spheres downstream the confluence originates most likely from the Mulde river. The largest concentrations of spheres can be found in the water samples closest to the confluence downstream on the left river side (**Figure 3**). With increasing distance to the confluence at the sampling sites of the cascade and net samples on the right sight of the Elbe, the influence of the Mulde decreases and the abundance of microplastics is comparable to that found upstream of the confluence. Similarly, a larger number of spheres on the Mulde-dominated left river bank supports the hypothesis of spheres originating from the Mulde (**Figure 4**). Therefore, the influence of the Mulde as one of possible sources for microplastic spheres in the Elbe is very likely. On the other hand, the Mulde seems to contribute few fibres only. While upstream at both sides and downstream on the right side fibres abound, the strongly Mulde-influenced water sample downstream on the right side contains far lower amounts of fibres (**Figure 3**).

The pyr-GC-MS analysis of the isolated particles reveal on the one hand that these spheres consists of PS or PS-DVB. This accords with the high PS content of the sediment sample downstream of the Mulde confluence (**Figure 4**). However, water samples with a large content of spheres do not show a higher concentration of PS. This indicates a certain degree of incomparability between visual and chemical analyses, and also between water and sediment samples. A possible explanation for this discrepancy could be that the spheres do not consist of 100% PS, but are made of another polymer and eventually only coated with PS. A clear categorization of single particles based on pyr-GC-MS remains challenging, especially because the differentiation between PS and PS-DVB is complicated due to the weak DVB signal. Therefore, PS-DVB is often identified as PS (Mani et al., 2019; Scherer et al., 2020).

A potential source and original function of these spheres could be so-called ion-exchange resin (IER) beads that are commonly used e.g., in industrial waste water treatment plants for softening and desalination of water (or aqueous solutions), a phenomenon known from several other German rivers (Mani et al., 2019). An increased occurrence of the spheres in the environment is commonly attributed to one or several point sources (Mani et al., 2019), which are often formed by the discharge of industrial (micro-)plastic production plants (Lechner and Ramler, 2015) or the above-mentioned wastewater treatment plants (Browne et al., 2011; Kay et al., 2018; Schmidt et al., 2020). However, our data set is too limited to clearly trace the origin of these particles. Further studies are in preparation.

Because the utilised pyr-GC-MS method is limited to the quantification of PS, PP and PE, only those polymers could be identified in our study. Other frequently used polymers such as PMMA, PET or PBT remained undetected. Furthermore, due to sieving no particles smaller than 20 µm could be analysed in the sediments. However, small microplastics are often abundant in river waters, sediments and the hyporheic zone and therefore of great relevance for microplastic research (Frei et al., 2019).

Additionally, the sampling methods and possible local peculiarities of the sampling site affect the detection of microplastic particles (Hidalgo-Ruz et al., 2012; Klein et al., 2018; Lenaker et al., 2019; Stock et al., 2019). For example, the smallest size of detectable particles in the water samples is limited by the mesh size and varies between the cascades (fractions of 50–100 and > 100 µm) and the nets (fractions of 150–300, 300–500 and > 500 µm). Consequently, our sampling methods cover different size ranges of particles and the fraction smaller than 50 µm remains undetected, affecting the overall number of microplastics that we can identify (Mai et al., 2018; Prata et al., 2019). The cascades contain slightly higher particle concentrations than the nets, in particular, more small fibres.

Moreover, the sampling depth of 30 cm below the water surface might have an impact on the abundance of detected polymers and particle shapes (Eriksen et al., 2013; Löder et al., 2017). Due to the specific density of the polymers, the particles' size and shape and the turbulent flow in the river, higher concentrations might have been detected at a greater water depth or directly at the surface (Waldschläger and Schüttrumpf, 2019a; Lenaker et al., 2019; Wurpts and Shiravani, 2019). Therefore, the total load of the Elbe might still be underestimated and a comprehensive sampling over the entire water column across the river would be desirable.

Furthermore, it should be noted that the sediment samples were dry-sieved. Compared to wet-sieved samples, microplastic particles could be distributed differently between the size fractions. However, the total number of particles remains the same. Additionally, the relatively high total recovery rate indicates a certain accuracy of our sample preparation and analysis.

4.2 Comparison With Other Parts of the Elbe and European Rivers

The high share of fibres in all water samples accords well with the results of several other studies on abundance of microplastics in

the Elbe (Scherer et al., 2020) and other European rivers (Frei et al., 2019; Lenaker et al., 2019; Napper et al., 2021). Due to their low density, small fibres tend to occur rather close to the surface, which is reflected especially in our cascade samples. Numerous potential sources can contribute microplastics to the river, e.g., urban and industrial areas within the catchment in general and waste water treatment plants as typical effluents for fibres in particular (Kay et al., 2018; Haberstroh et al., 2021).

The abundance of the most frequent polymers also reflects, to a certain extent, the findings of other studies. Especially the large share of PP and PS in the single analysed particles agrees with studies, e.g., on Swiss lakes (Faure et al., 2015), different sites from the Rhine and Main areas (Klein et al., 2015; Mani et al., 2016), the United Kingdom (Sadri and Thompson, 2014) and other places across Europe and Asia (Browne et al., 2011). Although the amounts of PP measured by pyr-GC-MS in our samples were small, one should keep in mind that the mass-based pyrolysis results give no information on the number of particles, which is well shown by Scherer et al. (2020) for the Elbe.

Scherer et al. (2020) worked on several sites along the Elbe. In their study, the amounts of PP also remained quite low in all sediment samples which were taken along the Middle, Lower and Outer Elbe (between 1.7 and 7.8 mg kg^{-1}). However, the share of PE varied between ca. 2 and 80 mg kg^{-1}. The concentration of PS varied even stronger. It remained mainly low (between 0 and 2 mg kg^{-1}), but increased to over 150 mg kg^{-1} in Geesthacht, where the Lower Elbe begins (Scherer et al., 2020). This sudden change in microplastic concentrations can be related to tidal influence and the barrage in Geesthacht. Close to our research area (further downstream), Scherer et al. (2020) measured concentration of 34 mg kg^{-1} PE, 3.25 mg kg^{-1} PP and 1 mg kg^{-1} PS. While the latter one accords roughly with our findings, the concentrations of PE and PP are both several times larger in the study by Scherer et al. (2020) compared to our samples.

Local conditions at sampling sites restrict the comparison of the absolute abundance of microplastics in the Elbe with other sites or rivers. We analysed one sample upstream and downstream of the confluence, respectively. The exact location of the samples (possibility of sedimentation of microplastics) might strongly influence the results. Indeed, flow patterns have a major impact on the transport, settling, deposition and remobilization of microplastics (Waldschläger and Schüttrumpf, 2019b). A larger transport capacity, for example, might impede deposition at riversides or in the hyporheic zone (Boano et al., 2014; Frei et al., 2019). Furthermore, a larger transport energy might (re-)mobilize coarser sediment that could crush microplastic particles and contribute to their physical degradation (Ding et al., 2019). The difference in sample preparation, especially for the sediment samples with dry-sieving, must also be considered. However, our results accord well with Scherer et al. (2020) who found comparably large amounts of primary PS-DVB spheres close to the Mulde confluence.

Scherer et al. (2020) estimated the concentration of microplastics in the Elbe water samples to be rather low compared to rivers that contribute a large global share of microplastics to the oceans (Meijer et al., 2021). On a regional scale, the studies by Mani et al. (2016) in the Rhine and Wagner et al. (2014) in the Elbe, Moselle, Neckar, and Rhine rivers, Weber and Opp (2020) in the Lahn, Horton et al. (2017) in the Thames, Constant et al. (2020) in the Rhone and Lechner and Ramler (2015), Pojar et al. (2021) in Austrian and Romanian parts of the Danube would be some of only a few eligible comparisons. Compared to these rivers, the microplastic concentrations of the Elbe estimated by Scherer et al. (2020) are lower in the water samples, while the concentrations in the sediments are comparable. However, the microplastic concentrations that were measured in this study remain at remarkably low levels.

A comparison with rivers on a global scale remains challenging as well. Besides the complexity of fluvial dynamics, different climatic and geomorphological conditions, the occurrence and distribution of microplastics may vary strongly (Kay et al., 2018; Haberstroh et al., 2021). Nevertheless, several studies showed that rivers draining large, densely populated and industrialized catchments carry considerable loads of microplastics (Lebreton et al., 2017; Gerolin et al., 2020; Napper et al., 2021) and discharge an amount of approximately 1.15–2.41 million tonnes of (micro and macro) plastic per year into the world oceans (Lebreton et al., 2017). Because Southeast Asia, e.g., in India and People's Republic of China, are such densely populated regions with an advancing industrialization, it is not surprising to find particularly large microplastic loads and concentrations there (Zhao et al., 2014; Lebreton et al., 2017; Yan et al., 2019; Napper et al., 2021).

5 CONCLUSION

We detected microplastics in all sediment and water samples taken from the Elbe close to the Mulde confluence by optical microscopy and pyr-GC-MS. In all water samples, we found numerous fibres. Although it is challenging to compare the results of the visual and chemical analyses, this large number of fibres roughly coincides with high PE concentrations. In contrast, we detected large numbers of PS (or PS-DVB) spheres in the sediment sample directly downstream of the Mulde confluence only. Although a clear identification of possible source area(s) remains challenging, the distribution pattern suggest that the Mulde contributes microplastics (especially spheres) to the Elbe. These findings reinforce the argument that tributaries may be important sources of microplastics in larger rivers, and might be applied to other catchments as well. However, a comparison with other sampling sites along the Elbe and other (European) rivers remains tentative due to different sampling and analytical approaches. In our study, the differences between water samples collected with Apstein nets and the filter cascades confirm this challenge. Nevertheless, our results may serve to better understand the different contributors and microplastic occurrence in a fluvial catchment.

DATA AVAILABILITY STATEMENT

Data for the calibration of the pyr-GC-MS analysis is provided in the **Supplementary Material**. Data and code necessary to reproduce the analysis presented in the study are published on

Zenodo, https://doi.org/10.5281/zenodo.5691239. Original photographs of filtered water and sediment samples can be obtained from the first authors upon request.

AUTHOR CONTRIBUTIONS

HL, FS, JK, CB, and GR conceived the study; field work was performed by FS, DS, and CS; JK prepared the water and sediment samples for analysis with contribution by HL, FS, GD, and CF; CF and GD measured and analysed the pyr-GC-MS; JK and CB provided the graphics; HL, CF, FS, JK, and CB wrote the manuscript; all authors critically discussed the results, commented and reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.794895/full#supplementary-material

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COVID-19 Generated Personal Protective Equipment: Sources of Microplastics and Pathogen Vectors in Marine Environments?

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The world has already experienced the severe adverse effects of COVID-19 at every level. When it became understood that the COVID-19 infection is spread in the community *via* respiratory transmission from humans, then the widespread use of plastic-made personal protective equipments (PPEs) like face masks and hand gloves tremendously increased throughout the world. Although it has reduced the spreading of virus, however, careless disposal or mismanagement of these single use PPEs has created another major concern for the environment, as plastics are a known source of environmental contamination. On one hand, they are infected with SARS-CoV-2, while on the other, they act as a carrier or vector or pathway for other pathogens or diseases, and hence can increase the degree of continuing the pandemic. Besides, there might be a chance that plastics or microplastics may be responsible for introducing new pathogenic viruses or bacteria to humankind. As such, it is clear that more research needs to be conducted to clarify this fact, and its underlying mechanisms. In this review, we briefly explored how PPE used in the COVID-19 pandemic aggravated existing microplastic pollution, how they could act as disease routes or vectors, and how they could introduce new pathogens to the terrestrial and marine environment. Addressing these questions may create awareness of plastic use, waste management, and enact relevant policy which may protect our environment and health.

Keywords: COVID-19, personal protective equipment (PPE), face masks, microplastics, plastisphere, human health

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INTRODUCTION

On 11th March 2020, the World Health Organization (WHO) declared COVID-19 (SARS-CoV-2) as a global pandemic (Puttaswamy et al., 2020). Various preventive measures have been taken worldwide to prevent this virus, such as lockdown and social distancing for restricting virus transmission. On an individual level, the measures are washing hands and using personal protective equipment (PPE), including face masks and gloves (Fadare and Okoffo, 2020). Among them, it is highly recommended or mandatory to wear single-use disposable surgical masks and gloves

against the ongoing pandemic to prevent transmission of the virus. This preventive measure has led to the massive production of face masks and gloves during this pandemic (Benson et al., 2021). Polymers are the major components of PPE like masks and gloves (Fadare and Okoffo, 2020). Therefore, the massive production of PPE was responsible for introducing tonnes of plastics to the environment (Benson et al., 2021; Yousefi et al., 2021). Before the pandemic, it was estimated that global plastic production may reach 33 billion tons by the year 2050 (PlasticsEurope, 2018), but the recent addition through PPE will be a substantial contribution to that amount. However, plastics and microplastics (plastics that are smaller than 5 mm) have already endangered the environment and human health. By providing a microhabitat for microorganisms, the plastic's surface could be a vector for disease transfer (Chin et al., 2020). Besides, those microplastics can be transferred to humans through the food chain and inhalation and impact human health (Cox et al., 2019; Mammo et al., 2020).

A good number of previous studies have already reported the occurrence of PPEs in the terrestrial and marine environment (Kassam, 2020; Benson et al., 2021; Dybas, 2021). Over the years, a huge amount of PPE and resulting microplastics will be added to the environment. However, it is still unknown how many microplastics are being added to the environment or, if there is any possibility that microplastics can transfer unknown pathogenic viruses or bacteria to humans from the wild environment. Based on this hypothesis, this review aims to give an idea of how and what possible amount of microplastics are being released into nature through the use of PPE during the COVID-19, the possibility of carrying other viruses in the microplastics biofilm, and describe the likelihood of introducing new virus or bacteria by vector plastics.

THE PANDEMIC PRECAUTIONARY MEASURES ARE PRODUCING VAST AMOUNT OF PLASTICS/MICROPLASTICS

Firstly, PPE, including face masks and gloves, are made of plastic polymers and have been used as precautionary measures to prevent the transmission of COVID-19. The vast production of these PPE, particularly face masks, have given rise to the tremendous amount of plastics and microplastics in the environment (Benson et al., 2021). For example, a study in South Korea revealed that if 70% of the country's urban population wear a single mask every day, at least 1,381 million microplastics fibers could be released per day in total in South Korea (Dissanayake et al., 2021). Therefore, it has increased the plastic demand attributable to medical waste by 370%, while the plastic demand for the packaging industry has increased by 40%. Polymers like polypropylene, polyurethane, polyacrylonitrile, polystyrene, polycarbonate, polyethylene, or polyester are the components of PPE, such as disposable face masks and gloves (Fadare and Okoffo, 2020). Non-woven materials (e.g., melt-blown fabric) are the core components of most disposable face masks; polypropylene and polyethylene are their major components. Various plastic materials such as low–density polyethylene, latex, vinyl, and nitrile are the components of gloves; those are highly persistent in the environment due to mechanic and chemical resistance. Like other plastic wastes, PPE-derived waste that is not treated as expected can be spread into the environment (Binda et al., 2021). Some of these materials are destined to waterways and ultimately reach the freshwater and marine environments, adding plastics into the aquatic medium. There are many reports about microplastics in the municipal wastewater and sewage effluents (Mason et al., 2016; Wang et al., 2021), and they are suspected as the most significant source of microplastics in the environment (Carr et al., 2016). Consequently, the present protection of masks mandates and human treatment procedures for COVID-19 might have added significantly to terrestrial and aquatic microplastic pollution. Besides releasing microplastics and nanoplastics, disposable face masks and other untreated equipment may release lead, cadmium, antimony, and various organic species through leaching (E&T, 2021). Similarly, it could be one reason for *SARS-CoV-2* genetic material to be in the vicinity of wastewater and the drainage of COVID-19 isolation centers and hospital (Ahmed et al., 2021), as one of the transmission routes is many face masks used worldwide by the general public, patients, and health workers (Tran et al., 2020).

MICROPLASTICS COULD BE A POTENTIAL VECTOR OF PATHOGEN TRANSMISSION

Secondly, what could the connection be between plastics and pathogens? It is known that plastics or microplastics provide new microbial niches in aquatic environments (Yang et al., 2020). Besides, there are many reports on the plastisphere community in sewage and wastewater containing human pathogenic bacteria and antibiotic resistance genes (Mason et al., 2016; Wang et al., 2021), because the surface of microplastics acts as a fertile micro-habitat for the rapid colonization of bacteria and viruses (Harrison et al., 2014; Moresco et al., 2021). However, it was hypothesized that human viruses frequently come into contact with plastics, therefore could increase the transfer of infectious viruses in the environment (Moresco et al., 2021). The possibility of the concurrent COVID-19 pandemic is much higher as the *SARS-CoV-2* virus has a higher life expectancy on plastic than other surfaces like paper (Corpet, 2021), and the existence of the COVID-19 virus in water and wastewater is not insubstantial (Tran et al., 2020). However, there are no studies about the co-existence of viruses like *SARS-CoV-2* in the plastisphere or their transmission route through microplastics to humans. Not only the virus transmission, but also the antibiotic resistance gene and pathogenic bacteria, might be responsible for immune dysregulation and disease in the human body (Zheng et al., 2020), and a dysfunctional immune response in COVID-19 patients can cause severe lung infection and systemic pathology (Tay et al., 2020). Therefore, is it possible that the plastics we use for the treatment unconsciously accelerate the frequency of virus

transmission or disease? The possibility is not negligible because from our toothpaste to most remote arctic seafloor, microplastics are everywhere. According to a report, an individual consumes between 74,000 and 121,000 plastic particles per year, including exposure *via* inhalation, with an additional 90,000 particles for those who drink bottled water (Cox et al., 2019). As a result of their potential entry into the food chain, microplastics pose a threat to both terrestrial and aquatic life (Mammo et al., 2020).

THE PLASTISPHERE MAY INTRODUCE UNKNOWN PATHOGENIC VIRUSES OR BACTERIA FROM THE WILD ENVIRONMENT

Finally, the marine environment is the ultimate sink of microplastics and is widely distributed in beaches, seawater, sediments, and rivers (Li et al., 2020). Rivers and surface runoff are significant plastic transport pathways into the sea and carry about 80 to 94% of the total plastic load to the sea. Numerous field surveys reported the highest abundance of microplastic debris in rivers, harbor areas, tourist beaches, and nearby industrial areas (Li et al., 2020). However, human and animal pathogenic bacteria and viruses in the marine plastic microbiome are not mere. The presence of *Vibrio* species on microplastics or other plastics in water has been frequently reported (Mammo et al., 2020). For example, potentially human pathogens such as *V. parahaemolyticus, V. mimicus, V. vulnificus, V. cholera, V. anguillarum, V. harveyi, V. pectinicida, and V. xiamenensis* were confirmed in biofilms attached to plastics recovered from the Barzil and Western Mediterranean Sea (Dussud et al., 2018; Silva et al., 2019). Other potentially human pathogenic microbes such as *Aeromonas, Haemophilus, Acinetobacter*, *Pseudomonas monteilii*, *Pseudomonas mendocina*, and pathogenic *E. coli* strains were also reported from microplastics recovered from different marine environments (Mammo et al., 2020). Furthermore, *SARS-CoV-2* can be released to the marine environment *via* human effluent, possibly present in coastal marine waters connected to sewage effluent (Mordecai and Hewson, 2020). Therefore, we hypothesize there could be a strong possibility that *SARS-CoV-2* present in the plastic biofilm of that coastal area has a connection with sewage and wastewater effluent and could be a secondary route of transmission to coastal people, anglers, and tourists.

CONCLUSION AND RECOMMENDATIONS

Overall, based on the above-interconnected evidence, we hypothesize that humankind is in a crisis condition concerning COVID-19 and plastic pollution. Although we have to use PPE for our protection, in return, plastics produced from PPE are posing a threat to us. Therefore, to clarify whether pre-existing and newly introduced microplastics in current disease treatments potentially threaten us or not, and to tackle this fact, we recommended some research directions as follows:

1. Can *SARS-CoV-2* be present in the plastisphere community?
2. As the genetic material of *SARS-CoV-2* is present in wastewater or drain, do they exist on the plastic biofilm of that wastewater or drain?
3. Is there any existence of *SARS-CoV-2* on the coastal and marine water plastic's microbiome?
4. What is the potentiality of plastic's microbiota in transferring any new contagious virus-like *SARS-CoV-2* from the wild environment?
5. Reusable PPE could be a better strategy; therefore, their efficiency should be well examined.

AUTHOR CONTRIBUTIONS

MN: conceptualization, data curation, writing—original draft, and writing—review. JS: resources, funding acquisition, and supervision. MH: writing—review, editing, and formal analysis. All authors contributed to the article and approved the submitted version.

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Roadmap for Long-Term Macroplastic Monitoring in Rivers

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Macroplastic pollution in and around rivers negatively impacts human livelihood, and aquatic ecosystems. Monitoring data are crucial for better understanding and quantifying this problem, and for the design of effective intervention strategies. However, current monitoring efforts are often of short duration, or study single river compartments. We present a "Roadmap" to overcome the challenges related to the design and implementation of long-term riverine macroplastic monitoring strategies. This "Roadmap" can help accelerating the process of achieving structural monitoring through providing a stepwise approach, which links monitoring goals and research questions to the data and methods required to answer them. We identify four monitoring goals: 1) policy, 2) knowledge development, 3) operations, and 4) solutions. Linked to these, we provide a non-exhaustive list of 12 globally common research questions that are important to answer to reach these goals. The "Roadmap" takes these questions and links them to development levels of monitoring methods for each river compartment: 1) method development, 2) baseline assessment, and 3) long-term monitoring. At each level, specific questions can only be answered if the level is achieved for specific river compartments. For questions at higher levels, the previous levels need to be achieved first. This creates a clear stepwise approach to solve open challenges. With the "Roadmap", we provide a new tool to support decision-making and planning of specific projects by policy makers. The "Roadmap" is a clear and stepwise, yet flexible framework that allows to add and remove elements based on new insights, available resources, and other relevant changes.

Keywords: litter, water, pollution, data collection, hydrology, marine debris, microplastic, monitoring strategy

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INTRODUCTION

Macroplastic pollution (plastic items >0.5 cm) in riverine environments is an emerging environmental risk, as it negatively impacts ecosystems, endangers aquatic species, and cause economic damage (van Emmerik & Schwarz, 2020; Meijer et al., 2021). To better quantify riverine macroplastic pollution, and effectively reduce its negative effects, a thorough understanding of sources, transport, fate, and effects of riverine macroplastic pollution is crucial. Macroplastics have been observed in all compartments of the river system (van Emmerik and Schwarz, 2020; Morales-Caselles et al., 2021). Known sources of riverine macroplastic pollution include sewage outlets from wastewater treatment plants, recreational activities in the vicinity of riverbanks, adjacent industrial areas, and areas with high urban activities (Hoellein and Rochman, 2021). Monitoring macroplastic in river systems is crucial to quantify the magnitude of the problem, to identify and quantify inputs from all sources, identify accumulation zones, and to observe temporal trends.

Methods to quantify riverine macroplastic pollution differ per river compartment (e.g., floating, water column, riverbank, sediment, and biota; van Emmerik and Schwarz, 2020) in terms of their

level of technological readiness. Floating macroplastics and macroplastics on riverbanks have been studied to a greater extent, which has led to these methods to be more developed compared to those for the other compartments. For example, multi-year monitoring strategies for riverbank litter have been carried out in Germany and Netherlands (Kiessling et al., 2019; van Emmerik et al., 2020). Floating macroplastic has been quantified across Europe using the same methodology as presented by the European Union Joint Research Centre in the RIMMEL project (González-Fernández et al., 2021). Other river compartments such as the water column and riverbed are studied less frequent and systematically (Blettler et al., 2018; Vriend et al., 2021). Work has been undertaken to develop and harmonize guidelines for monitoring macroplastic in freshwater environments (e.g., Wendt-Potthoff et al., 2020). However, knowledge on how to convert these efforts into long-term monitoring strategies that integrate multiple river compartments is lacking.

Data gathered through long-term and wide-scale monitoring that includes all river compartments are needed to answer the relevant policy, knowledge, operational, and solution-related questions, that are key to solving the problem of macroplastic pollution. However, a structured approach on how to advance from the current short-term and temporary measurements, to an integrated monitoring strategy for riverine macroplastic is currently missing. In this paper we provide the "Roadmap", which can be used by governments, scientists, and practitioners to structure the development of an integrated monitoring strategy. The ideal strategy is highly dependent on local context such as river typology, available resources and the level of pollution (Vriend et al., 2020a). The "Roadmap" can help develop and implement important long-term monitoring in a faster, more reliable, and cost-effective manner. This framework can further be used to determine what type of monitoring is required to answer specific research questions (*Goals for Long-Term River Plastic Monitoring*) concerning riverine macroplastic pollution.

GOALS FOR LONG-TERM RIVER PLASTIC MONITORING

Macroplastic monitoring strategies are often set up with different goals. For example, monitoring projects can be undertaken for knowledge development on riverine macroplastic pollution (Kiessling et al., 2021), to aid the development of policy through identifying frequently found items and possible sources (González-Fernández et al., 2021), and or to guide site selection of intervention strategies (Helinski et al., 2021). We identified four overarching goals for monitoring, these being 1) policy, 2) knowledge development, 3) operations and maintenance, and 4) solutions. These four goals were formulated based on literature, and our own experience with relevant stakeholders from academia, (inter)governmental stakeholders, and practitioners.

Goal 1: Policy Development and Implementation

The first goal of riverine macroplastic monitoring is to support the development of policy aimed at reducing pollution. There has been an increase in new guidelines and regulations related to plastic litter in aquatic environments, such as the EU Marine Strategy Framework Directive (Galgani et al., 2013), the EU Water Framework Directive (Directive 2000/60/EC), and the EU Single-Use Plastics Directive (Elliott and Thomsom, 2020). Monitoring is necessary to design effective policy aimed at the reduction and mitigation of macroplastic pollution, as well as to determine whether policy goals are achieved. Furthermore, macroplastic monitoring will support the development of item or material specific policies. For example, the persistent occurring of small bottles (<500 ml) during monitoring of macroplastic on land has led to the introduction of deposits on these bottles in Netherlands (van Veldhoven, 2020). As many large rivers are transboundary systems, monitoring has to be done in collaboration with neighboring regions (as shown by Schulz et al., 2013 for monitoring of beach litter in the OSPAR region).

Goal 2: Fundamental Knowledge Development

The second goal relates to all actions for knowledge development. To date, the understanding of macroplastic sources, sinks, pathways, effects, retention times, degradation, and fragmentation is limited. Such knowledge is crucial for optimizing prevention, mitigation, and reduction strategies. We identify three urgent knowledge gaps that require monitoring. The first gap concerns the limited knowledge on the sources of riverine macroplastic, its distribution throughout river systems and how it may affect source reduction and removal strategies (Helinski et al., 2021). The second knowledge gap considers that most riverine macroplastic items do not reach oceans (Meijer et al., 2021; Tramoy et al., 2021). Finally, understanding the effects of extreme events on the leakage, mobilization, and transport of macroplastic through rivers can support better preventive measures (Roebroek et al., 2021). Fundamental knowledge development on these three knowledge gaps will advance prevention, mitigation, and reduction strategies.

Goal 3: Operations and Maintenance

Governmental organizations may also include the monitoring of plastic pollution in their responsibility for the operation and maintenance of the public works and waterways, including the maintenance and clean-up of infrastructure such as locks, weirs, and levees (van Emmerik and Vriend, 2021). Operations and maintenance of these assets requires a thorough understanding of the presence and magnitude of possible risks. These risks include damage to, or blockage of infrastructure caused by macroplastic pollution (Honingh et al., 2020). To effectively mitigate these risks, managers require a thorough understanding of the effects macroplastic pollution on the infrastructure they are managing. Such understanding has to be generated through monitoring.

Goal 4: Solution Design and Implementation

Finally, monitoring is important to support the development, implementation and evaluation of best sollutions to prevent, mitigate, and reduce macroplastic pollution in (aquatic) ecosystems. Monitoring provides quantitative data, which allow to assess the efficacy of policy changes such as measures to reduce pollution sources (e.g., consumers, industry, and sewage leakage), improved waste infrastructure, or a specific macroplastic collection strategies such as litter traps. Insights gained from this can then be used to design best practices for macroplastic pollution prevention, mitigation, and reduction. For example, data on plastic loads are needed for site selection for the installation of macroplastic traps and to determine the efficacy of these traps (Tramoy et al., 2019; Helinski et al., 2021). Furthermore, these data can be used to forecast during what periods most litter is expected to be transported and what possible sources for this are (van Emmerik et al., 2019). Effects of targeted policy measures can also be evaluated. Long-term data allow for trend analyses to asses the effect of discouraring or banning specific products on leakage of those products into the (aquatic) environment.

Linking Goals to Research Questions

Based on these four goals and previously published literature, a non-exhaustive list of 12 universally relevant questions was distilled that can be answered to reach the monitoring goals set out in the previous sections (**Table 1**). This list includes fundamental questions such as how to measure macroplastic pollution in a specific river compartment. Moreover, it consists of questions that require long-term monitoring, such as how to determine the impact of measures taken to reduce macroplastic. This list can be expanded to include other open research questions that may stem from previously mentioned research goals. The "Roadmap" presented in the next section is a tool to aid the design of monitoring strategies which can answer this full range of questions.

TABLE 1 | Overview of research questions that can be answered with a large scale, integrated monitoring strategy for macroplastic pollution in rivers.

Nr.	Question
1	How can macroplastic be monitored in each river compartment?
2	How to determine the plastic mass balance in rivers?
3	What are the emissions of macroplastic from rivers into the ocean?
4	What are standard measuring units for each river compartment?
5	Where are macroplastic accumulation zones in rivers?
6	What are the sources of riverine macroplastic?
7	What are the most abundant macroplastic polymers and items?
8	How is macroplastic distributed over the river compartments?
9	What are the effects of specific prevention and reduction measures?
10	What are the long-term trends of riverine macroplastic transport?
11	What are transport pathways of plastic pollution though river systems?
12	What is the role of floods on macroplastic transport in rivers?

THE "ROADMAP" FOR LONG TERM-MONITORING OF MACROPLASTIC

The "Roadmap" is a tool that connects any envisioned future river plastic monitoring strategy with the steps that should be taken to reach this and aligns these actions with selected research goals. In this regard the "Roadmap" is inspired by the backcasting principle, which is a tool used in planning to deal with uncertainty of reaching a desired future by tracking back the steps that can be undertaken to reach it (Dreborg, 1996; Holmberg and Robèrt, 2000).

The "Roadmap" is structured around the twelve open questions identified in the previous section using a three-level framework. In the end, each question is related to data, though at a different level: 1) method development, 2) baseline assessment, and 3) long-term monitoring. The first level (method development) relates to all technical and methodological developments that are the foundation for a suitable monitoring strategy. For example, no standard method is available to monitor macroplastics in the water column (Collas et al., 2021). To answer in depth question for this compartments a standard method first has to be developed. The first step to solving questions for this compartment therefore starts at level one. The second level (baseline) focuses on establishing a baseline measurement, and can include rapid assessments of macroplastic in a specific compartment. Baselines are crucial to get a first sight on the magnitude of the problem and to provide insights for developing the final long-term monitoring protocol (Nurhati and Cordova, 2020). Macroplastic flux can vary more than five orders of magnitude around the world (van Calcar and Van Emmerik, 2019). A rapid assessment will reveal the approximate local pollution level of a river system. Each river may require a specific monitoring strategy, depending on the level of pollution, relevant research questions, and available resources (Vriend et al., 2020a). Finally, the third level relates to the actual long-term monitoring strategy. At this level, questions about trends, and effects of policy changes on the level of pollution can be answered. This is not possible at one of the lower levels. This creates a clear stepwise approach to solve open challenges. For example, to evaluate the effect of measures, insights on all levels are required (method development, baseline assessment, and long-term monitoring). In contrast, specific questions related to methods and protocols remain on the first level.

The presented three-level structure can be used to assess the current and desired state of knowledge of monitoring for specific compartments. Current monitoring strategies for riverine macroplastic may only include the quantification of it on riverbanks, which results in data that can be used to answer research questions for only this compartment (e.g., Kiessling et al., 2019; van Emmerik et al., 2020). In this case, level one

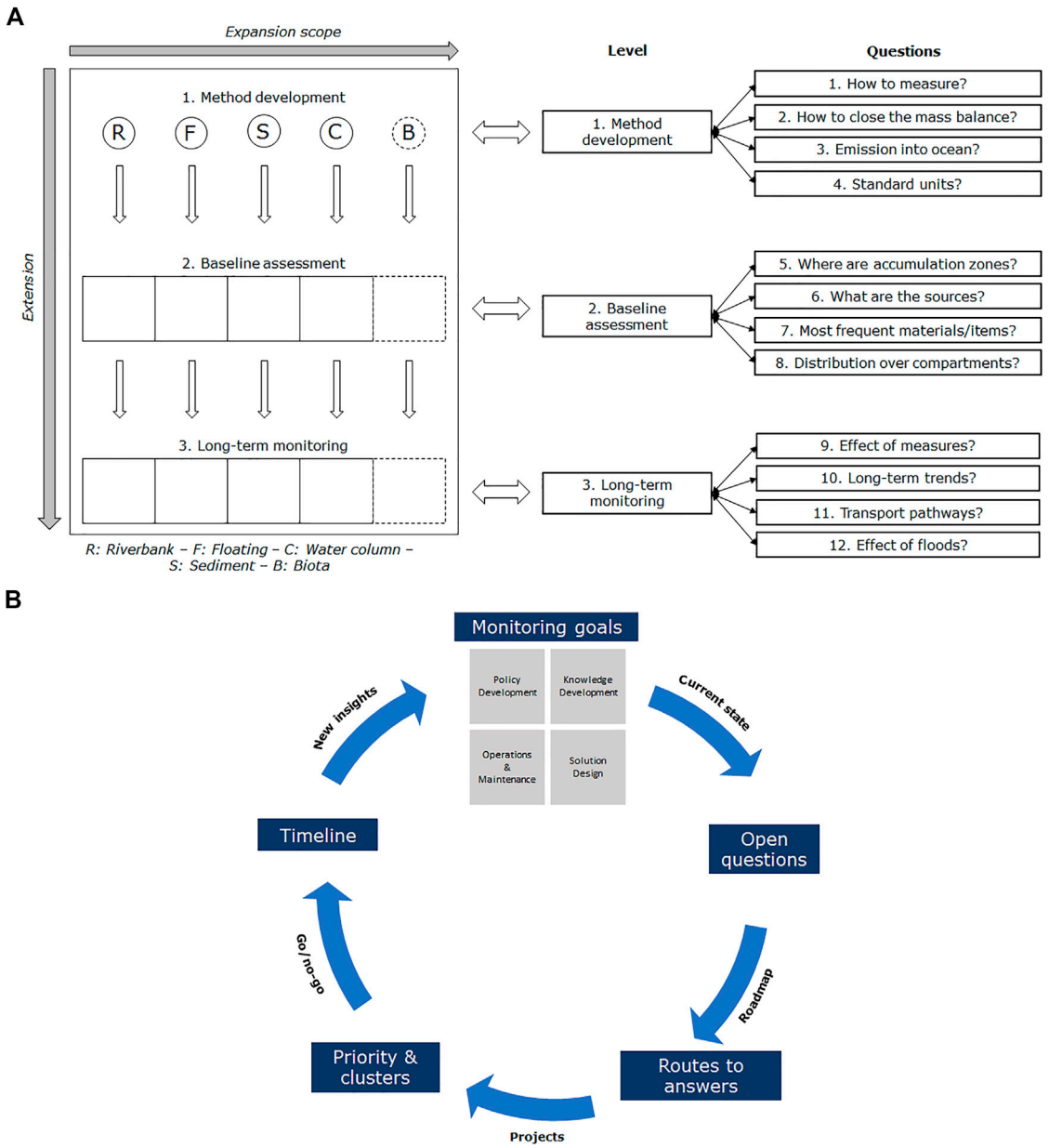

FIGURE 1 | Overview of the "Roadmap" presented in this study, with **(A)** the "Roadmap" with 3 development levels (Method development, Baseline assessment, and Long-term monitoring) for each river compartment (R = Riverbank, F = Floating, S = Sediment, C = Water column, and B = Biota), the level of questions that can be answered for each development level, and the option to expand the scope of monitoring by adding river compartments (dotted line around Biota), and **(B)** the iterative cycle of the "Roadmap" for long-term monitoring. Adapted from van Emmerik and Vriend (2021), published under a CC BY 4.0 license.

(method development) has been fulfilled since suitable monitoring methods have been developed and tested. Moreover, these methods have been applied at a large scale for both cases, indicating that a first efforts have been made for a baseline. Once the baseline study is finished, the values that are found provide the first statistics on the abundance of riverbank macroplastic pollution, the spatial distribution, and frequently found item types. However, only after the continuation of the monitoring over a longer period of time, the data allow for trend analysis, and observed variations can be attributed to policy measures with a higher degree of confidence.

The "Roadmap" framework is flexible in two dimensions. As discussed, the stepwise approach facilitates extension of the current or future monitoring strategies. Once levels 1 and 2 (method development and baseline assessment) are reached, the strategy can be extended to the next level. The framework also shows how to expand the scope of the strategy by also considering other river compartments besides riverbanks (e.g., floating and water column). **Figure 1A** shows the result of expanding and extending a strategy. At level 1 (method development), each compartment requires specific technology

and protocols (e.g., visual counting for floating macroplastics, net sampling for macroplastic in the water column). At the second level (baseline assessment), the compartments form an integrated strategy to allow for a holistic approach for the monitoring protocols, data collection, analysis and dissemination. At the third level (long-term monitoring) the compartments remain connected and integrated for an extended period of time.

The framework allows for a flexible and iterative approach, as individual components can be added, removed, or changed. If a new technology for water column measurements is developed (e.g., echo sounding; Broere et al., 2021), a new "compartment" can be added to the framework. However, here one starts again at level one, as the measurement method needs to be developed, and tested. Another possibility is to add or remove complete river compartments, based on new findings. For example, observations may show that macroplastic in biota is not a relevant compartment for the questions that the monitoring strategy is trying to answer compared to macroplastic on riverbanks and floating macroplastic. As a consequence, the biota "compartment" can be removed from the long-term strategy. Similarly, new compartments (e.g., floodplains) can be added through the expansion of the scope.

Iterative Cycle of Long-Term Monitoring

The "Roadmap" is not a linear tool. It offers a flexible approach that allows for the incorporation of new insights, monitoring goals, priorities, and data (**Figure 1B**). Design and optimization of a national riverine macroplastic monitoring strategy requires an iterative approach, the "Roadmap" is therefore designed as an iterative cycle (**Figure 1B**). First monitoring goals are set and the current state of knowledge is assessed. This leads to the identification of questions cannot be answered yet. The "Roadmap" can then be used to identify the routes that should be taken to develop a fitting monitoring strategy. These routes set out specific projects that should be carried out and the development levels provide guidance on in which order these projects should be executed. After the routes to answers have been finished it can be assessed whether the previously set monitoring goals have been achieved or are still relevant. After this a new cycle can start with new or revised monitoring goals, new open questions, and a new "Roadmap".

CASE STUDY—APPLICATION OF THE "ROADMAP" IN THE NETHERLANDS

The "Roadmap" is used by *Rijkswaterstaat* (RWS, Ministry of Infrastructure and Water Management, Directorate-General for Public Works, and Water Management, Netherlands) to advise the Dutch government on the development of a long-term integrated monitoring strategy for riverine macroplastic in the main rivers of Netherlands (Rhine and Meuse; van Emmerik and Vriend, 2021). This case study illustrates how RWS has used the "Roadmap" to plan the long-term monitoring strategy for Dutch rivers.

Monitoring Goals

Monitoring for policy is important since the Dutch government is in the process of implementing policy to reduce plastic pollution (van Veldhoven, 2020). Data gathered through monitoring can be used to facilitate policy implementation and to monitor the efficacy of measures after implementation. Moreover, monitoring macroplastic pollution for the effective operation and maintenance of waterways and hydraulic infrastructure is important (van Emmerik and Vriend, 2021). Last, RWS has been experimenting with removal technologies through small scale pilots to determine the effectiveness and cost-efficiency of these technologies (van Veldhoven, 2018). Data gathered through monitoring can be used to determine the main sources of pollution that should be reduced and show the efficacy of riverine macroplastic removal technologies.

Open Questions

The research questions that extend from these goals include:

- How much macroplastic is in the main Dutch waterways?
- What is the composition of macroplastic pollution in relevant river compartments?
- What is the efficacy of measures aimed at reducing riverine macroplastic pollution?

Routes to Answers

RWS included three river compartments in the first iterative cycle of developing a monitoring strategy: floating macroplastic, macroplastic on riverbanks, and macroplastic suspended in the water column (**Figure 2**). They made an inventory of the development levels of the monitoring methods for each compartment and used this inventory to decide on the routes required for answers (van Emmerik and Vriend, 2021).

Previous research efforts in Dutch rivers had mainly focused on riverbank macroplastic and on floating macroplastic. Riverbanks had previously been quantified on a large scale for multiple years, though a baseline for RWS was missing (van Emmerik et al., 2020). Floating macroplastic have also successfully been monitored on multiple occasions (e.g., van der Wal et al., 2015; Vriend et al., 2020b), though long term measurements were lacking. Macroplastic in the water column had not yet been quantified, though first tests with trawls, and larvae nets deployed from boats were tested (Collas et al., 2021; Oswald et al., 2021). It was therefore decided that the riverbank compartment and the floating compartment passed development level 1 (method development) and still needed work for passing level 2 (baseline assessment; **Figure 2**). The water column compartment needed more development to pass level 1 (method development).

Priority and Clusters

RWS subsequently uses the levels of the compartments to prioritize projects that have to be undertaken to develop a monitoring strategy that can answer their research questions. Methods to quantify macroplastic in the water column are

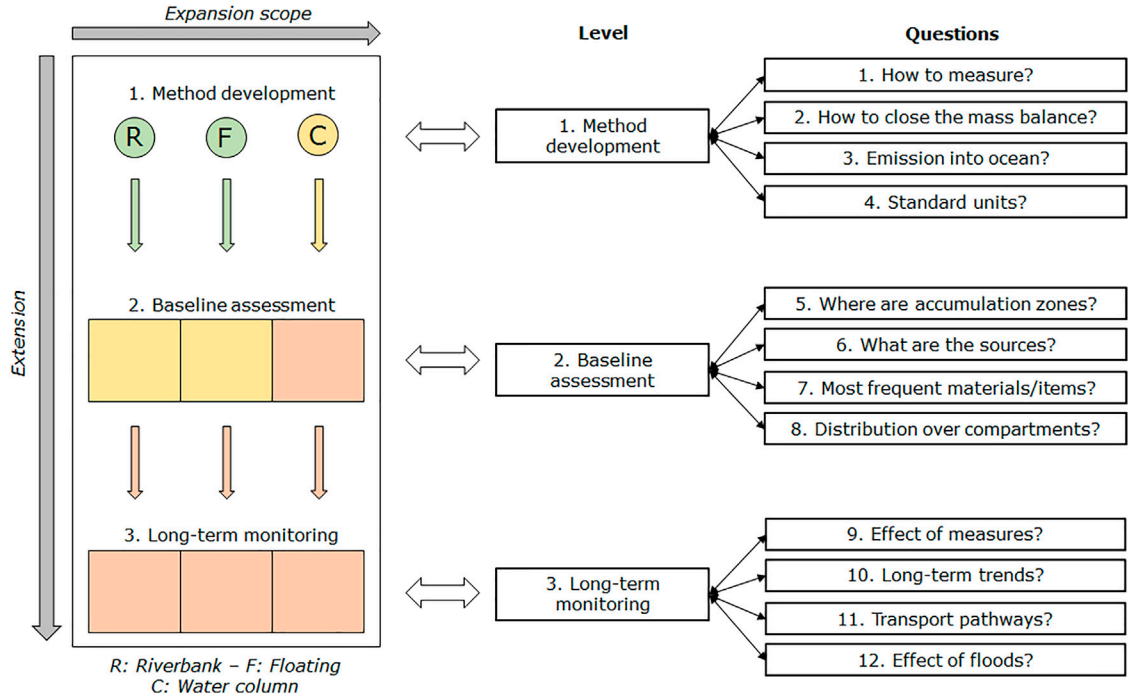

FIGURE 2 | Example in which the "Roadmap" is used to indicate the progress of developing a monitoring strategy by RWS for three river compartments (R = Riverbank, F = Floating macroplastic, and C = Water column). Green indicates the development level has been passed, orange indicates that the development level is in progress, and red indicates the development level has not been started yet since previous levels have not yet been passed. Adapted from van Emmerik and Vriend (2021), published under a CC BY 4.0 license.

relatively underdeveloped. RWS is therefore exploring options to further develop these methods through pilot projects. Besides, baseline measurements are undertaken for floating macroplastics in Netherlands (van Emmerik and de Lange, 2021). Last, the previously developed method for riverbank macroplastic (van Emmerik et al., 2020) is developed further so it can be applied in a standardized way. Once these goals are achieved, the results can be evaluated to determine whether the monitoring goals are being met, or changes need to be made.

CONCLUDING REMARKS

With the "Roadmap" a practical tool for the design of a national riverine macroplastic monitoring strategy is presented. We emphasize that there is no single solution or path forward. Depending on the defined goals, guidelines and new insights, the actual selection of projects and their respective timelines may change. The "Roadmap" shows what steps are required to arrive at an answer to a specific question.

The "Roadmap" defines four goals for the national riverine macroplastic monitoring strategy: 1) policy development, 2) knowledge development, 3) operations and maintenance, and 4) solutions. A non-exhaustive list of research questions that may stem from these monitoring goals is presented. This list can be expanded by the user of the "Roadmap" to include other open research questions.

The "Roadmap" consists of three levels: 1) method development, 2) baseline assessment, and 3) long-term monitoring. At each level, specific questions can only be answered if the level is achieved for specific river compartments. For questions at higher levels, the previous levels need to be unlocked first. This creates a clear stepwise approach to solve open challenges.

The "Roadmap" can be used by policy-makers to define and prioritize specific projects that are necessary to answer the locally relevant questions. The specific questions and projects are not exhaustive, and the "Roadmap" is a flexible framework that allows to add and remove elements based on new insights, the available resources, and other relevant changes. Riverine macroplastic monitoring remains an iterative process, and with the "Roadmap" we aim to provide a tangible starting point for policy-makers, scientists and practitioners Boonstra et al., 2021, Schmidt et al., 2017.

AUTHOR CONTRIBUTIONS

TvE conceived the idea, TvE, PV, and EC created the framework, PV prepared the initial draft. All authors wrote the final manuscript.

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Micro(nano)plastics Prevalence, Food Web Interactions, and Toxicity Assessment in Aquatic Organisms: A Review

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Plastic pollution is a fast-rising environmental catastrophe. Microplastics and nanoplastics (MNPs) are ubiquitous components of most aquatic environments, and their burgeoning prevalence is endangering aquatic organisms. Recent studies have documented the entanglement of marine and freshwater biota by plastic litters, particularly ghost fishing gear, resulting in suffocation, drowning, or starving to death. Numerous reports have shown that aquatic organisms readily ingest and accumulate these emerging contaminants in their digestive systems. Given experimental evidence that contaminants-laden MNPs can persist in the gastrointestinal tract for considerable durations, investigations have documented a high probability of lethal and sublethal toxicological effects associated with direct and indirect MNPs ingestions. These include chronic protein modulation, DNA damage, embryotoxicity, gastrointestinal toxicity, genotoxicity, growth inhibition toxicity, histopathotoxicity, liver toxicity, neurotoxicity, oxidative stress, reproductive toxicity, and tissue damage. Today, reports have proven the transfer of MNPs across the aquatic food web to humans. However, the mechanisms of multiple contaminants-laden MNPs-induced toxicities, size-dependent toxicity, and the comprehensive mode-of-action and alterations of digestive, reproductive, and neurological systems' functionality in marine organisms are still unclear. Thus, this review mainly addresses the prevalence, food web interactions, and toxicity assessment of micro(nano) plastics in marine and freshwater organisms. It summarizes documented studies based on the following broad objectives: (1) the occurrence and prevalence of micro(nano) plastic particles in marine and freshwater environments; (2) the ingestion of MNPs by aquatic biota and the food web exposure routes and bioaccumulation of contaminated MNPs by higher trophic entities; (3) the adsorption and desorption of persistent organic pollutants, metals, and chemical additives on/from micro(nano)plastics; and (4) the probable ecotoxicological effects of micro(nano)plastics ingestion on aquatic biota.

Keywords: microplastics, nanoplastics, emerging contaminants, food chain, trophic transfer, ecotoxicological effects

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INTRODUCTION

Our oceans are littered with plastics originating from both terrestrial and marine sources. It is estimated that about 80% of ocean plastics originate from land-based emissions, while the rest comes from marine sources (Li et al., 2016). Plastics are synthetic organic polymers made through addition or condensation reactions of substituted or unsubstituted hydrocarbons, which possess stable, lipophilic, and water-resistant structures. They are used in many commercially available products due to low cost, durability, and flexibility leading to a geometric increase in production. Numerous single-use plastics, particularly those used in beverage, food, and consumer product packaging, have a short useful life and are readily discarded into the environment. Millions of single-use COVID-generated plastics, including face masks, protective medical aprons, gloves, medical test kits, hand-sanitizer bottles, and takeout plastics, and medical test kits have been improperly discarded into the terrestrial environment, potentially increasing the amount of plastic washing up on ocean shorelines, beach resorts, and exacerbating the plastic pollution problems (Benson et al., 2021a,b). Particulate plastics generated on land are mostly carried by run-off, soil erosion, and stormwater through drainages into rivers, lagoons, and eventually to the sea and ocean. Additionally, fine plastics particles may reach freshwater and marine ecosystems *via* other land-based processes such as wastewater treatment plant effluent as well as shipping operations (Li et al., 2018; Bradney et al., 2019). Mismanaged plastics originating primarily from land-based sources continue to pervade the environment, and up to 12.7 million metric tons are estimated to enter the ocean annually (Jambeck et al., 2015). Additionally, plastic debris from anthropogenic sources into the marine and freshwater ecosystems could come from commercial fisheries that abandon fishing gears and equipment including lines, nets, lines, plastics lures, ropes, and occasionally abandoned trawlers (Macfadyen et al., 2009). The vast majority of these ocean plastics are in the form of macroplastics, mesoplastics, microplastics, and nanoplastics which have washed ashore, been buried or littered along the coastlines, or been transported to offshore environments *via* hydrological influences.

Particles of plastics are often categorized according to their size. Despite the absence of agreement on how to describe and categorize plastic particles, this report considered the following classifications. Macroplastics, mesoplastics, microplastics, and nanoplastics are plastic particles with size diameter range that are >200 mm, 4.76–200 mm, 0.01 μm–1 mm, and less than 0.1 μm, respectively (Eriksen et al., 2014). However, Hartmann et al. (2019) categorize plastic particles as macroplastics (>1 cm), mesoplastics (1–<10 mm), microplastics (1–<1,000 μm), and nanoplastics (1–<1,000 nm). Although there is no consensus on categorizing plastic particles, in this manuscript, microplastics (MPs) are regarded as the category of plastics with a diameter of 1 nm–<5 mm, and nanoplastics (NPs) are characterized as plastic particles having a lower size less than 1 nm. MPs which are classified into primary and secondary based on their sources are considered as an emerging persistent micropollutant threatening our global aquatic and terrestrial environments. While primary microplastics are originally manufactured for use in cosmetics, toothpaste, or pharmaceutical drugs (Mintenig et al., 2017), secondary microplastics, on the other hand, exist from the degradation of larger plastics into smaller pieces under different chemical, physical, and biological conditions (Horton et al., 2017a; Padervand et al., 2020; Agboola and Benson, 2021). The steady rise in the production and use of plastics led to an increase in presence of microplastics in the environment (Cole et al., 2011). MPs are found in a variety of everyday products, including facial scrub cleansers, sea salts, and toothpastes (Chang, 2015; Zhang et al., 2020a; Agboola and Benson, 2021). MPs enter aquatic ecosystems by a variety of routes, the most common of which being surface runoff, air currents, and wastewater treatment plants (WWTPs) effluent (Dris et al., 2015; Turan et al., 2021). The most common types of microplastics detected in WWTPs are fibers and microbeads. Wastewaters from domestic, commercial, and industrial sources are usually treated at municipal WWTPs and their effluents constitute a major source of MPs entering freshwater systems, most commonly rivers, from which they are transported to the ocean (Iyare et al., 2020; Zhang et al., 2020a). However, the introduction of microplastics and nanoplastics (MNPs) into the terrestrial and aquatic environments through WWTPs processes could potentially exacerbate the plastic pollution problem. Inappropriate management and disposal have led to a higher concentration of microplastics in freshwaters including lakes (Eriksen et al., 2013; Lenaker et al., 2019; Pico et al., 2020; Felismino et al., 2021) or rivers (Leslie et al., 2017; Rodrigues et al., 2018), and sand beaches (Benson and Fred-Ahmadu, 2020; De-la-Torre et al., 2020a). Researchers have reported an abundance of microplastics in the ocean which is both an aesthetical eyesore and an endangerment to marine life. According to estimates using different datasets, the amount of plastics in the aquatic ecosystems has increased by 7,000 (Cózar et al., 2014) and 250,000 tonnes (Eriksen et al., 2014) in the last few years, and is projected to reach 270 million tonnes by 2060 (Lebreton and Andrady, 2019). They are also reported to be enormously present in seawater including the Arctic (Morgana et al., 2018) and the Antarctic (Waller et al., 2017). Nevertheless, the precise volume of plastic waste particles floating around the surface of global freshwater and marine ecosystems remains unknown.

To the best of our knowledge, several landmark ecotoxicological investigations on the occurrence, ubiquity, and toxicity of micro (nano) plastics in marine and freshwater organisms have been conducted. In this manuscript, we conducted a meticulous review of peer-reviewed publications on the prevalence, food web interactions, and toxicity investigations of micro(nano)plastics in aquatic animals, and also identified future research priorities and constraints. The objectives of this review are to (1) highlight the sources, prevalence, and fate of micro(nano)plastics in aquatic ecosystems, (2) explicate food web interactions between micro(nano)plastics and aquatic organisms, and (3) review the toxicity and respective health risks associated with ingestion of micro(nano)plastics by marine and freshwater animals. The main aim of this review is to present relevant research findings and effects regarding the prevalence, food web interactions,

and toxicity assessments of micro(nano)plastic particles on aquatic species.

METHODS OF LITERATURE REVIEW

The aim of the literature search was to compile a list of core studies that addressed the research questions for the present review. Relevant databases including Elsevier's ScienceDirect[1] and Clarivate's Web of Science[2] were used to select peer-reviewed papers for the literature review conducted between 8th and 24th June of 2021, with an updated search on October 23rd, 2021. Advanced and basic selection techniques were utilized for the literature search, which involved keywords combination in forming queries for keywords and title elements. The following steps were used to narrow the search: "microplastics" OR "nanoplastics" AND "marine" OR "freshwater" were consistently retained as the basis in all bibliographic searches with keywords incorporated with the Boolean connector AND. Keywords used in this literature review were: "trophic transfer," "trophic interactions," "trophic web," trophic chain," "toxicity," "abundance," and "impact" to expand the search by using Boolean connector AND, OR. Afterward, each query was further expanded with word combinations including "microplastics in marine ecosystems," "microplastics in freshwater ecosystems," "nanoplastics in marine ecosystems," and "nanoplastics in freshwater ecosystems." The review was limited to research papers published in any year and on the research subject matter. The literature review identified 932 and 484 scholarly journal articles for MPs studies in marine and freshwater, respectively, published between 2011 and 2021 (as of October 23rd). **Figure 1** illustrates a progressive increase in studies conducted for marine ecosystems MPs (R^2 = 98%), as well as those carried out for freshwater ecosystems MPs (R^2 = 96%) since 2011 when the first original research article meeting the selected search criteria was identified in the two databases used. The significant increase in research papers throughout the reviewed period reflects the scientific community's growing interest in the occurrence, trophic food web interactions, and toxicity of MPs and NPs in marine and freshwater ecosystems.

MICRO(NANO)PLASTIC PARTICLES PREVALENCE, TOXICITY, BIOAVAILABILITY, AND FACTORS INFLUENCING FOOD WEB UPTAKE

According to van Sebille et al. (2015) there are an estimated 51 trillion particles of microplastics floating on the surface of the ocean, and have been established as vectors for the transfer of persistent organic and inorganic contaminants including plastic additives (Benson and Fred-Ahmadu, 2020; Torres et al., 2021), organochlorine pesticides (O) (Shi et al., 2020), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) (Frias et al., 2010; Hirai et al., 2011; Fisner et al., 2013; Tan et al., 2019), and heavy metals (Turner and Holmes, 2015; Brennecke et al., 2016; Fred-Ahmadu et al., 2020a; Xiang et al., 2021). Microplastics and nanoplastics are composed of a variety of polymers that are hydrophobic, having varied molecular structures and characterized with the capability to adsorb organic pollutants and metals on interaction with them.

Microplastics are remarkably variable in terms of particle size, morphology, color, material type, and density, and are regarded as a complex aggregation of emerging micropollutants of concern (Rochman et al., 2019). As a result of these unique particle characteristics, MPs can enter aquatic food webs at a variety of trophic levels and eco-niches with greater ease. However, in aquatic ecosystems, the distribution, fate and transport mechanisms, bioavailability, and trophic transfer among primary, secondary, and tertiary aquatic biotas are all influenced by these unique characteristics of microplastics (Sharma and Chatterjee, 2017; Bank and Hansson, 2022). Currently, there is a dearth of study on plastic particles transfer through freshwater food webs, and impacts of MPs exposure to freshwater organisms is limited. Thus far, our understanding of MPs uptake and potential toxicity has come mostly from laboratory studies that employ uncomplicated contact regimes with minimal environmental applicability. In particular, MPs in the marine environment can be transported from the top epipelagic and mesopelagic zones to mixed and deep layer depths by the continual vertical sinking of suspended organic matter, which may increase MPs bioavailability to bottom-dwelling organisms (Porter et al., 2018). It has been reported that 99.8% of plastic contamination in aquatic ecosystems since the 1950s has sunk to the seafloor in 2016, with an incremental 9.4 million tonnes accumulating yearly, according to modeling estimates by Koelmans et al. (2017).

Furthermore, due to MNPs small sizes and varied particle characteristics, those that are in suspension in the water column, floating on water surfaces (buoyant particles, <1.0 g cm^{-3}), and sedimenting plastic particles (benthic or seafloor materials, density >1 g cm^{-3}) are often mistaken for food by aquatic organisms, especially fishes (Galloway et al., 2017; Steer et al., 2017), or ingested by filter-feeding organisms, like bivalves (Scherer et al., 2018). Uptake of microplastics by fishes block their guts and intestines – depending on their size (Savoca et al., 2019), and increases the chance of organic pollutants sorbed onto microplastics to be leached into fish organs, thus causing health defects. The process of ingestion of microplastics and leaching of pollutants influences the transport of both through the food web and poses a threat to aquatic life (Betts, 2008; Wright et al., 2013a). A study testing the hypothesis of accumulation of polyethylene (PE) microplastics in animals, their possible cytotoxicity and effects on behavior and mutagens at upper trophic levels, recorded that accumulation of PE in *Danio rerio* was associated with behavioral disorders observed at upper trophic levels. The study showed the adherence, absorption and translocation of MPs through the aquatic food chain (da Costa Araújo et al., 2020). Direct and indirect ingestions of organic pollutants interfere with growth, fertility, and lifespan while dosage could alter metabolism (Jovanović, 2017), immunity

[1]https://www.sciencedirect.com

[2]https://www.webofscience.com

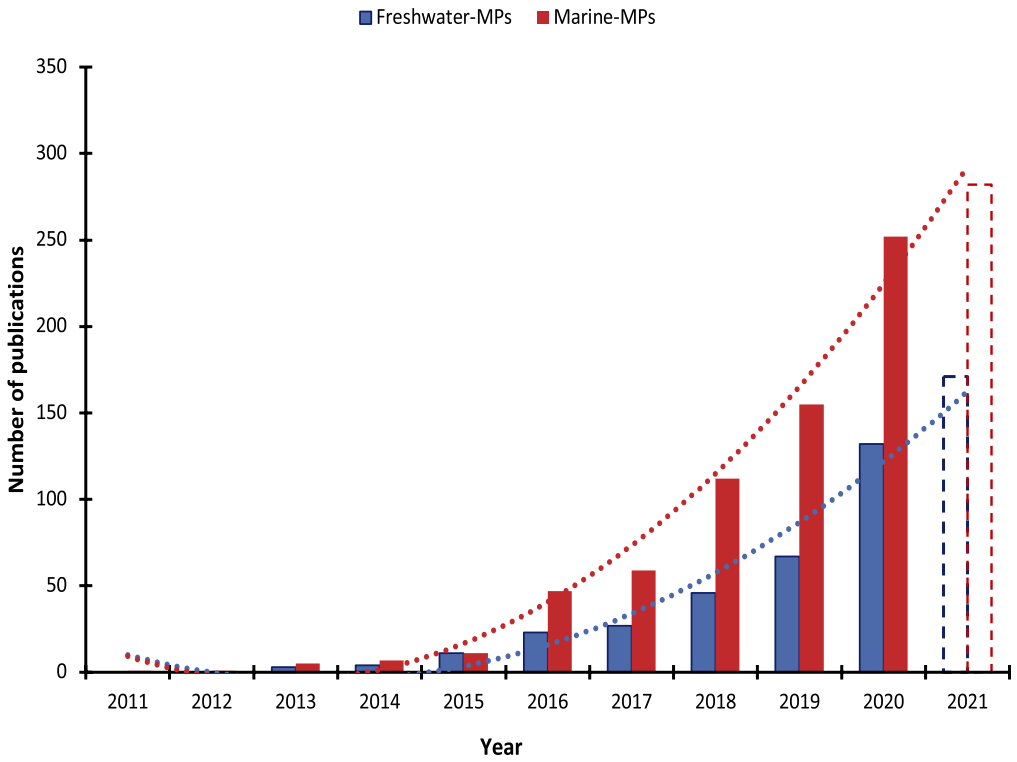

FIGURE 1 | Yearly published research articles on micro- and nano-plastic particles in marine and freshwater ecosystems. The total for 2021 represents the research articles as at October 23, 2021.

(Veneman et al., 2017), behavioral pattern (Gambardella et al., 2017), and energy budgeting (Wright et al., 2013b).

According to contemporary ecotoxicological investigations, the ingestion of microplastic particles by marine animals could culminate in both sublethal and lethal effects such as toxicosis in freshwater and marine fish species (Veneman et al., 2017; Jin et al., 2018; Lei et al., 2018; LeMoine et al., 2018; Hamed et al., 2020; Malafaia et al., 2020; van der Hal et al., 2020; Zhang et al., 2020b; Tongo and Erhunmwunse, 2022), oysters (Green, 2016; Sussarellu et al., 2016), mussels (Browne et al., 2008; von Moos et al., 2012; Jang et al., 2016; Paul-Pont et al., 2016; Ribeiro et al., 2017; Faggio et al., 2018; Pittura et al., 2018; Magni et al., 2019; Revel et al., 2019), clams (Ribeiro et al., 2017; Luan et al., 2019), and sea urchins (Della Torre et al., 2014). In the same vein, the ingestion toxicity effects of nanoplastics on fish (Greven et al., 2016; Brandts et al., 2018a; Pitt et al., 2018a; Sökmen et al., 2020), shrimps (Chae et al., 2019), and mussels (Brandts et al., 2018b; De-la-Torre et al., 2020b) have also been reported.

Multiple adverse effects (**Figure 2**), on marine and freshwater animals have been reported by several researchers, including changes in fertility (Sussarellu et al., 2016), reproduction abnormalities (Della Torre et al., 2014; Sussarellu et al., 2016), inflammatory responses (von Moos et al., 2012), gut inflammation (Jin et al., 2018), metabolism disorder (LeMoine et al., 2018; Yu et al., 2018; Banaee et al., 2019; Magni et al., 2019), immune system disabilities (Jang et al., 2016), endocrine disruption (Rochman et al., 2014), reduction in mortality rate (Green, 2016; Rist et al., 2016), hepatic stress (Greven et al., 2016; Brandts et al., 2018a,b), cytotoxicity (von Moos et al., 2012; Ribeiro et al., 2017; Hamed et al., 2020; Xia et al., 2020; Ali et al., 2021), particle transfer into the cardiovascular system (Browne et al., 2008; Ribeiro et al., 2017), and pathological impairment of respiratory and gastrointestinal tracts (Hämer et al., 2014; Mattsson et al., 2014; Jeong et al., 2016; Lu et al., 2016). Other effects of plastic ingestion by aquatic animals include the obstruction and damage of the digestive system (Camedda et al., 2014), gastrointestinal blockage, constriction, and injuries (Parga, 2012; Di Bello et al., 2013), immune system depression (Limonta et al., 2019; Huang et al., 2020; Hu et al., 2021), reduced growth rates, fecundity, reproductive success, and late sexual maturation (Hoarau et al., 2014; Vegter et al., 2014; Sussarellu et al., 2016; Liu et al., 2022), growth and reproductive abnormalities (Naidoo and Glassom, 2019; Pannetier et al., 2020; Liu et al., 2022), and metabolism, oxidative stress, and gastrointestinal dysfunction (Qiao et al., 2019a; Li et al., 2022).

Food Web Interactions of Microplastics

The food web is a complex feeding relationship among species within an ecosystem. It is a collection of interconnected food chains that illustrates the transfer of energy and nutrients from plant sources through herbivores to carnivores (Hui, 2012). Organisms in the aquatic ecosystem food web belong to different trophic levels including the (i) producers, (ii) primary consumers, (iii) secondary consumers, (iv) tertiary consumers, and (v) decomposers. Producers are autotrophs that create their food through photosynthesis utilizing sunlight, carbon

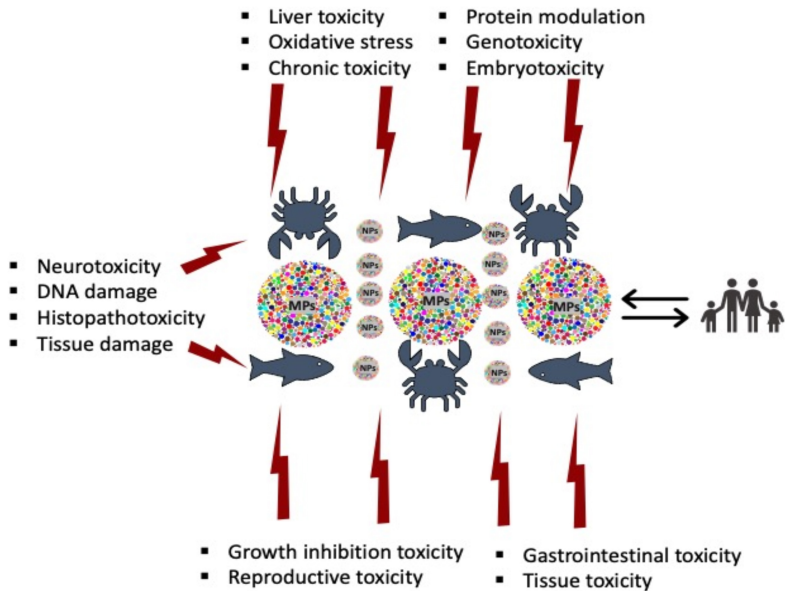

FIGURE 2 | Toxicological effects of microplastics and nanoplastics to aquatic animals.

dioxide, and water. Autotrophs such as algae and plankton are eaten by primary consumers like zooplankton, small fishes, and crustaceans. Turtles and sea urchins are also primary consumers. Secondary consumers such as sea otters feed on the herbivorous primary consumers and tertiary consumers feed on the secondary. The apex predators like large sharks, dolphins, and whales are top predators. The last trophic level in the food web comprises the decomposers (fungi and bacteria). They turn organic wastes, such as decaying plants and animals, into nutrients. They complete the cycle of life by returning nutrients to the soil or oceans for use by autotrophs (National Geographic Society, 2021).

In a healthy food web, autotrophs are present in higher abundance than herbivores therefore, biomass and energy production decrease with each trophic level. Conversely, toxic chemicals such as persistent organic pollutants and microplastics increase with each trophic level because organic chemicals can store in the fatty tissues of animals while microplastics have been shown to be stored in the guts, gills, liver, and brain of fish (Ding et al., 2018). The toxic chemicals and microplastics can be transferred from one trophic level to another upon ingestion leading to bioaccumulation in the food web.

Figure 3 shows how microplastics attached to phytoplankton and zooplanktons are ingested by small fishes, which in turn are fed upon by other primary consumers that are prey for secondary consumers at the higher trophic levels. Subsequently, microplastics spread through the aquatic food web. Microplastics discovered in fur seals were suspected to have been present due to the consumption of a pelagic fish *Electrona subaspera* that ingested microplastics (Eriksson and Burton, 2003). Microplastics are not biodegradable, thus remain in the digestive tracts of marine organisms across the food web, having biological and physical impacts on marine lives. The effect of chemically-laden contaminated microplastic ingestion might not be obvious immediately in big fishes, but the continuous accumulation of these microplastics could eventually be lethal.

Various researches on the prevalence and suffusive contamination of microplastic particles in the aquatic environment have concentrated on marine systems more than freshwater ecosystems (Isobe et al., 2017, 2019; Blettler et al., 2018; Zheng et al., 2019; Mataji et al., 2020; Nematollahi et al., 2020; Saeed et al., 2020; Ain Bhutto and You, 2022). Microplastics enter freshwater ecosystems (rivers, lakes, and streams) through a variety of ways, including sewage and landfill effluents, urban run-off, atmospheric deposition, wastewater treatment plants, and improper waste disposal (Horton et al., 2017b; Wu et al., 2019; Müller et al., 2020; Castro-Castellon et al., 2021). Rivers have nonetheless been identified as the primary route through which plastic waste enters the marine environment (Li et al., 2020). Climatological conditions and hydrodynamic processes in rivers, lakes, and streams such as current, waves, wind, river discharge rate, and geographical location have been identified as the primary factors that influence the occurrence and distribution (sinking and resuspension rates) of microplastic particles in freshwater ecosystems (Rodrigues et al., 2018; Bellasi et al., 2020; Dahms et al., 2020). Furthermore, microplastic particles concentration and bioavailability to planktonic, nektonic, and bottom-dwelling freshwater biotas (Meng et al., 2020; Krause et al., 2021), as well as their widespread relative abundance in freshwater compartments, can inevitably lead to ingestion by multiple biological organisms including diatoms, planktonic crustaceans, fish, mussels, and zooplankton.

Microplastics ingestion by biotas in the freshwater ecosystems are largely influenced by multiple interactions involving abiotic (e.g., temperature) and biotic factors (e.g., feed abundance, type of diet, size of feeding substance, availability of competitors,

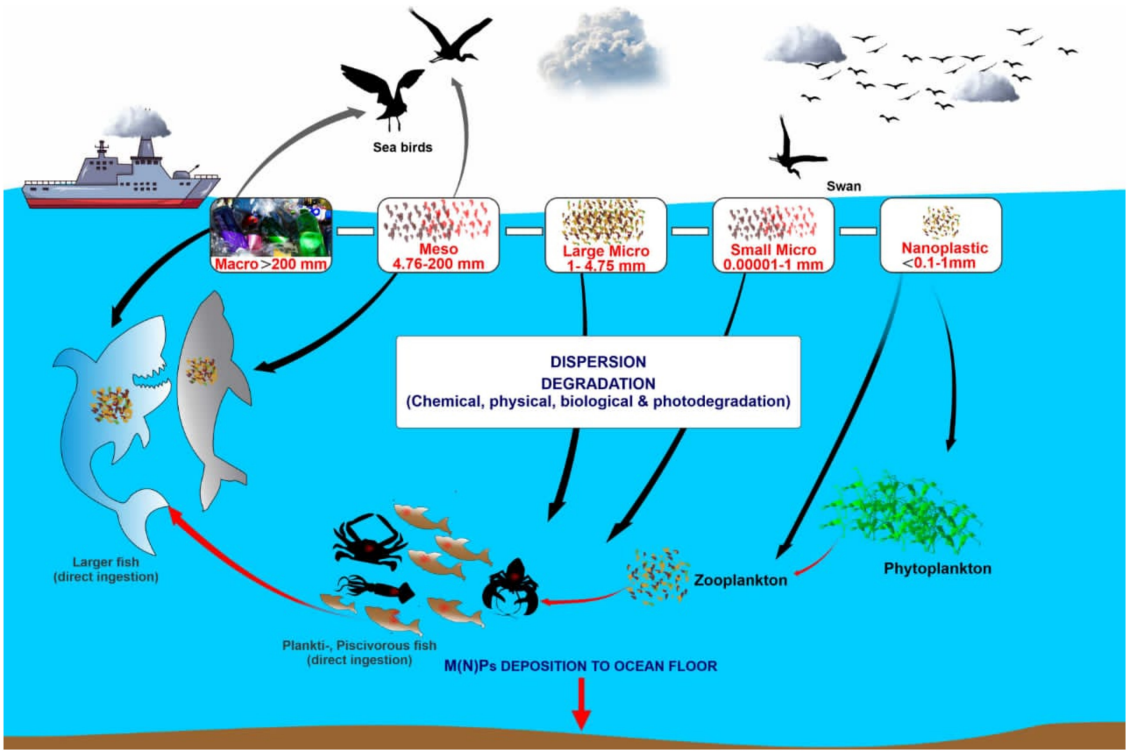

FIGURE 3 | A pictorial representation of the transport of microplastics across the marine aquatic food web.

and physiological condition) (Scherer et al., 2018), as well as feed behavior, morphology of MPs, and exposure route (Lambert and Wagner, 2018; Elizalde-Velázquez et al., 2020; Filgueiras et al., 2020; Garcia et al., 2020). It is well known that organisms in freshwater ecosystems are constituents of complex trophic food chain, feeding on a plethora of different types of food items, using a variety of distinct feeding methods including filter, fluid, suspension, sediment, and suction feeding. Filter and suspension feeders including planktonic crustaceans (e.g., Daphnids), mussels, protozoans, and rotifers are particularly susceptible to microplastics intake due to their frequent ingestion of living (bioseston e.g., nekton and plankton) and non-living (tripton e.g., plant debris) particulate matter suspended in freshwater bodies (Thouvenot et al., 1999; Browne et al., 2008; Scherer et al., 2018; Koelmans et al., 2022). These interactions and trophic processes are poorly understood and further complicate our understanding of their MPs impacts and toxicosis to freshwater biota (Castro-Castellon et al., 2021; Ma and You, 2021; Ain Bhutto and You, 2022).

Furthermore, a couple of studies have reported on the spatial distribution of MPs and their impacts on freshwater food webs. However, the mechanistic routes of MPs and sorbed additives exposure, bioaccumulation, and biomagnification through food chains and the associated toxicities on higher trophic level biota in freshwater ecosystems are still unknown. On the other hand, scanty studies have been conducted on the fate of nanoplastic particles in freshwater environments, owing to a lack of standardized analytical and reliable methods for sampling, detection, and characterization of NPs. Little is known about the toxicity of NPs in freshwater ecosystems. However, the few documented studies are largely lab-based reports that might not reflect similar biological toxicity if replicated in the field (Zhang et al., 2022). These laboratory studies employed polystyrene nanoplastics, and the toxicological effects reported include reproductive abnormalities (Li et al., 2020,a,b), oxidative stress and gastrointestinal dysfunction (Chae et al., 2018; Pitt et al., 2018b; Huang et al., 2020; Li et al., 2020,a), increased mortality (Liu et al., 2019; Liu et al., 2020; Zhang et al., 2020c), growth inhibition and disorder (Liu et al., 2019; Lin et al., 2020; Liu et al., 2020; Zhang et al., 2020c), and cardiovascular dysfunction and neurotoxicity (Chen et al., 2017a,b).

In aquatic ecosystems, microplastics are known to disintegrate further into nanoplastics when exposed to ultraviolet radiation, facilitated by shear forces of tidal currents and chemically or biologically driven degradation mechanisms, thus resulting in deleterious endpoints for freshwater and marine food webs. When micro(nano)plastics reach the aquatic environment, they may be directly ingested by zooplankton, planktivorous, and piscivorous fish in the aquatic food web (Abbasi et al., 2018; Ain Bhutto and You, 2022), and then transferred through the food chain, where they are finally consumed by humans. The ingestion of particulate plastics by humans is largely from consuming contaminated seafood, sea salts, and water (Kim et al., 2018; Schymanski et al., 2018; Bradney et al., 2019; Wong et al., 2020; Selvam et al., 2020). Toxicological studies have shown that plastic particles in the gastrointestinal systems of humans could pose

severe biological effects to the digestive systems and impair immune functioning (Chang et al., 2020).

Studies on the prevalence, exposure, and transfer of micro(nano)plastics from the lower trophic levels across the food web to humans are emerging. Almost all of these investigations are concerned with the plastics consumed by phytoplankton, zooplankton, oysters, crustaceans, and fish. A few studies have also reported the transfer of MNPs across different trophic levels (Nelms et al., 2018; Ribeiro et al., 2019). Furthermore, Farrell and Nelson (2013) reported the trophic level transfer of microplastics in *Mytilus edulis* (L.) to *Carcinus maenas* (L.), while the ingestion and biotransfer of MPs in the planktonic food web has been documented (Setälä et al., 2014). However, the bulk of food web dynamics comprises multiple organisms with modest and significant interactions among organisms and animals in other food webs, thus complicating risk and ecotoxicological assessments (Bellas et al., 2016; Walkinshaw et al., 2020). An in-depth investigation of the transfer of MNPs and sorbed organic and inorganic pollutants through aquatic food webs to humans has been reported (Bradney et al., 2019). According to documented toxicological studies, microplastics and nanoplastics are readily absorbed by human epithelial (Magri et al., 2018) and cutaneous (Rubio et al., 2020) tissues when exposed to particulate plastics. Bioaccumulation of polymeric particles, as well as absorption/desorption of MNP-bound hydrophobic organic compounds and trace elements when they reach the gastrointestinal and respiratory systems, could have long-term negative impacts on human health (Cole et al., 2015; Pozo et al., 2019; Rubio et al., 2020; Zazouli et al., 2022).

In recent times, food web interactions of ingested microplastics and nanoplastics by aquatic organisms are the subject of laboratory and field comparative studies. MPs are ingested by a number of aquatic organisms, and these particulate plastics have been found to be transferred through the food chain in several laboratory studies. The majority of published studies indicate the presence of microplastics in organisms' gastrointestinal and respiratory systems (Chua et al., 2014; Kaposi et al., 2014). The transfer of ingested microplastics from aquatic organisms' gut to the adjoining tissues, as well as excretion and trophic transfer, have largely been investigated in controlled laboratory experiments (Browne et al., 2008; Hesler et al., 2019; Cousin et al., 2020; Kong et al., 2020). Several laboratory studies have reported the ingestion of primary microplastics by organisms across the aquatic food web like zooplankton (Cole et al., 2013; Setälä et al., 2014) and other macro-sized invertebrates (Setälä et al., 2016; Gray and Weinstein, 2017), as well as fishes (Rochman et al., 2013; Batel et al., 2016). Setälä et al. (2014) reported the ingestion and planktonic trophic transmission of fluorescent polystyrene (PS) microspheres (10 μm) from zooplankton to the mysid shrimp *Mysis relicta*. Furthermore, the food chain interspecies transfer of fluorescently labeled MPs between mussels (*M. edulis*) and shore crabs (*C. maenas*) has been reported (Farrell and Nelson, 2013; Watts et al., 2014). Microplastic intake has also been studied in various field investigations, primarily using marine organisms. However, no distinction between direct ingestion of MPs and trophic transfer is apparent considering such field investigations (Duis and Coors, 2016). On the other hand, limited data exists on freshwater organisms' ingestion of microplastic particles in field research studies (Sanchez et al., 2014; Hurley et al., 2017; Nel et al., 2018; Bertoli et al., 2022; Buwono et al., 2022). Furthermore, nanoplastic particles could be transferred trophically up the planktonic food chain through multiple secondary aquatic organisms at the higher trophic level. Moreover, our contemporary knowledge of the interactions between lower trophic level species and nanoplastic particulates remains relatively sparse (Latchere et al., 2021; Zhu et al., 2021; Sendra et al., 2020; Haegerbaeumer et al., 2019).

Factors Influencing Microplastics and Nanoplastics Bioavailability and Uptake

Plastic particles in the aquatic environment experience variations in physical properties which in turn influence their deposition, transport, retention dynamics, and bioavailability (Krause et al., 2021). The bioavailability and uptake of microplastics in the aquatic food web can occur at all trophic levels as evidenced in the detection of microplastics in organisms ranging from benthic planktons to large fishes in the water column (Lima et al., 2014; Digka et al., 2018). The bioavailability and uptake are predicated on several factors such as density, color, size, and the abundance of the polymer particles and hydrodynamic conditions such as flow dynamics of water.

Density

The density of plastics is an important characteristic that determines whether the microplastic particles will be suspended in the water column or sink to the benthic sediment. This will subsequently determine the type of organism that might ingest such plastic particles. Low-density plastics such as polyethylene with specific gravity 0.91–0.94 are likely to be ingested by filter feeders and suspension feeders which reside in the upper column of seawater. Annelids feed on microplastics in intertidal beach sediment (van Cauwenberghe et al., 2015) and seabirds pick up floating particles on seawater. Biofouling of MPs changes the buoyancy of plastics by increasing their mass and density causing the low-density plastics to sink and become available to benthic organisms for ingestion (Long et al., 2015). Other factors that affect the buoyancy of plastics include adsorption of minerals to plastic surfaces (Corcoran et al., 2015), microplastics ability to combine with fecal pellets (Cole et al., 2016), and the types and amount of additives included during the manufacture of plastics. Polymers with density lower than that of seawater ($\sim$1.02 g/cm^3) are expected to float.

Color

For visual predators, the color and sometimes the shape of prey is an important characteristic. A study by Shaw and Day (1994) reported that some commercially important fish and their larvae fed on small zooplankton which is usually white, tan, or yellow in color; the chances of mistaking these classes of plastic particles as prey and ingesting them selectively are high. The researchers found a consistent decrease in the number of white plastic particles in fish sampled from the North Pacific and concluded that marine organisms showed a preference for

certain colors, sizes, and shapes of plastic particles (Shaw and Day, 1994). Similarly, Ory et al. (2017) investigated the ingestion of blue microplastics by Amberstripe scad (*Decapterus muroadsi*) in the South Pacific. According to their findings, Amberstripe scads unintentionally ingested microplastics that resembled their natural prey (a blue copepod). Other than fish, holothurians (sea cucumbers) are another class of sea organisms that ingest microplastics of specific colors and shapes (Ivar and Costa, 2014).

A variety of fish larvae (meroplankton) have been reported to have ingested microplastic particles in the western English Channel, with blue fibers accounting for 66% of the total ingested microfibres (Steer et al., 2017). Desforges et al. (2015) also reported that two foundational zooplankton species *Neocalanus cristatus* and the euphausiid *Euphausia pacifia* showed selective ingestion for black, blue, and red microplastic particles. A recent study by Gurjar et al. (2022) indicates that different pelagic and demersal fish species showed selective preference for black [Bombay duck (*Harpodon nehereus*), Malabar sole fish (*Cynoglossus macrostomus*), and shrimps (*Metapenaeus dobsoni*)], blue [Belanger croakers (*Johnius belangerii*) and white sardine (*Escualosa thoracata*)], and green and yellow (Malabar sole fish, Belanger croaker, and Bombay duck). Further, a documented study by Renzi et al. (2018) revealed that the European anchovy's feeding habit of preferentially swallowing dark prey materials could be responsible for the mostly black and blue colored microplastics identified in their digestive systems. Also, a penaeid shrimp (*Penaeus monodon*) has been reported to dominantly ingest black colored microplastic particles while also swallowing four other different colors (blue, green, red, and white) of plastic particles found in its gastrointestinal tracts (Hossain et al., 2020). However, more research is needed to establish whether freshwater and marine organisms exhibit preferential behavior toward ingestion of microplastic particles of varying color.

Size

The small size of microplastics increases the likelihood of their ingestion by lower trophic organisms which are less selective in their feeding behavior as they take up anything of the appropriate size (Moore, 2008). Higher trophic organisms, on the other hand, may mistakenly ingest microplastic particles as food (Fossi et al., 2012; Hartmann et al., 2017). Quinn et al. (2017) reported the ingestion of both micro-and macro-plastics in demersal flatfish and pelagic fish species harvested from the East and West coasts of Scotland. Similarly, turtles have been reported to ingest micro-, meso-, and macroplastics in several studies due to their size and feeding habits (Clause et al., 2021), while small invertebrates, such as annelids, are prone to ingest microfibres a few hundreds of micrometers in size (Gusmão et al., 2016).

Abundance

Enhanced quantity of plastic particles in the marine and freshwater environments increases the likelihood of organisms to encounter them, making them available for ingestion. The continuous introduction from land-based sources and subsequent fragmentation under environmental conditions in aquatic ecosystems will ensure a steady supply of MPs and NPs to marine and freshwater organisms (Browne, 2015). Due to their ubiquity, microplastics in particular are unequivocally interacting with the marine food web as indicated by several scientific evidence showing the detection of microplastics in many environmental matrices and biota. According to Gurjar et al. (2022), the mean abundance of microplastics in demersal fish species varied between 5.62 ± 2.27 and 6.6 ± 2.98 items/species, whereas in pelagic species the results ranged from 6.74 ± 2.74 to 9.12 ± 3.57 items/species. As a result, the study concluded that microplastic abundance was higher in pelagic flesh-eating types of fishes than in bottom-feeding fish species. Additionally, James et al. (2020) showed that microplastics were more abundant in pelagic fish species than in benthic species. In general, aquatic fishes existing and feeding in shallow coastal waters have a greater probability of ingesting microplastic particles directly or accidentally through their feeding behaviors than bottom-dwelling species (Pozo et al., 2019; James et al., 2020). This might likely be attributed to the increased introduction of macro-, meso-, and microplastics from land-based anthropogenic activities into the aquatic coastlines.

On the other hand, relatively low ingestion of microplastic particles by aquatic species has been reported in various regions of the world, including south-east Bay of Bengal (Karuppasamy et al., 2020), southwest coast of India (Robin et al., 2020), Mediterranean Sea (Fossi et al., 2018; Giani et al., 2019), and Adriatic Sea (Pellini et al., 2018), all of which showed a reduced abundance of MPs. Moreover, Davison and Asch (2011) estimated that the annual ingestion rate of plastic debris by mesopelagic fishes in the North Pacific Ocean ranged between 12,000 and 24,000 tonnes. Generally, the relative abundance of MNPs in freshwater and marine organisms may be a function of many factors including trophic level, habitat, feeding habits, type and size of species, size of microplastic, type of season, and anthropogenic emissions.

Hydrodynamic Conditions

The influence of local hydrodynamic conditions such as current intensity, seawater influx and velocity, tidal currents induced by winds (Sadri and Thompson, 2014; Tan et al., 2019; Fred-Ahmadu et al., 2020b), and depositional environment (Wilson et al., 2021) on the abundance, distribution and dispersion of microplastics have been reported. Beaches with low current intensity or low energy were found to accumulate more microplastic particles on their coastlines. This accumulation allows MPs to enter the food web through uptake by filter-feeding crustaceans such as crabs on shoreline sediments (Horn et al., 2019). In freshwater ecosystems, models have been developed for MPs transport and retention using Stoke's settling velocity which is a function of particle density, size, and shape (Krause et al., 2021). Hyporheic exchange caused by flow interactions with sediment and benthic algal beds results in the availability of MPs to benthic organisms (Drummond et al., 2020). The remobilization of microplastic particles into the sediment-water interface during base flow and storm flow releases MPs into the water column. However, the dynamics of remobilization of sediment-sorbed MPs due to disturbances and turbulent mixing

in the marine environment is yet to be fully understood. Further research is required in this aspect.

ADSORPTION AND DESORPTION OF MICROPLASTICS AND NANOPLASTICS-BOUND POLLUTANTS

Microplastics as Sources of Persistent Organic Pollutants Through Chemical Additives

Microplastics have been found to be sources of organic pollutants like polycyclic aromatic hydrocarbons (PAHs) which are present in petroleum; a primary ingredient of synthetic plastic. Plastics also include chemical additives like bisphenol-A (BPA), heavymetals, phthalate esters (PAEs), alkylphenols, and polybrominated diphenyl ethers added to them to enhance flexibility and durability (Barnes et al., 2009; Benson and Fred-Ahmadu, 2020; Hajiouni et al., 2022). These chemical additives are not strongly bonded to polymers and could eventually desorb and get leached into tissues of marine organisms upon ingestion (Koelmans, 2015). A study by Collard et al. (2017) reported the leaching of additive chemicals from microplastics into the blood and other organs of European anchovies (*Engraulis encrasicolus* L.). Likewise, the study by Tanaka et al. (2013) showed the possibility of leaching of chemical additives from plastics into biological tissues of African seabirds and North Pacific seabirds. The release of chemicals from microplastics occurs in a process called desorption. Studies have reported desorption to occur in tissues and organs of marine organisms after ingestion. PAHs sorbed on fluorescent microplastic particles were found to have desorbed in the intestine of Zebrafish before being transferred to the intestinal epithelium and liver (Batel et al., 2016). The ability of microplastics to release sorbed Persistent organic pollutants (POPs) and potentially toxic trace metals into various environmental media and organisms' tissues have been shown to have detrimental effects.

Microplastics as Sinks and Vectors for Persistent Organic Pollutants and Metals

While microplastics are sources of organic pollutants through the chemical additives incorporated in them during production, they also act as sinks for organic pollutants and metals through adsorption process. The sorption process depends on the physicochemical characteristics of both the POPs and the microplastics (Fred-Ahmadu et al., 2020c). Generally, the hydrophobicity of POPs and microplastics is the major factor that contributes to the interaction between them as well as sorption. Rubber-like feature of polypropylene (PP) and polyethylene (PE) (Bakir et al., 2012), glass-like property of polyvinylchloride (PVC) (Rodrigues et al., 2019), π-π electron system (Nakano, 2010), and amorphous structure of PS, the crystalline structure of polyethylene terephthalate (PET) (Miandad et al., 2018) are all properties that govern their sorption capacity. Aging influences the sorption capacity of microplastics and surface area to volume ratio is another property of microplastics that influence the sorption of organic pollutants and metals to them.

A report by Bakir et al. (2012) showed that microplastics made of polymers like PE, PVC, PP, and PS have a high sorption capacity for polycyclic aromatic hydrocarbons, hexachlorocyclohexanes, polychlorinated benzenes, and dichlorodiphenyltrichloroethane. A study by Turner and Holmes (2015) showed the adsorption of trace metals; silver (Ag), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) onto both virgin and aged microplastics with the aged plastic having higher sorption capacity. This sorption increases the long-distance transport of toxic chemicals in the marine environment. Due to electrostatic interactions and complexation on the surface of plastics such as PVC, preferential adsorption of bivalent metals (Pb^{2+}, Cu^{2+}, and Cd^{2+}) was observed by Zou et al. (2020). Furthermore, adsorption of metals on MPs surfaces also occurs *via* surface charge created through weathering and organic matter precipitation which allows the binding of metal ions to active sites on the microplastics (Maršić-Lučić et al., 2018). The capacity of microplastics to act as adsorbents for metals and POPs requires further research in order to maximize this property for the remediation of wastewater and contaminated soils and sediment.

ECOTOXICOLOGICAL IMPACTS OF PLASTIC POLLUTION AND CONTAMINATED MICROPLASTICS ON AQUATIC ORGANISMS

Physical Impacts of Plastic Pollution on Aquatic Organisms

The impacts of microplastics on aquatic organisms could be physical, biological, or chemical. Physical impacts include entanglement, blockage of sunlight, and ingestion. There are recorded cases where marine animals like seabirds, sea turtles, and marine mammals have been trapped by plastic debris (Nelms et al., 2016; Hiemstra et al., 2021). Ghost nets, which are usually made of synthetic, non-biodegradable fibers like nylon, can persist for several years in the aquatic ecosystems, entangling, and in most cases killing an uncountable number of marine species (Stelfox et al., 2014; Wilcox et al., 2014; Nelms et al., 2016). The entrapment limits mobility for marine life which could eventually lead to discomfort that later results in death by starvation of marine animals caught in such situations (Schuyler et al., 2014). An increase in cases of entrapment has led to movements like "Skip the straw" and "Save the turtles Campaign," as consequences of entrapment have included a decline in species of sea turtles around the globe (Friends of the Sea, 2009). Gall and Thompson (2015) study showed that almost 700 species interact with microplastics and an increase in this estimation is reported every year.

Microplastics are light weighted enough to float on water bodies, thus hindering the passage of adequate sunlight when excessively present. The hindrance of sunlight has been shown to

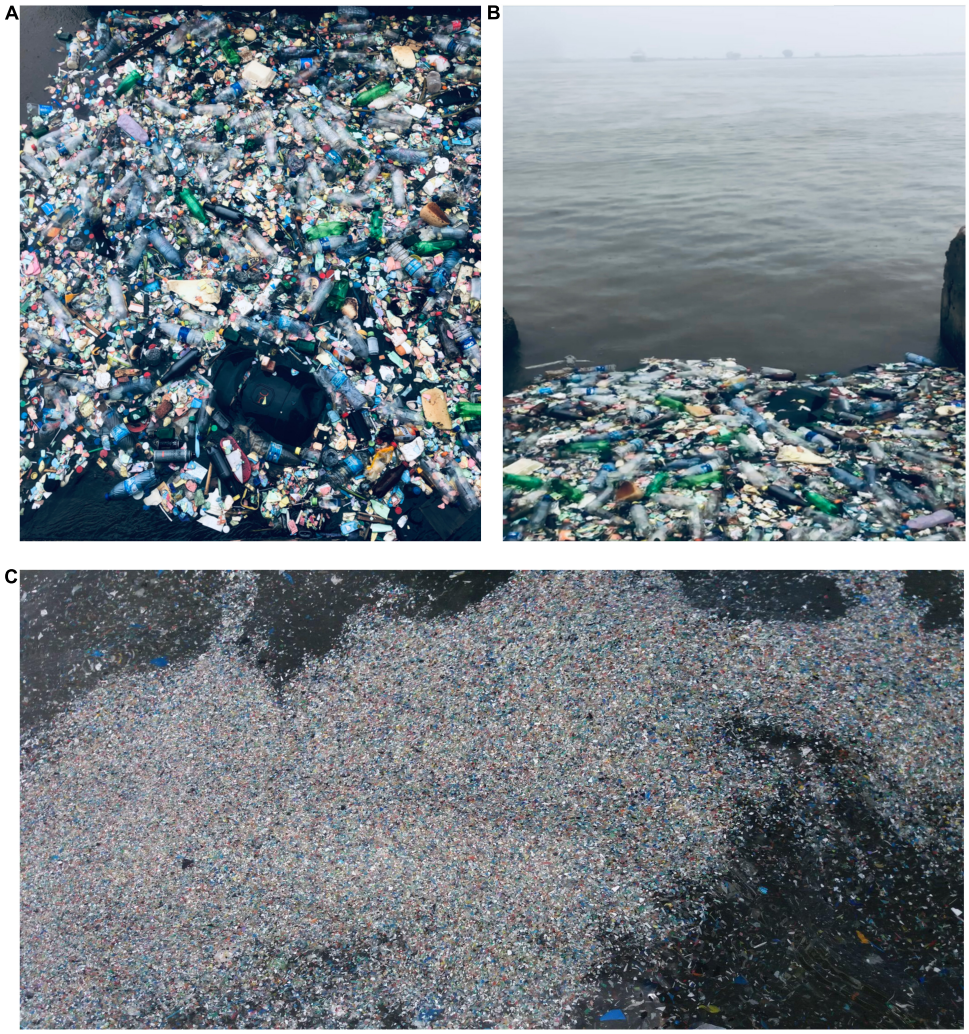

FIGURE 4 | Example of thousands of floating macro(meso)plastic **(A,B)** (Nigeria) and micro(nano)plastic **(C)** (Lima Peru) particles possibly blocking sunlight penetration at extremely polluted coastal sites.

alter photosynthetic activity (Zhang et al., 2017; Mao et al., 2018) which influences a decrease in chlorophyll content (Zhang et al., 2017; Prata et al., 2018). The altered photosynthetic activity and chlorophyll content could stunt the growth of phytoplankton and algae which form the bases of aquatic food webs, thus resulting in shortage of food for primary and then secondary consumers in aquatic biota. These scenarios are likely to occur in areas where microplastics pollution is severe enough to limit sunlight penetration. **Figure 4** shows an example of an extremely polluted site, possibly hindering sunlight penetration.

Physical impacts of microplastic that arise from ingestion include malnutrition, intestinal blockage and internal injury, increased buoyancy, and dietary dilution (Nelms et al., 2016). More so, a study by Lei et al. (2018) showed that ingestion of microplastic particles by Zebrafish led to intestinal damage. Inflammation, malnutrition, depleted energy reserves as well as prolonged stay of MPs in the guts of *Arenicola marina* on exposure to PVC were some of the negative effects recorded (Wright et al., 2013b). Reduced feeding activity leading to a decrease in energy available for growth was detected in *Carcinus maenas* on exposure to polypropylene fibers (Watts et al., 2014). However, Chen et al. (2020a) in their study observed hyperactivity in adult zebrafish which was evident in an increase in their swimming distance and suggested it was probably as a result of up-regulation of estrogen contents in the fish on exposure to PS. However, physical injuries and adverse impacts of plastic debris to marine organisms can be avoided by regular beach cleaning, public awareness, and proper regulation of activities on and around water bodies.

Chemical Impacts of Contaminated Microplastics on Aquatic Organisms

The chemical impacts of microplastics usually originate from the ingestion of microplastics contaminated with heavy metals and organic pollutants. It is well established that microplastics

can act both as sources and sink for organic pollutants and metals. These pollutants upon ingestion can pose threats to the health of marine organisms that consume them and can as well be transported through the aquatic food web thus posing a direct and/or indirect threat to all entities across the trophic level of the food web. The toxicity of PAH on ingestion by humans include nausea, diarrhea, confusion, kidney, and liver damage. Organohalogenated compounds cause reproductive problems (Sweeney et al., 2015) and phthalate esters (PAEs) disrupt metabolic systems (Diamanti-Kandarakis et al., 2009). The acute effects of POPs and metals on marine organisms have not been widely investigated. However, some studies have given an insight into the accumulation and toxic effect of contaminated microplastic on marine organisms. Nickel (Ni) contaminated polystyrene led to abnormalities like immobilization and changes in the morphology of *Daphnia Magna* (Kim et al., 2017). Copper-contaminated polystyrene accumulated in the tissue of Zebrafish and was found to be toxic to guts and the liver (Qiao et al., 2019b). The feeding activity of Lugworm was disrupted after it ingested polychlorinated biphenyl-contaminated polyethylene (Besseling et al., 2017). Mercury (Hg) adsorbed on fluorescence red polymer microsphere was found to have accumulated in the brain and muscles of European seabass (*Dicentrarchus labrax)*, resulting in neurotoxicity, oxidative stress and damage, and causing changes in the activities of energy-related enzymes in juveniles of the species (Antao Barboza et al., 2018). The leaching of chemical additives and pollutants from microplastics into the blood and other organs of European anchovies (*Engraulis encrasicolus,* L.) enhanced vascular thrombosis (Collard et al., 2017).

Biological Impacts of Microplastics on Aquatic Organisms

Few studies have been carried out to investigate the biological impact of microplastics on the marine ecosystem. Geographical transfer of microorganisms is one of the biological roles that microplastics play in the marine environment, due to microorganisms' ability to inhabit the surface of microplastics and be transported around with them (Oberbeckmann et al., 2015; De-la-Torre et al., 2021). The colonization of microplastics by microorganisms (biofouling) leads to changes in the physical properties of microplastics. Some of these changes include an increase in the density of MPs which causes them to sink to the bottom of the sea making them available to benthic organisms for ingestion and a decrease in hydrophobicity rendering them less accessible for contamination by organic pollutants (Fred-Ahmadu et al., 2020c). While the colonization of microplastics by microorganisms reduces chances of contamination by metals or organic pollutants, it increases their chances of being mistaken for food by other marine life in higher trophic levels, which primarily feed on microorganisms attached to the microplastics. Fractions of microplastics that adhere to planktons and algae can be ingested by other aquatic organisms like fish, shrimp, invertebrates and bivalves as reported by De-la-Torre et al. (2021), Kasamesiri and Thaimuangphol (2020), Jabeen et al. (2017), Rehse et al. (2016).

Biological effects of microplastic ingestion by marine organisms include disruption of oxidative balance, energy metabolism, antioxidant capacity, DNA makeup, immunological, neurological and histological impairment (Prokić et al., 2019). Direct and indirect consumption of contaminated microplastics could result in biological responses like inflammation, reduced feeding and body weight, and mortality stemming from bioaccumulation (Wright et al., 2013b). The study by Chen et al. (2020b) showed that interaction of marine model fish *Oryzias melastigma* with PS led to alteration of heartbeat, delay in hatching time, and decrease in hatching rate of embryos. According to the transcriptome result, exposure of these embryos to PS led to an increase in diseases as immune responses, genetic formation processing, and metabolism pathway were negatively impacted. Reproductive disruption was detected in *Crassostrea gigas* on exposure to polystyrene and significant impacts were also observed in offspring (Sussarellu et al., 2016). Polystyrenes were found in the circulatory system of mussels 3 days after they were first found in the guts (Browne et al., 2008; Carbery et al., 2018). A reduction in isocitrate dehydrogenase-IDH, a metabolic biomarker was observed in the gall bladder of Juvenile goby, *Pomatoschistus microps* fish after exposure to polyethylene (Oliveira et al., 2013). Mortality of brine shrimps was observed after they were exposed to polystyrene microplastics (Suman et al., 2020). Recently, Buwono et al. (2022) demonstrated that MPs (0.0001–1.0 mm) abundance has both direct and indirect biological impacts on oxidative damage on the gills and digestive tract of *Gambusia affinis*.

CONCLUSION AND FUTURE PERSPECTIVES

There is a wide range of biological, chemical, and physical effects resulting from direct or indirect ingestion of microplastic and nanoplastic particles by aquatic biota because of the relatively diminutive size of these emerging contaminants. The effects are variable and impact critical organs of aquatic organisms leading sometimes to increased morbidity and mortality. Small fishes and lower trophic marine organisms are the most impacted by contaminated micro(nano)plastics because they have the most direct contact with them. Biological impacts of microplastics on marine organisms influence the marine food web the most. This is due to the adherence of primary microorganisms on micro(nano)plastics, which are then fed upon by higher marine animals. Physical impacts include intestinal blockage, internal injury which could result in disturbed mobility, stunted growth, and death. The presence of microplastics that persist in the digestive tracts results in starvation which eventually could lead to death. The chemical effects of ingested microplastics on marine organisms have not been fully understood, because only accumulation has been studied the most. Most of the studies conducted to date have reported mainly on microplastic particles occurrence and prevalence in marine biota. The toxicity and mechanistic interactions of organic chemicals and metals on getting into the marine organisms is not fully understood. Thus, we recommend the following:

i There are currently limited studies on the prevalence and toxicity of MNPs on freshwater organisms, as well as their occurrence, fate, and transport in freshwater ecosystems. More research is needed to clearly understand the gastrointestinal-neuro-endocrine mechanisms of micro- and nanoplastic-induced adverse effects associated with

MNPs ingestion, absorption, metabolism, and excretion by freshwater organisms.

ii Physiological responses and broad toxicological effects in marine organisms, particularly adult marine species, are the focus of the majority of published studies. Furthermore, a relatively limited study has been conducted on the bioaccumulation and bioavailability of micro(nano)plastics. Understanding how microplastics are transferred from one trophic level to the next is essential in determining how long they remain in the digestive tract and how much they accumulate. Therefore, accurate, open and reliable data on the occurrence and ingestion/egestion rate of micro(nano)plastics, as well as the intake of hydrophobic micropollutants by freshwater and marine organisms, are required across a broader variety of intermediate and higher-trophic animals. This will provide a framework for establishing sound and effective risk assessment across a wider population and ecosystem scales.

iii Further studies should be focused on nanoplastics to enhance our understanding of size-dependent toxicity mechanisms.

iv In-depth investigations on the modes-of-action of microplastic and nanoplastic particles ingestions by marine animals, and the toxicological effects of these emerging contaminants' sizes on the oxidative status, digestive systems changes, neurological system damage, and reproductive alterations should be carried out to gain explicit understanding.

v It would be imperative to conduct further research to understand the pathways of chemicals leached from microplastics and nanoplastics into marine organisms upon exposures and the associated effects.

vi More research is required to explore the metabolic pathways in marine biota after ingestion of MNPs/plastic debris taking into consideration the chemical and physical properties of the MNPs, exposure time, and concentration of MNPs.

vii Finally, the biological mechanisms by which microplastic and nanoplastic particles affect marine and freshwater organisms are still poorly known, and therefore necessitate extensive research.

AUTHOR CONTRIBUTIONS

NB: conceptualization, investigation, supervision, project administration, funding acquisition, and writing—original draft, review, and editing. OA: conceptualization, investigation, and writing—original draft, review, and editing. OF-A: conceptualization and writing—original draft, review, and editing. GD-l-T, AO, and AW: conceptualization and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Microplastics in Sediment of Kuakata Beach, Bangladesh: Occurrence, Spatial Distribution, and Risk Assessment

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Kuakata beach, known as Daughter of Sea in Bangladesh, has drawn a growing number of tourists from all over the world, leading to the higher use of single plastic products. This study was a first attempt to describe the occurrence, spatial distribution, and ecological risk of microplastics (MPs) in Kuakata beach sediments. A total of 24 surface sediment samples were collected from the intertidal zone of the beach, and MPs were extracted using the density separation method and a stereomicroscope. Fourier transform infrared (FTIR) spectroscopy was used for qualitative and quantitative identification. The results revealed that the average MPs in the beach sediment were 232 ± 52 items kg^{-1} dry weight, which was much higher than many other sandy beaches throughout the world. Analyses of variance showed a significant ($p < 0.01$) difference among the mean abundance of MPs in sampling points. Fibers were dominated in every sampling point with an average of 123 ± 27 item kg^{-1}. Most of the MPs observed were colored (60%), and the rest were transparent (40%). It was found that the size range of 1–5 mm MPs constituted over half (55%) of total MPs covering an average value of 127 ± 34 items kg^{-1}. Three polymer types were identified in the sediment samples through FTIR analysis which followed the decreasing order of polyethylene terephthalate > polyethylene > polypropylene. Correlation analysis showed a positive relationship between the abundance of MPs and the finer grain size of sediment ($p = 0.055$; $r = 0.7$), indicating grain size-controlled the density of MPs. The pollution load index was assessed to estimate the ecological risk and found that the beach sediment of Kuakata belonged to the risk category I of the pollution index. This investigation provided preliminary information on MPs pollution in the marine ecosystem that the policymakers can use to take appropriate management approaches.

Keywords: microplastic pollution, beach sediment, sand grain size, ecological risk assessment, Bangladesh

INTRODUCTION

Microplastics (MPs) (<5 mm in size) were first reported in 1972 as an aquatic pollutant in marine environments, and now it has become a global concern for potential harmful effects not only on ecosystems but also on human health (Carpenter and Smith, 1972; Crew et al., 2020). Most of the MPs float on the ocean surface except high-density polymers, such as polyvinylchloride (PVC), polycarbonate (PC), and polyethylene terephthalate (PET). These floated MPs remain in the marine environment for longer periods due to their longevity, ubiquity, and impact resistance properties and cannot be easily removed (Geyer et al., 2017). Almost 367 million tons of plastic products were produced worldwide in 2020, from which 2–5% ended up in the oceans (Plastics Europe Market Research Group [PEMRG], 2021). If proper action is not taken to reduce the use of plastic products, the amount of plastic entering the ocean each year will be increased to 16 million tons by 2030 and approximately 32 million tons by 2050. It was estimated that in terms of weight, there will be more plastics in the ocean than fish by 2050 (Neufeld et al., 2016).

Plastic materials that have been widely used as virgin pellets, pharmaceuticals, cosmetics, or exfoliating scrubs (glitters and microbeads) in the plastic industry and cleaning products are generally referred to as primary MPs (Hahladakis et al., 2018; Camacho et al., 2019). The larger amount of plastic items are degraded into small, microscopic elements (<5 mm) due to mechanical abrasion caused by wave action, photochemical oxidation generated by UV-B radiations as well as biological processes (Corcoran et al., 2015), which are commonly known as secondary MPs. Plastics and their degraded products enter into the oceans from some point and nonpoint sources, such as incorrect disposal, sewage systems, loss during maritime activity, tourists activity, industrial effluents, roadside dust (vehicles tires, grease, etc.), beach adjacent hotels, motels, and restaurants, which are driven by riverine output, atmospheric outfall, and stormwater activity (Corcoran et al., 2015; Li et al., 2020). The key sources of synthetic fibers in aquatic ecosystems are from the washing process of synthetic textiles, which contribute about 35% to the worldwide release of MPs to the oceans (Boucher and Friot, 2017).

Microplastics are bioaccumulated into marine organisms and then infiltrated into the human food web *via* direct or indirect ingestion (Van Cauwenberghe and Janssen, 2014). Though some toxic pollutants and coexposure of MPs have health hazards, many ecotoxicological studies suggested that the physiological activities of marine organisms may not be significantly affected by a representative number of MPs (Rist et al., 2016; Canniff and Hoang, 2018). Furthermore, MPs alone has no effects on biochemical biomarkers in mussel. Still, the combined effects of MPs and triclosan, an antimicrobial agent, have enhanced the superoxide dismutase activity as well as lipid peroxidation and caused oxidative stress (Webb et al., 2020). Usually, some of the persistent organic pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, and medicinal agents, as well as heavy metals (e.g., Cu, Pb, Hg, Cd, Cr, etc.), are carried by MPs biofilms which can cause adverse health issues on aquatic organisms and humans (Wang and Wang, 2018; Camacho et al., 2019; Duan et al., 2020; Mahfooz et al., 2020). However, these toxicants are sorbed onto MPs following several mechanisms driven by the physicochemical properties of the MPs, toxicants, and the intermediate substances where the sorption takes place (Yu et al., 2019; Fred-Ahmadu et al., 2020). Plastic products, such as plastic bags, bottles, and other wrapping substances release polybrominated diphenyl ether (PBDE), nonylphenol, and bisphenol A, which are responsible for cardiovascular disease, reproductive disorders, and breast cancer in humans (Glausiusz, 2014; Weidemann et al., 2016; Ortiz-Villanueva et al., 2018). Hence, it is indispensable to estimate the ecological and environmental risks posed by MPs due to the versatility of numerous physiochemical features. The ecological risks which may usually drive-by MP particles can be illustrated by different initial assessment methods (Li et al., 2020; Wang et al., 2021). For instance, to calculate an index of MPs polymer types Xu et al. (2018) merged the hazard scores of plastic polymers and the pollution load index (PLI) which are discovered by Tomlinson et al. (1980) and Lithner et al. (2011).

However, the occurrence, distribution, and impacts of MPs had already been studied and detected in marine habitats from different parts of the world (Lo et al., 2018; Rodrigues et al., 2018; Anela et al., 2019; Botterell et al., 2019; Wieczorek et al., 2019; Li et al., 2020; Selvam et al., 2020; Wang Q. et al., 2020; Wong et al., 2020; Bhowmick et al., 2021; Gardon et al., 2021; Nithin et al., 2021; Sivagami et al., 2021; Taha et al., 2021; Wu et al., 2021; Yaranal et al., 2021). The presence of MPs was also documented in Bangladesh from sea salt (Parvin et al., 2022), fish species (Hossain et al., 2019, 2020; Ghosh et al., 2021; Parvin et al., 2021), and the beach sediment of Cox's Bazar (Hossain et al., 2021). Those studies, however, were only a brief description of MPs occurrence, and none of them focused on the hazards connected with MPs.

Kuakata sea beach, also known as Shagor Kannya (Daughter of Sea), is the second-largest sandy beach along the Bay of Bengal coastline of Bangladesh. About 115,000 tourists from home and abroad visit this beach in the peak tourist season (November–March) (Rahman et al., 2015). However, beachside hotels, restaurants, and tourists' activities produce many plastic wastes, often disposed of on the beach. The three main rivers in Bangladesh (The Padma, Meghna, and Jamuna) could enable that transport, although currents could also transport MPs from other parts of the Bay of Bengal into the coastline and beaches. Therefore, important to know the occurrence of MPs and understand the potential impacts of MPs on the environment and the health risk to humans and organisms therein. So far, no scientific study on MP contamination has been carried out along the entire coastal water and beach sediment in Kuakata beach. Therefore, this study was the foremost step toward elucidating the occurrence, spatial distribution of MPs particles in beach sediments from Kuakata, Bangladesh. Furthermore, ecological risk assessment of MPs and a relationship between sediment grain size and MPs distribution in Kuakata beach was studied for the first time in Bangladesh.

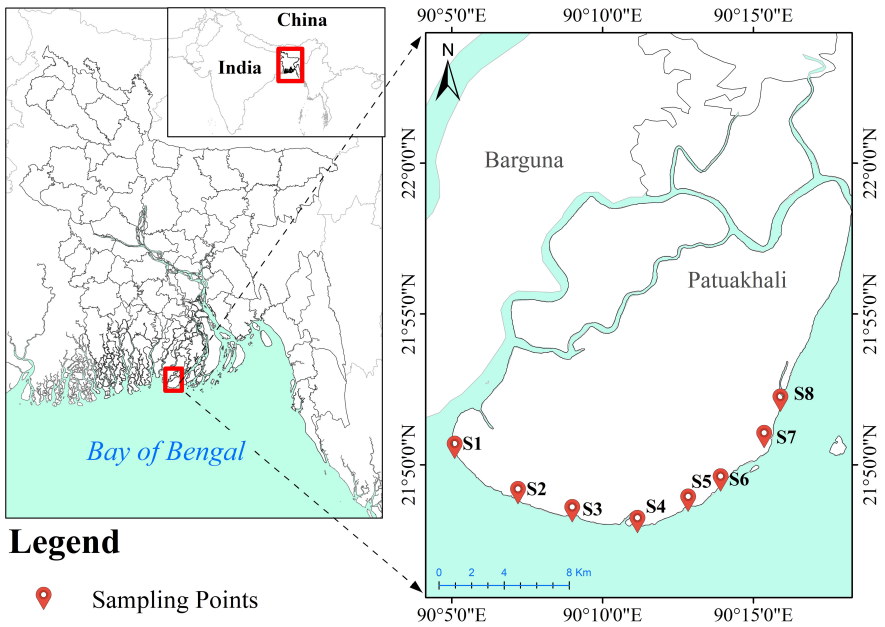

FIGURE 1 | Sampling points in Kuakata Beach along with the Bay of Bengal Coast, Bangladesh.

MATERIALS AND METHODS

Study Site

Kuakata sea beach (90° 7′ 0.0012″ E and 21° 49′ 0.0012″ N) of Patuakhali district is one of the attractive tourist places on the southernmost tip of Bangladesh (**Figure 1**). This beach area is about 65 km away from Patuakhali town and is situated in the middle of the Galachipa river and Andharmanik river estuary. It has an unbroken natural sandy beach (approximately 18 km long and 3 km wide) along the Bay of Bengal. The straight coastline of Kuakata lies 7 m above the mean sea level. The yearly mean temperature and rainfall are 25.9°C (78.70°F) and 2,590 mm where the average sea surface temperature (SST) is 27.30°C (81.14°F), respectively. Moreover, the average UV index is recorded at 7–12 (Bangladesh Meteorological Department [BMD], 2016) and such climate is categorized as tropical monsoon (Am) climate based on the Köppen–Geiger system. The current arrangement of the Bay of Bengal is recorded as clockwise from January to July whereas counterclockwise from August to December with a mean wind speed of 8.2 miles/h. The semidiurnal tide with two high and two lows has been noticed daily. The study area is generally flat and smooth with an average elevation of 65 cm AMSL and lies on the mid-southern coast of the old Ganges delta, covered with recent tidal deposits. In addition, most of the area is blanketed by tidal flats and these tidal flats are broad and nearly horizontal, which is detected by numerous tidal creeks and channels. The formation of beach ridge is a continuous linear mound of relatively coarser sediment close to the high-water mark, and a well-developed dune is present in Cower Char which is about 400 m away from the shoreline. One of the major factors is wave energy for controlling the beach development and changes across the Kuakata shoreline. There are well-generated longshore and rip currents in Kuakata beach, which develop within the surf zone by wave action. The maximum velocity of longshore and rip currents may exceed 1 ms^{-1}. This sandy beach with a gentle slope indicates that it has formed by faulting and down wrapping. The slope of Kuakata is 1–2° at Gangamatir char, 1–1.5° at Labur char, and 3–5° at Cower char which are the parts of Kuakata beach toward the Bay of Bengal (Rashid and Mahmood, 2011). Tourists usually enjoy the scenic beauty of Kuakata beach through scenic drives, boat tours, and bike tours. However, two forms of anthropogenic activities adversely affect the beach environment in this area: (1) the industrial and domestic waste discharge into Galachipa river in the East and Andharmanik river in the West and (2) exploration of tourists and the waste thrown by them.

Sample Collection

A total of 24 sediment samples were collected from eight sampling points, each having triplicates, in the pretourist season from September to October (postmonsoon) in 2019. These locations were S1 (90° 5′ 6″ E, 21° 50′ 34.8″ N), S2 (90° 7′ 12″ E, 21° 49′ 4.8″ N), S3 (90° 9′ 0″ E, 21° 48′ 28.8″ N), S4 (90° 11′ 9.6″ E, 21° 48′ 7.2″ N), S5 (90° 12′ 50.4″ E, 21° 48′ 49.68″ N), S6 (90° 13′ 55.2″ E, 21° 49′ 30″ N), S7 (90° 15′ 21.6″ E, 21° 50′ 56.4″ N), and S8 (90° 15′ 54″ E, 21° 52′ 8.4″ N) (**Figure 1**). All the samples were collected from strandline when there was ebb tide into the Bay of Bengal. Surface sand samples (top 5 cm) were collected using a metal quadrate (30 cm × 30 cm) following the methods of de Carvalho and Neto (2016) and Li et al. (2020). All the sands within the quadrate were carefully collected using the metal shovel and transferred into an aluminum foil bag. The sample bags were then carefully packed and shipped back to the laboratory for further processing.

Extraction Procedure of Microplastics

The extraction procedure of MPs in this study was conducted following the methodologies illustrated by Masura et al. (2015) and Hossain et al. (2020) with some modifications. First, wet sand samples of 400 g each were weighed and dried at 90°C using a hot air oven until their dryness. The dried samples were subsequently taken into an 800 ml glass beaker with 300 ml of $ZnCl_2$ (1.8 g cm^{-3}) salt solution (Coppock et al., 2017) and stirred with a spatula for a few minutes. Next, all solutions, e.g., H_2O_2 (Scharlab, Spain), $ZnCl_2$, $FeSO_4$, and NaCl (Loba Chemie, India), were filtered through a cellulose nitrate filter paper of 5.0 μm to remove indigenous MPs from them. After that, all the floating solids were sieved with a 0.3 mm sieve and moved into a 500 ml beaker. Then the beaker with the sample was dried at 90°C for 24 h. Finally, to eliminate organic matters from the dried sample, Fenton's reagent (30% H_2O_2 + $FeSO_4$) associated with 3 ml H_2SO_4 was added, and again heated to 75°C temperature on a hotplate for 30 min. A total of 6 g of salt (∼5 M NaCl) was added afterward per 20 ml of sample to intensify the density of the wet peroxide oxidation (WPO) solution and transferred to a density separator (Coppock et al., 2017) and kept overnight. After that, the floating solids from the separator were collected into a 500 ml beaker and filtered through a 5.0 μm of cellulose nitrate filter paper (Minipore, India) with 47 mm diameter (Bonello et al., 2018).

Visual Identification of Microplastics

A stereomicroscope (Leica EZ4E, Germany) with 8 to 35X magnification was used to quantify and identify the MPs from the filter paper with the method as in Hidalgo-Ruz et al. (2012), Cheung et al. (2016), and Catarino et al. (2018). For these, the filter paper was divided into four quarts pointing to the top clearly, and MPs were counted one by one quart from the filter paper (Lots et al., 2017). The images of MPs were taken with a high-resolution camera (DP-software) attached with the microscope, and measurements were done using ImageJ software (ver. 2.0.0) (Laglbauer et al., 2014). Besides, a hot needle test was conducted for suspicious plastic pieces (De Witte et al., 2014). The morphometric characteristics of MP particles were categorized into different types (microbeads, sheets, foams, films, fibers, and fragments) and shapes (irregular, elongated, rectangular, and cylindrical) (Hidalgo-Ruz et al., 2012; Lusher A. et al., 2017; Lusher A. L. et al., 2017; Frias and Nash, 2019), colors (Möller et al., 2020), and sizes (Zhang et al., 2016).

Polymer Type Identification

Comparatively larger particles were collected to Petri dish from filter papers to identify the polymer types of MPs. An FTIR 8400S manufactured by Shimadzu Corporation, Japan (wavenumber range of 4,000–400 cm^{-1}) and potassium bromide (KBr) pellet technique were used for the polymer characterization. Nearly 200 mg of KBr powder was mixed with around 1–3 mg of finely ground sample. The mixture was then pressed for 1 min in a pellet maker with a continuous pressure of 10 tons to form a transparent pellet using a Shimadzu (IR Prestige-21) hydraulic press. During pellet preparation, the system was kept under evacuation. The pellet was analyzed immediately using an FTIR spectrometer with resolution 2 cm^{-1} in 30 no. of a scan. The identification process is performed through an automated contrast with the extensive spectral libraries. However, depending only on automated libraries may lead to false identification. Therefore, the FTIR spectra have also been contrasted to absorption bands of polymers reported in the previous studies (Noda et al., 2007; Jung et al., 2018).

Sand Grain Size Analysis

Wet sand samples of 250 g were collected from each sampling point (S1–S8) at Kuakata beach and dried at 105°C in a hot air oven until sample dryness (Urban-Malinga et al., 2020). The average grain size was assessed by sieving 40 g of dry sediment through a sorted sequence of sieves (2, 1, 0.5, 0.25, 0.125, 0.063, and 0.002 mm) with a sieve shaker (Biobase BK-TS 200, China), and shake for 15 min (Wu et al., 2021). After that, all sediments on each sieve were collected and weighed for analysis. The grain sizes were ascertained based on the Wentworth scale (Wentworth, 1922).

Risk Assessment

The PLI is generally used to evaluate the ecological risk in terrestrial and aquatic environments (Tomlinson et al., 1980). In this study, the concentration of MPs was considered the pollutant to estimate the ecological risk in the beach sediment of Kuakata Beach. The PLI was evaluated using the following equations (Xu et al., 2018; Wang et al., 2021).

$$CF_i = \frac{C_i}{C_{oi}} \tag{1}$$

$$PLI = \sqrt{CF_i} \tag{2}$$

$$PLI_{zone} = \sqrt[n]{PLI_1 PLI_2 PLI_3 \ldots PLI_n} \tag{3}$$

where CF_i is the quotient (contamination factor) of the MP concentration at each sampling site, C_i is the MP concentration at each sample site, and C_{oi} is the background value of MP concentration in sediments before expanding the plastics industry. Nevertheless, there was no scientific study regarding MPs pollution in Kuakata to acquire the background values. Besides, there is no existing standard method to assess the risk of MPs. C_{oi} has been suggested to be denoted by the minimum MP concentration (153 items kg^{-1}) to assess the PLI (Isobe et al., 2014; Xu et al., 2018; Li et al., 2020). However, this approach has the potential that an unusually scattered minimum value can distort the PLI values. The MP concentration (154 items kg^{-1}) at 5% cumulative probability was used to avoid this. The advantage is that this value is determined by the entire probability distribution and hence not significantly affected by a single point value. The PLI value of MPs pollution was categorized according to Wang et al. (2021). It provides a clear extent of MP risk and raises concerns about managing MP contamination (Xu et al., 2018).

Control of Contamination

In this study, all the cautious steps were taken to avoid possible contamination. While working with a toxic hydrogen peroxide mixed solution, special care was taken, and all the reaction was carried out under a fume hood. Precautions were also taken to prevent cross-contamination, predominantly with aerial contaminants and synthetic fibers from clothes. All equipment was washed with distilled deionized water before and after use. Moreover, working surfaces were continuously wiped with distilled H_2O and 70% alcohol. Samples were kept retained, wrapping with aluminum foil. Two blank-control samples were run using the above procedure: one with pure sand and concentrated $ZnCl_2$ solution and the other with concentrated $ZnCl_2$ solution (Yu et al., 2016). The blank samples contained no plastics. As we sieved the sand salt ($ZnCl_2$) solution through 0.3 mm mesh, the size range of particles used in this study was between 0.3 and 5 mm.

Statistical Analysis

Significant variations in the mean abundance of MPs among the sites were analyzed using one-way ANOVA followed by pairwise comparisons using Tukey's HSD test. In all the cases, homogeneity of variances was tested with Levene's test, and the data were transformed using square root or logarithm when needed. The granulometric data were analyzed on normalized data using Ward's method. Statistical analyses were conducted using the computer package, PAST (PAleontological STatistics), Version 4.03.

RESULTS AND DISCUSSION

Occurrence and Spatial Distribution of Microplastics

Microplastics were recognized in every sampling point of Kuakata Beach sediment with an overall mean value of 232 ± 52 items kg^{-1} dry sediment. In our investigation, the highest mean value of MPs was observed in the estuarine S8 sampling point (311 ± 11 items kg^{-1}), followed by S7 (279 ± 8 items kg^{-1}), S1 (270 ± 9 items kg^{-1}), S4 (246 ± 8 items kg^{-1}), S5 (221 ± 9 items kg^{-1}), S3 (191 ± 11 items kg^{-1}), S6 (182 ± 6 items kg^{-1}), and lastly in the lowest interrupted zone S2 (157 ± 5 items kg^{-1}) (**Figure 2**). One-way ANOVA showed that the abundance of MPs significantly varied (F = 123.8, df = 7, $p < < 0.001$) among the eight stations. Tukey's pairwise comparisons showed significant differences between almost all sites pair, except for S1 and S4 (p = 0.12), S1 and S7 (p = 0.94), S3 and S6 (p = 0.75). In addition, the cumulative probabilistic distributions of all the sample values were calculated (**Figure 3**). The 5% and 95% values from the distributions were 154 and 318 items kg^{-1}, respectively.

The higher abundance in S1, S7, and S8 sampling points indicated that MPs load in the estuarine beach might be attributed to extensive river discharge along with tourism activities (Zhang et al., 2019). Tourist activity would yield an incredible amount of plastic waste, leading to MP pollution on the beach. It was proposed that wind-driven oceanic circulation could also influence MP accumulation at beaches with higher concentrations (Vianello et al., 2013). On the other hand, the S2 sampling point was a remote area for the tourists, and therefore the abundance of MPs was recorded lowest at S2. Previous studies indicated that MP contamination was significantly positively correlated with population density, river discharge, and the spread of industrial zones (Fetner and Miller, 2021; Zhu et al., 2021). However, present outcomes were compared with other countries that used almost similar extraction and detection methods, as well as the quantification unit (**Table 1**). The mean abundance of MPs in this study site was found to be higher than those in Small Island, Fuji (Ferreira et al., 2020), Brest Bay, France (Frère et al., 2017), and some other beaches (Graca et al., 2017). Our results depicted the MPs pollution in the surface sediments of the Kuakata beach was lower than the sediments from Chennai, India (Sathish et al., 2019), Lido di Dante, Italy

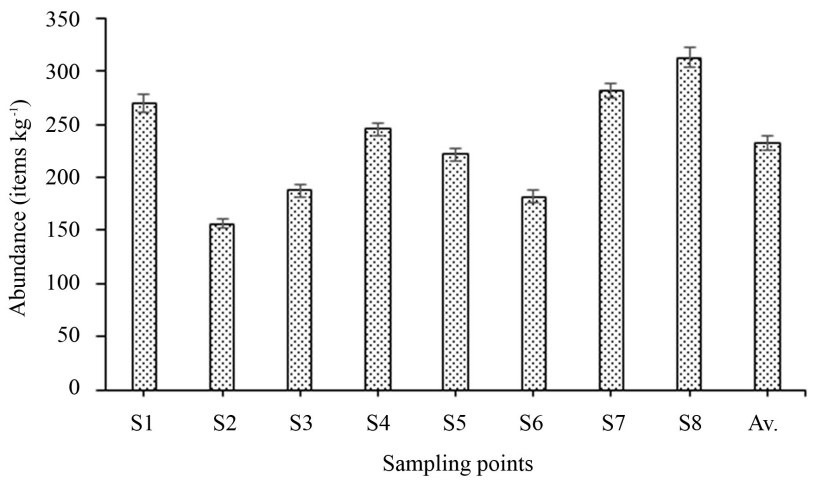

FIGURE 2 | The abundance of microplastics (MPs) in different sampling points.

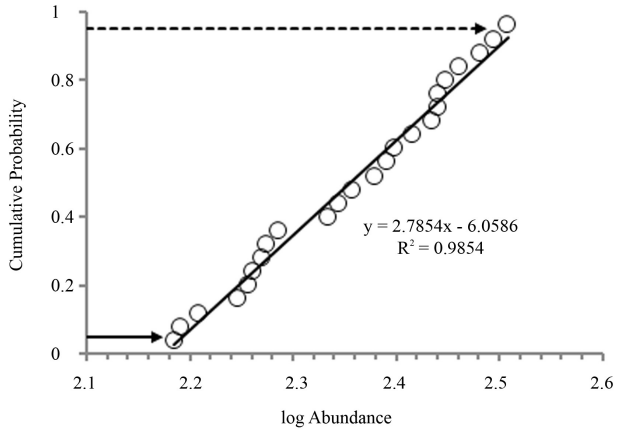

FIGURE 3 | Cumulative probabilistic distributions of all the sample values indicating 5 and 95% values.

(Lots et al., 2017), Bohai sea, China (Zhu et al., 2021), and Da Nang, Vietnam (Nguyen et al., 2020).

Characteristics of Microplastics

The investigation on characteristics of MPs in this experiment exposed the types and shapes, color, and size of MPs presented in the Kuakata beach sediment. A total of six different types of MPs were noticed in the examined sediment, namely microbeads, sheets, foams, films, fibers, and fragments (**Figure 4A**). Among these, fibers were predominant in every sampling point. They contributed about 55% of total MPs with an average of 123 ± 27 items kg^{-1}, followed by 15% fragments (35 ± 20 items kg^{-1}), 14% films (30 ± 11 items kg^{-1}), 10% microbeads (28 ± 30 items kg^{-1}), 5% sheets (12 ± 9 items kg^{-1}), and 2% foams (4 ± 6 items kg^{-1}), respectively. Fibers, fragments, films, and microbeads were documented at all sampling points, and most of the fragments and films (98%) were irregular in shape. In addition, a little elongated, rectangular, and cylindrical-shaped particle were also visualized. While comparing with other studies conducted worldwide, fibers were found to contribute a significant portion of most beach sediment (Graca et al., 2017; Sathish et al., 2019; Nguyen et al., 2020), showing a similar pattern to our present findings (**Table 1**). Among the MPs types, fibers were also observed to be dominant in the surface sediment from Belgium, Singapore, Slovenia, and South Africa (Nor and Obbard, 2014; Nel and Froneman, 2015). However, a larger amount of fiber found in the beach sediment of Kuakata, Bangladesh, may be originated from clothing materials and industrial fabrics, domestic laundry effluents through river discharge, fishing activity using nets and ropes in the Bay of Bengal. Besides, food and beverage packaging plastics disposed of by the tourists and locals might have caused the presence of MPs in the study area.

Most of the MPs were found colored (60%) in the sediment of Kuakata, whereas 40% were transparent. The highest number of MPs were purple (27%) in color with an average of 62 ± 24 items kg^{-1}, followed by red (12%; 29 ± 14 items kg^{-1}), blue (10%;

TABLE 1 | A summary of microplastic pollution in different coasts and beaches worldwide.

Country	Location	Sample type	Mean abundance (item kg^{-1})	Dominant type	Extraction method	Detection method	References
Bangladesh	Kuakata beach	Beach sediment	232.1 ± 52.43	Fibers (55%)	A: $ZnCl_2$+NaCl B. H_2O_2+Fe (II)	Stereomicroscope, FTIR	Present study
China	Bohai Sea	Subtidal sediment	458.6 ± 150.0	Fibers (77.1%)	A: NaI+NaCl B: H_2O_2	FTIR	Zhu et al., 2021
Fiji	Small Island	Surface sediment	19.8 ± 4.2	Fibers (60.2 ± 6.9%)	A: NaCl+KO_3P B: H_2O_2 + Fe (II)	Microscope, ATR-FTIR	Ferreira et al., 2020
France	Brest Bay	Surface sediment	0.97 ± 2.08	-	A: NaCl+Na_2WO_4	Dissecting microscope, micro-Raman spectroscopy	Frère et al., 2017
India	Chennai	Beach sediment	309 ± 184	Fibers (63%)	A: $ZnCl_2$ B: H_2O_2	Dissecting microscope, FTIR-ATR	Sathish et al., 2019
Italy	Lido di Dante	Beach sediment	1512 ± 187	Fibers (98.7%)	A: NaCl	Stereo-microscope, Raman spectrometry	Lots et al., 2017
Poland	Southern Baltic Sea	Beach sediment	39 ± 10	Fibers	A: NaCl	Stereomicroscope, μ-FTIR	Graca et al., 2017
Singapore	Coast	Mangrove sediment	36.8 ± 23.6	Fibers (72.0%)	A: NaCl B: Tween-80	FTIR-ATR	Nor and Obbard, 2014
Slovenia	Coastal beach	Beach sediment	133.3	Fibers (75%)	A: NaCl	Stereomicroscope	Laglbauer et al., 2014
Vietnam	Da Nang	Beach sediment	9238 ± 2097	Fibers (81.9%)	A: NaCl B: H_2O_2	Stereomicroscope, Raman spectroscopy	Nguyen et al., 2020

A: chemical used in flotation method; B: chemical used in organic removal method.

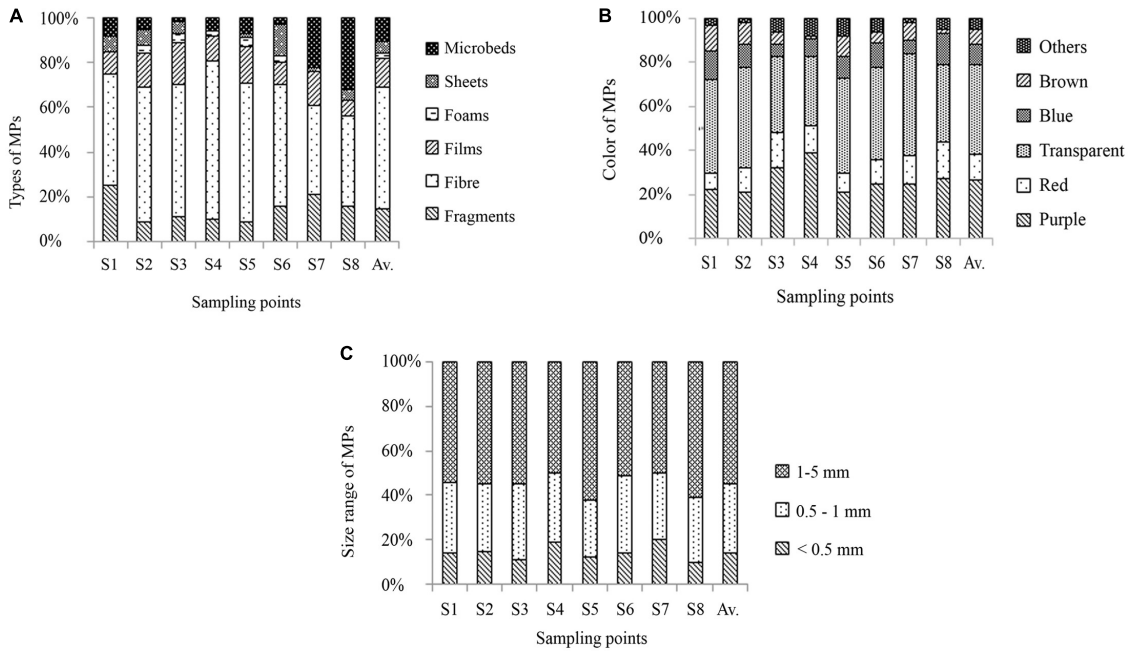

FIGURE 4 | Characteristics of microplastic, **(A)** types; **(B)** color; and **(C)** size.

23 ± 13 items kg^{-1}), brown (6%; 15 ± 11 items kg^{-1}), and others (5%; 12 ± 11 items kg^{-1}). The mean abundance of transparent MPs was 92 ± 25 items kg^{-1}. Colorful MPs were distributed on every sampling point of Kuakata Beach and highest (68%) in site S4 (**Figure 4B**), which might be degraded mainly from plastic food and beverage package. MPs' color may be attributed to their various sources (Patterson et al., 2019). However, the finding of our study was consistent with some other studies conducted worldwide (Peng et al., 2017; Robin et al., 2019; Yuan et al., 2019), who reported that most MPs were colored particles in the sediment. As bleaching processes happen in the marine ecosystem, it is difficult to comment on MPs' color (Stolte et al., 2015). Nevertheless, the color of the MPs is a significant factor as the marine organisms prey on colored MPs for resembling their prey, and the results have already been documented in many parts of the world (Ory et al., 2018; Botterell et al., 2019; Hossain et al., 2019, 2020; Hoellein et al., 2021; Liu et al., 2021; Muller, 2021; Wootton et al., 2021).

Microplastics ranging from 0.3 to 5 mm were the primary concern in this investigation. The observed MPs were thus categorized into three distinct groups among which size range between 1 and 5 mm comprised almost half (55%) of total MPs with an average of 127 ± 34 items kg^{-1}, followed by $0.5 - 1$ mm (31%; 72 ± 19 items kg^{-1}), and <0.5 mm (14%; 34 ± 15 items kg^{-1}) (**Figure 4C**). Most fibers were predominantly documented in a size range of 1–5 mm. Though our findings were similar to some authors (Zhang et al., 2016; Sagawa et al., 2018), many others disagree with these (Nor and Obbard, 2014; Klein et al., 2015; Lots et al., 2017; Peng et al., 2017; Urban-Malinga et al., 2020; Wang S. et al., 2020) who reported that MPs < 1 mm were more abundant. It was proven that MPs in sediment are accredited from large plastic particles and domestic laundry wash effluent (Yu et al., 2018). Approximately 6,000,000 microfibers ranging from 20 to 2,000 mm can be released from each 5 kg of polyester fabrics washing effluent (De Falco et al., 2017), which degrade to MPs (<5 mm) due to water turbulence, wave action, and high UV from sunlight (Auta et al., 2017).

Correlation With Grain Size Distribution

Beach sediments of Kuakata were primarily composed of very fine sands (39%) and mud (35%), followed by fine sand (24%), medium sand (1.5%), and coarse sand [0.5 (**Figure 5**)]. However,

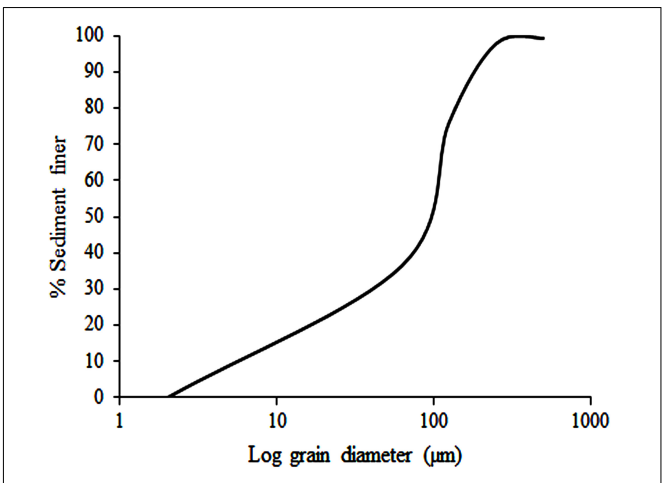

FIGURE 5 | Cumulative curve of sediment grain size of Kuakata Beach.

a positive correlation was found between the MPs abundance and the finer grain size distributions from each sampling point (p = 0.055; r = 0.7). McLachlan and Brown (2006) stated that beach sediments work as pollution traps that nonspecifically adsorb particles carried by tides and currents. It was proven that the finer the sediment is more effective to trap the particles. Besides, fine-grained sediments are typical for accumulation regions, therefore, may be susceptible to pollution. In this primary investigation, we have noticed higher MPs incidence in finer sediment grain size, which reinforced our assumption that finer sediments act as pollution traps for MPs on beaches. Nevertheless, the absence of the relationship between MPs occurrence and finer sediment grain size was previously observed from tidal beaches (Browne et al., 2013; Mathalon and Hill, 2014; Urban-Malinga et al., 2020) as well as shallow coastal sediment (Alomar et al., 2016). Such findings in our study might be due to the position of beaches in the river estuary (Galachiap river and Andharmanik river), where deposition happens with substantial domestic and industrial input.

Composition and Sources of Microplastics

A total of 12 representative samples of a size range between 1 and 5 mm were extracted for FTIR analysis, and 11 were found as plastic polymer, while 1 remained unidentified. Our investigation elucidated three types of polymers, namely, PET, polyethylene (PE), and polypropylene (PP), in the sediment samples of Kuakata, Bangladesh. PET was found to be the most abundant polymer type, contributing 45.5% of the total samples identified through FTIR analysis, whereas PP was found to be least (18.2%). The FTIR spectra of these polymers are shown in **Figure 6**. Due to the aging, natural weathering, and degradation of MPs, some identical peaks of those plastic polymers were not

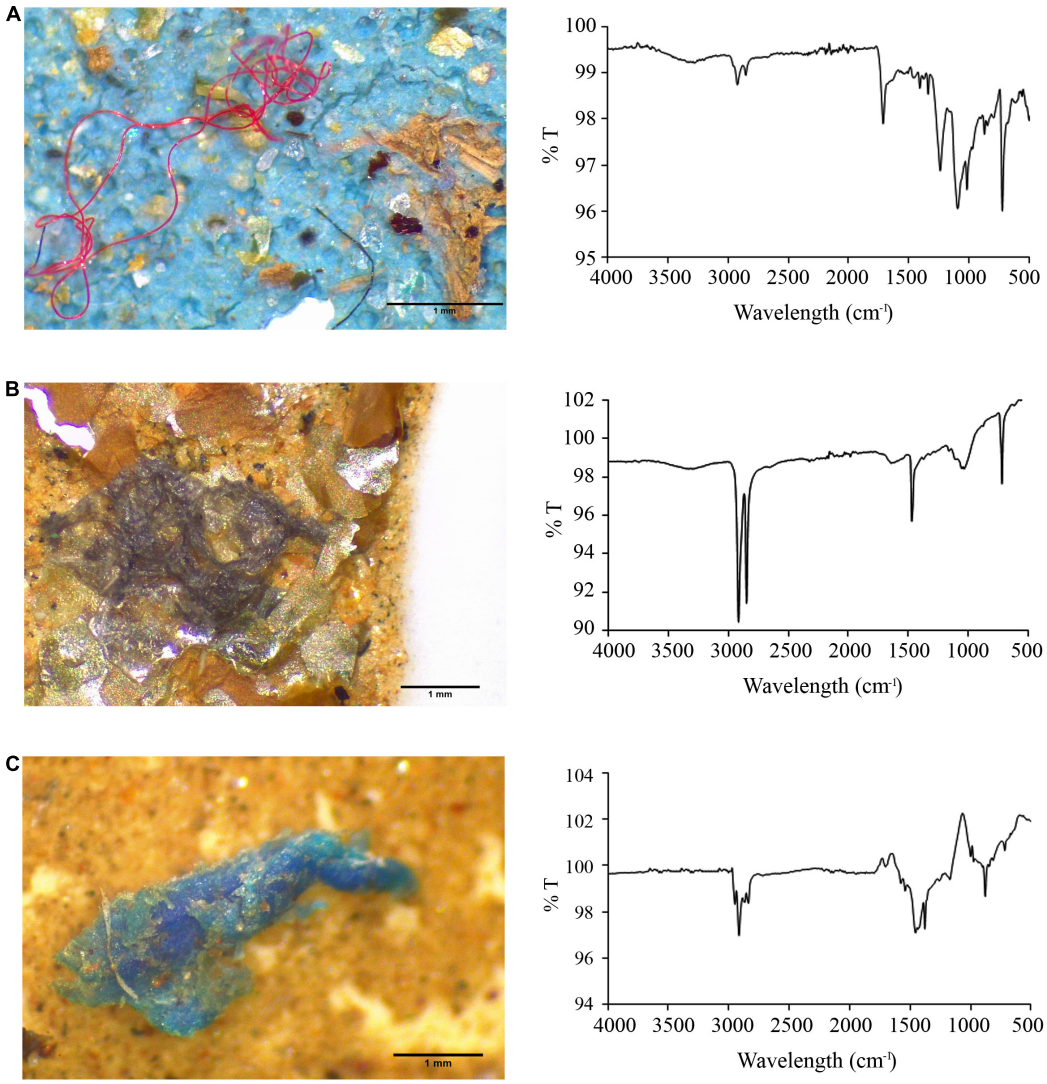

FIGURE 6 | FTIR spectrum of MPs in beach sediments of Kuakata, Bangladesh, **(A)** PET; **(B)** PE; **(C)** PP.

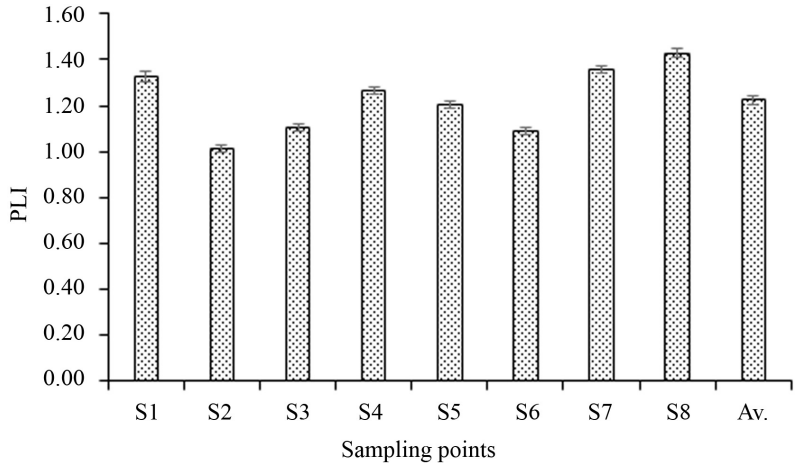

FIGURE 7 | PLI value of each sampling site in beach sediments of Kuakata, Bangladesh.

found in the FTIR spectrum (Wang et al., 2017; Sathish et al., 2019). However, these polymers were common MPs in the coastal ecosystems (Sathish et al., 2019; Godoy et al., 2020; Selvam et al., 2020; Wu et al., 2021; Zhu et al., 2021).

Recognized polymer type through FTIR analysis did not provide enough evidence to identify the exact sources of MPs origin (Claessens et al., 2011). We can only assume their potential sources based on the extensive discretion of our findings (Wang et al., 2019). The main source of MPs in the studied beach might be river discharge, surface runoff, and plastics deposited by tourists and locals. However, most of the fibers found in this study were PET and fragments were either PE or PP. The possible sources of PET and PP could be clothes and textile products as these polymers are extensively used industrially. PP is also used for packaging food, beverage, plastic containers, carpets, and pipes. PE is another type of polymer found in the studied area which is used widely as food-packaging film and containers for oil, shampoos, soap, etc. (Hossain et al., 2020; Wu et al., 2021).

Ecological Risk Assessment of Microplastics With Pollution Load Index

The PLI value of each sampling site (S1–S8) was calculated, and the findings were illustrated in **Figure 7**. The highest value of PLI was evaluated in the S8 sampling point (1.43 $\pm$ 0.02), followed by S7 (1.36 $\pm$ 0.02), S1 (1.33 $\pm$ 0.02), S4 (1.27 $\pm$ 0.01), S5 (1.20 $\pm$ 0.02), S3 (1.11 $\pm$ 0.02), S6 (1.09 $\pm$ 0.02), and S2 (1.01 $\pm$ 0.01). The PLI specified that the beach sediment of Kuakata belonged to the risk category I of the pollution index, indicating slightly polluted by the MPs. However, the results of one-way ANOVA showed that the values of PLI significantly varied ($F = 211.7$, $p < < 0.001$) among the eight stations.

The estuarine beach (i.e., S8, S7, and S1) possesses a higher PLI value due to massive river discharge from two main estuaries along with tourism activities (Zhang et al., 2019). Moreover, we mentioned earlier that the estuarine sediments of Kuakata mainly consist of fine sand, which acts as a more effective pollutant trapping agent. Tourist activity and beachside development programs would produce a significant number of plastic wastes which may dispose of on the beach. On the contrary, the S2 sampling point had a lower PLI value which might be due to the lower touristic and beach developmental activities. However, according to these findings, it can be concluded that the PLI value can assess the degree of MP contamination in an area, but it is not possible to calculate the precise MP concentration by the PLI values (Wang et al., 2021). The toxic effect of MPs is primarily associated with the hazard scores; therefore, the value of PLI is not a practical endpoint of health risk assessment. Besides, the evaluation of health risks allied with MPs exposure is quite deficient. MPs might be ingested through drink or food and inhaled (Rist et al., 2018; Cox et al., 2019; Vianello et al., 2019). Hence, advanced investigation regarding possible exposure pathways of MPs and their menace to humans is needed.

CONCLUSION

This investigation aimed to assess the occurrence and characteristics of MPs, for the first time, in beach sediments from Kuakata, Northern Bay of Bengal, Bangladesh. The results confirmed the presence of various types of MPs (fibers, microbeads, fragments, etc.) and polymer forms (PET, PE, and PP) in beach sediment samples with the highest density detected at sampling points near the estuary, which could be attributed to colossal river discharges along with tourism activities. The higher incidence of MPs was found in finer sediment grain sizes, which supports the assumption that finer sediments act on beaches as pollution traps for MPs. The abundance and nature of MPs indicate that these MPs are derived from land-based sources. PLI analyses showed the beach sediments of Kuakata were in category I of pollution index is slightly polluted. This finding can help inform improved management of local and regional plastic debris. More long-term and systematic studies on the impacts of MPs on marine life, habitats, and eventually on human health are recommended.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

PB contributed to sample collection, laboratory analysis, and draft writing. MH and JS contributed to conceptualization, design, writing, and supervision. A-AN contributed to data analysis and draft writing. TC and SL contributed to FTIR sample processing and analysis. JY contributed to writing and editing. MN contributed to formatting, writing, and editing. All authors contributed to the article and approved the submitted version.

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Sources and Leakages of Microplastics in Cruise Ship Wastewater

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To date, the contribution of sea-based sources to the global marine litter and plastic pollution problem remains poorly understood. Cruise ships produce large amounts of wastewater and concentrate their activities in fragile and ecologically valuable areas. This paper explores for the first time the sources of microplastics in cruise ship wastewater, as well as their pathways from source to sea. It thereto uses a novel approach for the identification of sources and pathways, based on scientific literature on microplastic sources and pathways, literature on cruise operations and wastewater management as well as a questionnaire among cruise lines. The study highlights personal care and cosmetic products, cleaning and maintenance products and synthetic microfibers released from textiles in laundry as relevant source categories. Untreated grey water and the overboard discharge of biosludge, resulting from the treatment of sewage and grey water, were identified as key pathways. Cruise lines can reduce microplastic emissions by adapting their purchasing policies for personal care, cosmetic, cleaning and maintenance products and professional textiles. In addition, the holistic management of all wastewater streams and resulting waste products is essential to prevent leakages of microplastics from cruise ships to vulnerable coastal and marine ecosystems. Furthermore, the approach can be used to guide company-level assessments and can be modified to address microplastic leakages in other maritime sectors.

Keywords: cruise ships, microplastics, wastewater, sea-based sources, marine litter, plastic pollution, marine pollution, shipping

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1 INTRODUCTION

Marine litter is a problem of emerging concern and research efforts as well as initiatives to address the problem are developing rapidly (UNEP, 2021). Recently, a breakthrough was achieved at the United Nations Environments Assembly (UNEA-5.2), where 175 nations committed to forge an international legally binding agreement to end plastic pollution by 2024, addressing the full lifecycle of plastic from source to sea[1]. Marine litter is defined as "any persistent, manufactured or processed

[1] https://news.un.org/en/story/2022/03/1113142

solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2021). While the term embraces different types of materials, plastics constitute the largest proportion (Galgani et al., 2015). Jambeck et al. (2015) estimated that in 2010, 4.8 to 12.7 million MT of plastics entered the ocean, and inputs are expected to increase over the coming decades (Lebreton and Andrady, 2019). Plastic can travel long distances and is found in all parts of the marine ecosystem, even in very remote locations such as in Arctic sea ice (Obbard et al., 2014) and the Mariana Trench (Chiba et al., 2018). Microplastics (MPs) are small pieces of plastic, with a size smaller than 5 mm. MPs comprise both manufactured microscopic plastic particles (primary MPs), such as microbeads with applications in the cosmetic industry and industrial pellets used for the production of plastics, and particles that result from the abrasion and degradation of larger items (secondary MPs) (Cole et al., 2011). MPs in the marine environment can be ingested or inhaled through the gills by a wide range of organisms (Wright et al., 2013; GESAMP, 2016; Hantoro et al., 2019). Once ingested, MPs may block or damage intestinal tracts (Cole et al., 2011; Wright et al., 2013). They can also be absorbed through the gut walls (Foley et al., 2018). In addition, MPs may leach toxic pollutants, including chemicals that are intentionally added during plastic production as well as organic contaminants and heavy metals that sorb to the MP surface (Teuten et al., 2009; Rochman et al., 2014). Impacts that have been associated with MP ingestion in marine biota include adverse effects on feeding (e.g. Wegner et al., 2012), growth (e.g. Au et al., 2015), reproduction (e.g. Della Torre et al., 2014) and survival (e.g. Luís et al., 2015). Besides the effects at the individual level, MPs as well as pollutants absorbed by MPs, can be transferred through food webs (Farrell and Nelson, 2013; Setälä et al., 2014) and induce ecological impacts (Rochman et al., 2016). Human health may also be affected by MPs in the marine environment through the consumption of contaminated seafood (Hantoro et al., 2019; Campanale et al., 2020).

In order to effectively address marine litter and (micro-)plastics, it is necessary to understand the contribution of individual sources and the pathways from these sources to the environment. Assessing the origin of MPs in the environment is complicated (Hardesty et al., 2017) and the relative contribution of different sources and pathways is strongly dependent on local conditions (Duis and Coors, 2016). While it is generally assumed that most marine litter derives from land-based sources, the contribution from sea-based source varies strongly by geographic location and could be substantial for specific locations (GESAMP, 2021). Knowledge about sea-based sources is still little developed compared to land-based sources; the GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) Working Group on sea-based sources of marine litter concluded that knowledge of the type, quantity and impact of sea-based sources is lacking (GESAMP, 2021), thus hindering the development of effective mitigation strategies. Ship-based sources contribute to MP pollution, e.g. through paints and coatings, abrasives used for the cleaning of ship hulls during maintenance, loss of cargo (e.g. plastic pellets) and discharges of wastewater (Boucher and Friot, 2017; Bray, 2019; GESAMP, 2021). In terms of wastewater, cruise ships would be of particular interest because of the large quantities of wastewater that are generated on board these ships (GESAMP, 2021). Vicente-Cera et al. (2019a) estimate that the world cruise fleet produced about 34.000.000 m^3 of wastewater in 2017; a production rate that is comparable to that of the country Cyprus[2].

Until the start of the COVID-19 pandemic, the cruise industry had shown a constant growth, from 17.8 million passengers in 2009 to 29.7 million passengers in 2019[3]: an increase of 75% in 10 years. The pandemic led to a complete halt of operations; however, the industry expects a full recovery compared to 2019 levels by 2023 and a growth of 12% by 2026[4]. Currently, the largest cruise ship in operation can carry up to 6988 passengers and 2300 crew members[5]. Besides a means of transportation and accomodation, cruise ships typically provide a wide array of onboard services and attractions to their passengers, such as swimming pools, spas, theatres and sports facilities. The main mainstream cruise destinations are located the Caribbean, the Mediterranean and Northwestern Europe; specialty "adventure" types of cruises attend extremely remote and vulnerable environments (Lamers et al., 2015) such as the Arctic and Antarctic. Around 70% of the cruise destinations are located in biodiversity hotspots (Lamers et al., 2015) and cruise ships frequently pass through fragile coastal and shallow areas as well as marine protected areas, especially when entering or leaving ports (Lloret et al., 2021). Caric et al. (2019) highlight that in the Mediterranean, cruise ships frequently anchor in close proximity of many marine protected areas (MPAs) and the heavily trafficked cruise port of Venice is even located within such a site. Considering that cruise activities typically concentrate in certain coastal areas and routes, these vulnerable areas are exposed to cumulative environmental impacts of these activities (Toneatti et al., 2020). With increasing cruise intensity, the impacts of the industry, including MP pollution, are likely to increase in the coastal and marine environment.

This study aims to highlight characteristics of the cruise sector that affect the potential for MPs being found in wastewater discharges, and provide recommendations to guide and set-up future research efforts as well as indicate general directions for mitigation. It thereto uses a novel approach for the identification of these sources and pathways, based on scientific literature on MP sources and pathways, literature on cruise operations and wastewater management as well as a questionnaire among cruise lines. First, an inventory was made of sources of MPs in the marine environment, based on general scientific literature. From this general inventory those sources were selected that are relevant to cruise operations and additional source categories were identified based on the characteristics of cruise operations and facilities. Subsequently, the identified sources were linked to the different wastewater streams and finally the management of each of these wastewater streams was evaluated.

[2] https://www.fao.org/aquastat/en/databases/maindatabase/

[3] https://www.statista.com/topics/1004/cruise-industry/#dossierKeyfigures

[4] https://cruising.org/en-gb/news-and-research/research/2022/january/state-of-the-cruise-industry-outlook-2022

[5] https://www.royalcaribbeanpresscenter.com/fact-sheet/34/wonder-of-the-seas/).

2 MATERIALS AND METHODS

Figure 1 presents an overview of the methodology for the identification of sources and pathways of MPs in cruise ship wastewater (detailed descriptions of the steps are described in the following paragraphs). Here, the term "sources" refers to the different applications of plastics and synthetic polymers on board cruise ships that have the potential to release. MPs to the marine environment. Through different release mechanisms, MPs find their way to the wastewater streams. Pathways are defined as the routes through which MP particles are transported to the marine environment, where the scope of this research is restricted to pathways through cruise ship wastewater discharges.

2.1 Literature Review of Microplastic Sources

Since cruise ships are often characterized as "floating cities", it was reasoned that MP sources on cruise ships have significant overlap with land-based urban sources of MPs. In addition, the maritime operations as well as any aspects that are unique to the cruise industry should be addressed. To identify and characterize sources of MPs, the research is based on the approach that was applied in different European countries, the European Union and the OSPAR region, as reported by Sundt et al. (2014); Lassen et al. (2015); Essel et al. (2015); Magnusson et al. (2016); Scudo et al. (2017); Verschoor et al. (2017) and Hann et al. (2018). These studies estimate MP emissions at a local or regional scale, based on the sources and pathways of MPs reported in general literature in combination with local data on plastic uses and other relevant local factors. Lassen et al. (2015) define eight categories of primary MP sources and six categories of secondary MP sources, and identified the pathways from these sources to surface waters. This structure was adopted and the list was complemented with the results of other studies, reflecting all reported land-based and sea-based MP sources and pathways at national or regional level. Next, sources were selected that could be relevant for cruise ship wastewater during normal operations.

2.2 Cruise-Specific Functionalities

In order to cover all sources of MPs that are specific to the cruise industry, the following overarching types of MP sources were considered, representing different functionalities of cruise ships: *cruise ship facilities, ship stores* and *people*. Cruise ship facilities were further divided into *hotel facilities* and *ship facilities*, in accordance with the structure proposed by Lois et al. (2004). The proposed facilities were supplemented by consulting Vogel et al. (2012) and Gibson and Parkman (2019), as well as by studying the deck plans of the ten largest cruise ships in the world, in order to cover the main facilities that are present on modern cruise ships. *Stores* comprise the different purchasing streams of cruise ships: fuel, corporate, technical and hotel purchasing (Véronneau and Roy, 2009). Finally, personal belongings of passengers and crew may act as MP sources; these are covered by the category "*people*".

2.3 Inventory of Microplastic Sources

Following the identification of the main MP source categories on board cruise ships, the inventory as derived from the literature study was further developed and supplemented to cover those categories that have relevance to cruise ships. This was done by crosschecking the identified categories as derived from literature on the one hand and the identified facilities, stores and people categories from the previous step on the other. This approach resulted in the elimination of some of the MP sources that were identified in the previous step, because of differences in the characteristics of these sources on board cruise ships compared to the general characteristics that are described in literature. On the other hand, cruise-specific sources were added to the general inventory. The contribution of specific facilities and stores to MP pollution is not always straightforward and requires a thorough understanding of operations, facilities and the types of stores. The details of many specific cruise operations are not extensively reported in literature, and only to a limited extent in grey literature. Therefore, in order to assess the relevance of the different facilities and stores, Google searches were used to

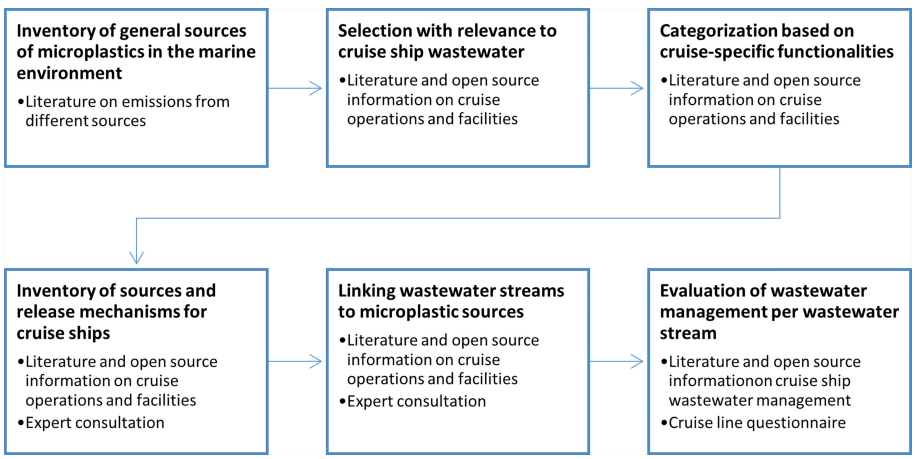

FIGURE1 | Research steps in the identification of sources and pathways of microplastics in cruise ship wastewater.

identify open access online resources, such as deck plans and pictures of the 10 largest cruise ships (e.g. to understand the application of artificial grass and the organization of laundry facilities) as well as blogs and YouTube videos, concerning the specific cruise ship operations and facilities such as laundry installations and engine room operations. In addition, experts were consulted to verify the findings (see below).

2.4 Linking Wastewater Streams to Microplastic Sources

In order to establish links between the sources on the one hand and wastewater streams on the other, the different sub-streams of the wastewater streams were identified based on literature. Then, the pathways from the identified sources to the different wastewater streams were assessed, by crosschecking each of the sources to the identified wastewater streams and vice versa.

2.5 Wastewater Management

The objective of this step was to map the main routes of the different wastewater streams and the key characteristics of treatment processes, where applicable, in order to identify potential pathways of MPs from the different wastewater streams. In addition, the characteristics of common treatment technologies were described. The assessment is based on scientific literature as well as grey literature. In order to verify the findings based on the grey literature, experts were consulted and a questionnaire was distributed among cruise lines.

2.6 Expert Consultation and Questionnaire

A preliminary version of the inventory of sources and pathways of MPs on board cruise ships was reviewed by experts in the fields of marine litter (3 experts) and MPs in onshore wastewater (1 expert). The typical practices and systems for wastewater management on board cruise ships were discussed with two experts in the field of maritime wastewater management and one cruise industry representative. In addition, a questionnaire was developed and distributed among cruise lines to verify the preliminary findings and collect additional industry-specific information. The questionnaire was distributed in February 2020 to the environmental managers of different cruise lines through the Cruise Lines International Association (CLIA). It consisted of a general section, where respondents could indicate the fleet size, a general wastewater management section and sections related to different wastewater treatment technologies. The final section concerned the measures and policies addressing MPs in wastewater.

2.7 Analysis and Interpretation of Results

This research involved different types of information and data, from different fields of research as well as use of a questionnaire and expert interviews. In order to organize these data, the research was structured around the existing frameworks from literature for the inventory of general MP sources as well as cruise facilities. In addition, the identified wastewater streams and related wastewater management practices were described in tabular form. These frameworks were then combined into matrices in order to structure the available information and to ensure that all relevant topics were covered through crosschecking. This structure guided the more detailed part of the research, and in particular the identification of cruise-specific MP sources. Where scientific literature was lacking, secondary resources were considered.

3 RESULTS

3.1 Literature Review of Microplastic Sources

Tables 1, **2** present the overview of main source groups of primary and secondary MPs in the marine environment, modified from Lassen et al. (2015), and extended with the results from other studies (indicated in the table, where applicable). The column on the right indicates whether the listed source groups were considered relevant for cruise ship wastewater. MP sources that were not considered relevant include raw materials for plastic production, industrial and professional handling processes of plastics, emissions from road traffic (tires, brake pads, bitumen and road paint), agricultural, aquaculture and oil and gas applications, typical onshore waste management issues (illegal waste burning, landfills and dumps), as well as the fragmentation of macroplastics in the environment due to natural processes. Also, the blasting of the ship hull during large scale maintenance with plastic abrasives is not further considered as blasting is not part of normal ship operations. Furthermore, Lassen et al. (2015) includes a separate category of primary MP emissions from paints through the washing of brushes. This source group was not considered applicable to cruise ships since this is mainly relevant for "do it yourself" and not for industrial practices (Verschoor et al., 2016). The category other includes plastic beads used in professional dish washing machines, plastic beads and ironing beads used by children, printer toner, specialty chemicals in wastewater treatment facilities (Scudo et al., 2017) and oil and gas industry (Sundt et al., 2014).

3.2 Cruise-Specific Functionalities

3.2.1 Facilities

Tables 3 and **4** give an overview of the typical hotel facilities and ship facilities as present on contemporary cruise ships, based on Lois et al. (2004). The overview is not exhaustive and may not be representative for all cruise ships but is indicative of the main systems and facilities present on ships, with the purpose to identify potential sources of MPs throughout the vessel.

3.2.2 Stores

Cruise ships carry stores of various types. Such stores include fuel and ship maintenance products for ship operations as well as food, potable water and detergents for hotel operations. Véronneau and Roy (2009) distinguish the following main purchasing streams of cruise ships: fuel, corporate, technical and hotel purchasing. Fuel purchasing covers fuel and other petroleum products for daily consumption, such as lubricants. Corporate items relate to office related materials such as office supplies and computers. Technical items include items for facility and ship maintenance, e.g. engine parts, electronic components and carpeting materials. Consumable items and food required for hotel operations fall under the category

TABLE 1 | Generic primary MP sources, modified from (Lassen et al., 2015) and indicating relevance to cruise ships.

Source group	Relevance
Raw materials for production of plastic items (plastic pellets)	No
Plastic particles used for cosmetics	Yes
Plastic particles in abrasive media	No
Plastic particles in cleaning and maintenance products	Yes
Plastic particles used in paints	Yes
Rubber granules and powder from recycling of tires	Yes
Expanded Polystyrene beads used for other applications than plastics production	Yes
Plastic particles used in medical and dentist products and in research	Yes
Plastic particles used in other applications	Yes

TABLE 2 | Generic secondary MP sources, modified from (Lassen et al., 2015) with descriptions and relevance to cruise ships..

Release mechanism	Source group	Relevance
Industrial activities	Plastic items	No
Particles released from plastic items during use	Plastic items used indoors and outdoors	Yes
	Textiles	Yes
		Yes
	Tires	No
	Automotive brake dust	No
	Polymer modified bitumen	No
	Artificial turfs	Yes
	Plastic film used in agriculture	No
	Plastic in fishing and aquaculture gear	No
Particles released from painted surfaces	Paint for indoor and outdoor applications	No
	Paint for marine applications	Yes
	Road paint	No
Waste handling	Plastic waste and waste contaminated with plastics	No
		Yes
		Yes
		No
	Illegal waste burning	No
	Landfills and waste dumps	No
Fires	Fires	No
Fragmentation of plastic waste in the environment	Terrestrial waste handling	No
	Maritime waste handling	Yes

of hotel purchasing. Furthermore, fresh water is a key resource on board.

3.2.3 People

Passengers and crew bring their personal belongings in their luggage. Significant categories are likely to include personal clothing, shoes, flipflops, personal toiletries and medication, electronics, books, suitcases and backpacks and snacks. People with children may bring plastic and inflatable toys. Furthermore, souvenirs bought ashore are brought on board after port visits.

3.3 Inventory of Sources and Release Mechanisms

Overviews of key MP sources and release mechanisms of both primary and secondary MPs on board cruise ships are displayed in **Tables 5** and **6**. The categories from Lassen et al. (2015) were revised to reflect both the general categories as found in literature as well as the relevance of these categories for cruise operations.

The main source groups for primary MPs (**Table 5**) are personal care & cosmetics, cleaning & maintenance and medical & pharmaceutical. Potential release mechanisms are mainly related to the use of products in "wet" applications, e.g. rinse-off bath and shower products, spa treatments, wet cleaning, dish washing, laundry and wastewater treatment. Other release mechanisms include medication use, medical and dental treatments, printing and damage of user products that contain primary MPs, e.g. polystyrene pellets or beads. In addition, certain shipboard wastewater treatment systems use flocculants (EPA, 2011; Chen et al., 2022), which could be polymer-based. The detailed assessment of cruise ship facilities led to the exclusion of rubber granules from artificial turfs as a source of primary MPs: no examples could be found of high impact sport facilities on board cruise ships that would require "third generation turfs" using a performance infill of (synthetic) rubber granules for shock absorption (Hann et al., 2018).

The identified release mechanisms for secondary MPs (**Table 6**) include the wear and damage of products during normal use, laundry and cleaning of textiles, wear and damage of painted surfaces, waste handling and littering. Sources embrace all plastic and synthetic items and surfaces on board the vessel, including paints and waste.

3.4 Linking Wastewater Streams to Microplastic Sources

The main wastewater streams that are produced on board cruise ships are sewage, grey water and oily bilge water. Sewage is the wastewater from toilets and primarily consists of human body wastes and water and may on some ships be mixed with wastes from medical facility sinks and drains (EPA, 2008). The International Convention for the Prevention of Pollution from Ships (MARPOL) covers the international regulations for sewage in Annex IV of the convention. According to these regulations, sewage may be discharged overboard without treatment outside coastal zones, provided that the ship maintains a minimum sailing speed of 4 knots. The average sewage generation rate is estimated at 68 l/person/day (Vicente-Cera et al., 2019a). Grey water consists of the wastewater streams from shower and bath, accommodation sinks, laundry, dishwashers and galleys (EPA, 2008). Wastewater from these sources is in practice often mixed with wastewater from other sources, such as drainage from drains and sinks in non-engine room spaces, food pulper effluents and wastewater from whirlpools (EPA, 2008). Unlike sewage, grey water discharges are not internationally regulated. Vicente-Cera et al. (2019a) estimate the average generation rate throughout the industry at 160 l/person/day. EPA (2008) defines oily bilge water as "the mixture of water, oily fluids, lubricants, cleaning fluids, and other similar wastes that accumulate in the lowest part of a vessel from a variety of different sources including engines (and other parts of the propulsion system), piping, and other mechanical and operational sources found throughout the machinery spaces of a vessel". International regulations, covered by MARPOL Annex I, allow discharges of oily bilge water at sea, provided that approved oil filtering

TABLE 3 | Hotel facilities on board cruise ships [adapted from Lois et al. (2004)].

Passenger facilities	Crew facilities	Entertainment facilities	Service facilities	Others
Staterooms/cabins	Cabins	Pools	Receptions and information desks	Medical center
Stairways and halls	Messes and bars	Whirlpools/solarium	Conference rooms	Shops
Public elevators	Recreation and internet areas	Spas	Offices	Beauty salon
Public areas (indoor)	Gym	Slides, flow-riders	Restaurants and bars	Nursery
Public areas (outdoor)	Galleys	Gym	Galleys, food preparation areas	Photo shop
	Launderettes	Sport fields, running tracks	Food storehouses	Print shop
	Store	Casino	Service elevators	Internet
	Crew office	Theaters	Bell box (room service)	Self-service launderettes
	Stairs and corridors	Cinemas	Housekeeping facilities	
		Nightclub	Laundry and dry-cleaning	
		Games area		
		Kids recreation areas		
		Mini golf		
		Climbing wall		
		Ice rink		
		Walkways and ziplines		
		Bowling		

TABLE 4 | Ship facilities on board cruise ships [adapted from Lois et al. (2004)].

Comfort system	Machinery	Tanks	Navigation	Decks and gear	Safety
Electricity infrastructure	Main engines	Fuel and oil	Bridge	Mooring equipment	Lifeboats
HVAC	Generators	Fresh water	Navigation equipment	Anchoring equipment	Life rafts
Fresh water generation and distribution	Transmission	Wastewater		Gangways	Fire-fighting system
Waste management systems	Control room	Ballast tanks		Helideck	Detectors and alarms
Stores of technical parts, paints etc.	Propellers/pods	Bilges		Open deck spaces	Low-level lighting
	Steering gear	Sludge tanks			Life jackets

equipment is used. The oil residue from the filtering process is to be stored in dedicated oil sludge tanks and delivered to port reception facilities (PRF). Vicente-Cera et al. (2019a) estimate that the average industry generation rate is 23 l per nautical mile.

In order to link the different wastewater streams to MP sources, the identified wastewater streams were divided into different sub-streams, each reflecting potential entry routes of MPs into wastewater. The left-hand side of **Table 7** summarizes the main sub-streams of which the wastewater streams consist. On the right-hand side, the primary MP source categories (as listed in **Table 5**), as well as the typical types of secondary MPs of relevance to these (sub-)streams are listed.

TABLE 5 | Primary microplastic sources and release mechanisms with relevance to cruise ship wastewater.

Source group	Sources	Release mechanism
Personal care & cosmetics	Stores: soaps and disinfecting agents in dispensers for hand washing/cleansing	Application of product
	Stores: spa, salon and nursery specialty products	Application of product
	Stores: products for sale in onboard shops	Application of product
	Stores: complimentary products provided in passenger cabins and showers in public facilities (gym, spa, etc.)	Application of product
	People: products brought on board by passengers and crew	Application of product
Cleaning & maintenance	Stores: cleaning products for wet cleaning of floors and surfaces	Use of products
	Stores: cleaning products for cleaning toilets	Use of products
	Stores: laundry detergents	Use of products
	Stores: dishwashing detergents	Use of products
	Stores: professional hand soaps	Use of products
	Stores: detergents for wet cleaning of specialty equipment and products	Use of products
	Stores: machinery and equipment maintenance products	Use of products for machinery and equipment maintenance
Medical & pharmaceutical	Stores: medical stores	Medical and dental treatments
	People: personal medication	Medication use
Other	Facilities: expanded polystyrene pellets in products	Damage of products
	Stores: wastewater flocculants and similar plastic-based products	Use of products
	Stores: printer toners	Spill of printer toner, printing dust
	Stores/people: beads	Loss of beads during use

The results demonstrate that the MP sources attributed to the different wastewater streams vary significantly. The MP content in sewage derives from pharmaceuticals and detergents used for the cleaning of toilets as well as larger items that are disposed in toilets. The MP sources related to grey water include personal care and cosmetic products (PCCP), detergents used for cleaning, dishwashing and laundry, fibers from synthetic textiles and the secondary MPs that are removed by wet cleaning. Finally, the MP sources attributed to oily bilge water mainly relate to engine room operations, which may involve various products for the cleaning, maintenance and operation of machinery that contain primary MPs. In addition, the different sub-streams of oily bilge water collect solid waste and dust, including plastics and secondary MPs, on their way to the bilges.

3.5 Wastewater Management

3.5.1 Sewage and Grey Water

There exist two categories of treatment systems that are relevant to sewage and grey water. Older ships are typically fitted with sewage treatment plants (STP), generally referred to as Marine Sanitation Devices (MSD), dedicated to the treatment of sewage. On these ships, grey water is typically not treated (EPA, 2008). MSD must be approved by the flag state of the vessel and comply with local effluent standards, if available. EPA (2008) reports that conventional MSD on board cruise ships treat sewage through biological treatment and chlorination, while some systems combine maceration and chlorination. Advanced Wastewater Treatment Systems (AWTS) comprise a range of relatively new technologies for treating sewage more effectively than the older MSD. For these systems to function properly, the influent of sewage is typically not sufficient. Thereto, (part of) the grey water streams are also routed through the AWTS. The use of these systems is becoming the standard in the cruise industry (King County, 2007) and newbuilds are typically fitted with such systems (Nuka Research, 2019). From the 2021 Cruise Report Card, published by Friends of the Earth[6] and covering the 18 major cruise lines and 202 ships, it can be derived that 75% of the cruise ships have an AWTS. According to Vard (2018), most AWTS on board cruise ships are of the Membrane Bioreactor (MBR) type, utilizing an activated sludge process in combination with membrane filtration. Systems of the Moving Bed BioReactor (MBBR) type consist of a bioreactor filled with plastic beads, supporting bacterial growth, in combination with a Dissolved Air Flotation (DAF) unit (Huhta et al., 2007). No complete overview could be retrieved of systems that are in use throughout the industry. However, the Alaska Department of Environmental Conservation annually reports which large cruise ships operated in Alaskan waters and which type of treatment system is used on

[6]https://foe.org/cruise-report-card/

TABLE 6 | Secondary microplastic release mechanisms and sources with relevance to cruise ship wastewater.

Release mechanism	Source group	Sources
Abrasion and weathering during normal use of plastic and synthetic products and textiles	Plastic items	Hotel facilities: outdoor facilities, incl. public spaces, entertainment areas, catering areas; indoor facilities, incl. passenger, crew, entertainment and service facilities, galleys, shops, medical center Ship facilities: outdoor ship facilities, incl. outdoor deck spaces and equipment, safety equipment, ropes; indoor ship facilities, incl. machinery and comfort system areas, control room and bridge, safety system Stores: disposable plastic items (e.g. cups, straws, personal protective equipment) and packaging People: plastic products brought by crew and passengers (e.g. toys, footwear etc.)
	Textiles	Facilities: permanent textiles in hotel and ship facilities (e.g. carpets, curtains, furniture, etc.) Facilities: professional textiles (e.g. towels, sheets etc.) Stores: cleaning cloths People: personal textiles (e.g. clothing, towels, etc.)
Laundry and cleaning of textiles	Textiles	Hotel facilities: professional textiles (e.g. towels, sheets, crew uniforms etc.) People: personal textiles (e.g. clothing, towels, etc.)
Particles released from painted surfaces	Painted surfaces	Hotel facilities: outdoor hotel facilities, incl. sun decks, pool areas, catering areas; indoor hotel facilities, incl. accommodation, hallways, catering areas, entertainment Ship facilities: outdoor ship facilities, incl. decks, superstructures, safety devices, equipment; indoor ship facilities, incl. indoor vessel structure, tanks, machinery spaces, bridge
Dust from the abrasion of turfs and fields	Artificial turfs, sports fields, artificial grass, playgrounds	Facilities: artificial grass (e.g. mini golf) and shock absorbing floors used in e.g. running tracks, sports fields and playgrounds
Waste handling and littering	Solid waste handling and compacting	Stores: (food) packaging materials Stores: single-use plastic items Stores: printed materials People: personal plastic and paper waste
	Food pulper	Stores: (food) packaging materials Stores: single-use plastic items
	Waste incineration	Plastic waste from various sources (stores/people)
	Plastic litter	Stores: (food) packaging materials Stores: single-use plastic items Stores: printed materials People: personal plastic and paper waste

TABLE 7 | Linking cruise ship wastewater streams to pathways and microplastic sources.

Wastewater stream	Sub-streams	Primary MPs types	Secondary MPs types
Sewage	Toilet flushing	Cleaning & maintenance	Plastic waste & litter
	Medical wastewater	Medical	
Grey water	Accommodation	Personal care & cosmetics	Plastic waste & litter
		Cleaning & maintenance	Dust, particles and fibers
	Laundry	Cleaning & maintenance	Textile fibers
	Galley	Cleaning & maintenance	Plastic waste & litter
Oily bilge water	Wash water	Cleaning & maintenance	Plastic waste & litter
	Leaks from machinery		Dust, particles and fibers
	Engine room spills		
	Condensates		
	Deck drainage		

board these ships. **Table 8** provides an overview of the different systems that were used on board the ships that operated in Alaskan waters in 2019 (ADEC, 2019), and indicates the number of ships associated with each system. Further information about these systems was collected from the AWTS brand websites, as well as ship-specific implementations, and added to the table. It follows that 18 out of 24 ships had an MBR type of AWTS, and 14 of these were of the brand Hamworthy. Six vessels operated an MBBR type AWTS of which 5 were of the brand Scanship.

The MBR systems all involve a pre-treatment filtering of the influent to remove coarse solids and prevent blocking of the membranes. The treatment itself involves the biological oxidation through an activated sludge process and ultrafiltration through membranes, where concentrates are generally fed back to the bioreactors and filtered effluents are collected in a permeate tank. The MBBR influents also pass filters to remove coarse solids. In the reactor, biological matter is removed through aerobic biological oxidation, and consequently DAF units separate particulate matter. Finally, the effluents pass polishing filters. All systems utilize UV disinfection to remove pathogens. Where available, mesh sizes of screens and filters are included. Since MBR systems are based on ultrafiltration, the mesh of the membranes is very fine with pore sizes below 100 nm.

Both grey water and sewage could be discharged to the marine environment without treatment. This applies to grey water for ships which do not have AWTS and ships which route only certain grey water streams through AWTS. Furthermore, it is possible that treatment systems are switched off at open sea, resulting in discharges of raw sewage and grey water. In 2021, 25% of the cruise fleet had no AWTS in place and thus discharged untreated grey water to the marine environment. Since these would typically concern older, and smaller cruise ships, the percentage of total grey water discharged through this route is likely smaller and this is expected to decrease in the future due to the increased use of AWTS. The MARPOL Convention allows the discharge of untreated grey water and, under certain conditions, sewage outside coastal zones. So theoretically, treatment systems could be switched off when the ship is on open seas. An EPA survey of four cruise ships fitted with AWTS reports that all vessels operate the system on a continuous basis (EPA, 2006a; EPA, 2006b; EPA, 2006c; EPA, 2006d) and therefore do not discharge raw sewage. This is in line with the CLIA waste management policy, which prohibits the discharge of untreated sewage on board member cruise lines[9]. One of the ships in the EPA survey (EPA, 2006b) only routes the grey water from accommodations to the AWTS and discharges galley and laundry wastewater overboard without treatment, demonstrating that discharges of untreated grey water also occur on vessels with AWTS. AWTS and MSD filtering and treatment processes separate the wastewater into treated effluents and waste products. Sewage is typically high in solids, such as toilet paper and sanitary items, which is removed before sewage enters the treatment system, leaving screening solids of various sizes in the sieves and membranes. Another waste stream is the formation of biosludge. Biosludge or excess biomass consists of organic material as well as bacteria, resulting from the biological consumption of sewage (EPA, 2008) and contains over 95% water (Avellaneda et al., 2011). It is separated from the treated effluents by filtration (EPA, 2008) and therefore would contain any solids such as MPs that have entered the bioreactor.

Literature provides some information on the disposal of waste products from cruise ship sewage and grey water treatment. Disposal options are incineration on board, landing at PRF and discharge at open sea (EPA, 2008; Klein, 2009; Avellaneda et al., 2011). The relevant findings from an EPA survey of four cruise ships with AWTS (EPA, 2006a; EPA, 2006b; EPA, 2006c; EPA, 2006d) are shown in **Table 9**, together with the details of a case study cruise ship, representing an average-sized cruise ship operating in the Caribbean, as described by Kotrikla et al. (2021). From this table it follows that three out of five ships discharge biosludge overboard. One of these ships also discharges the screening solids from the laundry and accommodation wastewater treatment system overboard, whilst solids from sewage are collected and incinerated on board. These data are in line with Klein (2009) who reports the overboard discharge of waste biosludge by 15 out of 16 ships in Washington State waters, with dewatering and incineration of biosludge on board one ship. Experts interviewed as part of this research stated that delivery of biosludge to PRF is currently not a common method on a worldwide scale as adequate facilities are lacking. This is also outlined by Avellaneda et al. (2011) who raise

[7] https://www.wartsila.com/waw/waste-treatment/wastewater/membrane-bioreactors

[8] https://www.environmental-expert.com/products/membrane-bioreactors-mbr-245436

[9] https://cruising.org/en/-/media/Sustain/CLIA_EnvInnovations_FS2019%20FINAL

TABLE 8 | Overview of characteristics of AWTS systems and processes on cruise ships operating in Alaskan waters during 2019.

Brand	No. of ships	Type	Pre-treatment	Main treatment steps	Effluent management	References
Wärtsilä Hamworthy	14	MBR	Aeration of buffer tanks, mixing, screen presses (400 µm)	Two stages of aerated bioreactors, external membrane ultrafiltration (40 nm)	UV disinfection	Wärtsilä[7], EPA (2006d)
Scanship	5	MBBR	Coarse drum filters (0.5mm)	Aerated MBBR bioreactor with addition of defoaming agent, DAF with addition of anionic polymer and flocculant	Polishing screens (0.03 mm), UV disinfection	EPA (2006c)
Zenon ZeeWeed	2	MBR	Mixing, coarse screens	Aerated bioreactors, submerged membrane ultrafiltration	UV disinfection	EPA (2006b); Celebrity (2013)
Hydroxyl CleanSea	1	MBBR	Mixing, fine screen rotating drums	Aerated MBBR bioreactor (no additives), DAF	Tertiary filtration, UV disinfection	Celebrity (2013); Headworks International (2018)
Rochem Bio-filt	1	MBR	Mixing, addition of antifoam chemical, vibratory screen filter (104 µm)	Aerated bioreactors, external membrane ultrafiltration	UV disinfection	EPA (2006a)
Triton Water	1	MBR	Not specified	Aerated bioreactors, submerged membrane ultrafiltration (0.05 µm)	Not specified	Environmental XPERT[8]

the logistic challenges of dealing with the large amounts of biosludge from cruise ships in ports without fixed reception facilities, rendering this scenario unrealistic. The available data indicates that for screening solids, incineration or delivery at PRF is more common.

3.5.2 Oily Bilge Water

As international regulations prohibit the discharge of untreated bilge water, there are two main methods used for the disposal of oily bilge waters: storage on board and delivery to onshore facilities, and onboard treatment. The treatment of bilge water is aimed at separating the oily constituents and water, such that the treated bilge water can be discharged overboard and the oily constituents are retained on board in sludge tanks for delivery to shoreside facilities (EPA, 2011). The systems used for the treatment of oily bilge waters are generally referred to as Oily Water Separators (OWS). EPA (2011) reports that contemporary OWS are comprised of a series of different separation methods and that all of the OWS systems for bilge waters that are approved by the US Coast Guard are a combination of gravity-based separation and one or more forms of polishing treatment. Oil and other contaminants that are contained from the bilge water are collected in sludge tanks. This oily sludge may be stored on board for discharge at shore reception facilities or incineration on board. **Table 10** summarizes representative options for wastewater treatment and the discharge and disposal of the resulting effluents and waste products.

3.6 Cruise Line Questionnaire

Since the questionnaire was distributed almost simultaneous with the first infections of COVID-19 on board cruise ships, the response was minimal. One CLIA member company responded and completed the questionnaire. However, with a fleet size of over 15 vessels, the responding company can be considered an important player in the industry and generally representative.

All ships of this company have holding tanks and MSD or AWTS systems for the treatment of sewage and grey water, with most ships having AWTS. In the case of MSD, grey water is stored on board and discharged at a minimum distance of 12 nautical miles from the nearest land. All ships are equipped with OWS for the treatment of oily bilge water, and also fitted with holding tanks for discharge at PRF when necessary.

All MSD operated by the company are using biological treatment in combination with chlorination. The screening solids captured by the treatment process are incinerated on board. The MSD are operated on a continuous basis. When the ships operate within 12 nautical miles from nearest land, treated effluents are contained in storage tanks and discharged later.

Most AWTS installed are of the MBBR type, and some are MBR. All sewage, accommodation, laundry and dishwashing wastewater streams are routed through the AWTS. The systems are operated on a continuous basis and effluents are discharged at a minimum distance of 3 nautical miles from the nearest land, confirming commitment to the CLIA zero-discharge policy for untreated sewage. Biosludge is either discharged to sea, incinerated or landed at PRF, where the chosen method depends primarily on the region of operation. Screening solids are typically incinerated on board and ashes are delivered to PRF.

TABLE 9 | Sewage sludge treatment and disposal on board four cruise ships (EPA, 2006a) (EPA, 2006b) (EPA, 2006c) (EPA, 2006d).

Ship	Treatment type	Waste streams	Disposal
Oosterdam (EPA, 2006a)	1. Chemical disinfection and reverse osmosis	Coarse screening solids from vibratory screen filters	Incinerated on board
		Spent bag filters from fine bag filters	Shredded and incinerated on board
	2. MBR	Screening solids from vibratory screen filters	Discharged overboard
		Waste biosludge	Discharged overboard
Veendam (EPA, 2006b)	MBR	Screening solids from coarse filters	Shredded and stored in collection tank, disposal ashore
		Waste biosludge from bioreactor	Discharged overboard
Norwegian Star (EPA, 2006c)	MBBR	Coarse drum filter solids	Stored in solids holding tank; dewatered, pressed and dried and incinerated on board; ashes are brought ashore.
		DAF solids (excess biomass from reactors)	
Island Princess (EPA, 2006d)	MBR	Screening solids	Collected in plastic bags which are incinerated on board; ashes are brought ashore
		Spent bag filters from interstage bag filters	Incinerated on board with screening solids
		Waste biosludge	Discharged overboard
Case study ship (Kotrikla et al., 2021)	Biological decomposition and disinfection	Screening solids and biosludge	Disposed in PRF

In terms of policies, the company reports the initiation of the phasing out of "discretionary single use plastics on our ships". Additionally, onboard gift shops and spas do not sell products containing microbeads. No measures were reported regarding the use of synthetic textiles or the application of microfiber filters in laundry installations.

4 DISCUSSION

This article explored for the first time the sources and pathways of MPs in cruise ship wastewater, using a novel approach, based on general literature on MP sources in the marine environment as well as literature and industry information on cruise operations and wastewater management practices on board cruise ships. An overview was presented of the main source groups and release mechanisms of primary and secondary MPs on board cruise ships. Pathways of MPs were identified by linking the identified sources to the main wastewater streams on board cruise ships and an assessment of typical wastewater management practices.

4.1 Inventory of Sources

An overview was presented of the main source groups of primary MPs on board cruise ships, each reflecting the types of products and operations that are relevant to MP releases: personal care & cosmetics, cleaning & maintenance, medical & pharmaceutical and miscellaneous. PCCP are generally considered a key source of MPs in onshore wastewater treatment plants (e.g. Carr et al. 2016; Mason et al. 2016). There is no reason to assume that this would not be the case on board cruise ships. Moreover, the use of sun protection products and presence of spa and beauty facilities could result in even higher loads. Both fragrances and UV-filters linked to PCCP have been detected in cruise ship wastewater (Westhof et al., 2016; Vicente-Cera et al., 2019b), with concentrations of fragrances at similar levels as those in onshore domestic wastewater and concentrations of UV-filters exceeding those (Vicente-Cera et al., 2019b). It should be noted that the data reported in the latter study were collected under maintenance conditions and could be an underestimate for normal operations with passengers on board. This suggests that cruise ship wastewaters contain concentrations of PCCP constituents that are similar or exceeding those of onshore wastewater. Several studies (Sundt et al., 2014; Lassen et al., 2015; Magnusson et al., 2016) assessed medical and pharmaceutical products as a minor source of MPs to the environment. Both Westhof et al. (2016) and Vicente-Cera et al. (2019b) found concentrations of pharmaceutical compounds in cruise ship wastewater at similar levels compared to domestic wastewater, suggesting no substantial differences in their use on board cruise ships and on land.

Literature reports MPs and synthetic polymers in various products used for industrial cleaning and care. These include hard surface cleaners, toilet cleaners and blocks, stainless steel cleaners, bathroom acid cleaners, oven cleaners, laundry detergents and stain removers (Scudo et al., 2017), commercial hand-cleaning products (Lassen et al., 2015; Scudo et al., 2017) and synthetic waxes in floor

TABLE 10 | Summary of identified representative wastewater management options per wastewater stream.

Wastewater streams	Treatment	Subtype	Liquid effluents	Discharge of effluents	Waste products	Disposal of waste products
Sewage	No treatment	–	Untreated sewage	Disposal at PRF	–	–
				Overboard discharge	–	–
	MSD	Biological and chlorination	Disinfected effluent	Overboard discharge	Waste biosludge	Incinerator/PRF/overboard discharge
	AWTS	MBR	Disinfected effluent	Overboard discharge	Screening solids	Incinerator/PRF/overboard discharge
					Waste biosludge	Incinerator/PRF/overboard discharge
		MBBR	Disinfected effluent	Overboard discharge	Screening solids	Incinerator/PRF/overboard discharge
					DAF sludge	Incinerator/PRF/overboard discharge
Grey water	No treatment	–	Untreated grey water	Overboard discharge	–	–
	AWTS	MBR	Disinfected effluent	Overboard discharge	Screening solids	Incinerator/PRF/overboard discharge
					Waste biosludge	Incinerator/PRF/overboard discharge
		MBBR	Disinfected effluent	Overboard discharge	Screening solids	Incinerator/PRF/overboard discharge
					DAF sludge	Incinerator/PRF/overboard discharge
Oily bilge water	No treatment	–	Untreated bilge water	Disposal at PRF	–	–
	OWS	–	De-oiled effluent	Overboard discharge	Oily sludge	PRF

agents (Essen et al., 2015). Most of the listed product types could be relevant to cruise ships. However, no studies could be identified that address concentrations of detergents and other maintenance products in cruise ship wastewater, nor about the presence of MPs in products used for specific ship operations. Scudo et al. (2017) estimated that industrial hand-cleaning soaps used for the removal of grease, paints etc. account for more than half the tonnage of all applications of MPs in rinse-off products. Considering the nature of cruise ship operations, this could be an important source as well. In addition, considering the wide range of applications of MPs in industrial cleaning products, the use of MPs in specialty maritime and cruise cleaning and maintenance products cannot be ruled out.

The identified release mechanisms for secondary MPs include laundry, waste handling and littering as well as the general wear and tear of products, painted surfaces and other surfaces and facilities. The source products encompass a broad array of products and materials. Many of these concern facilities such as painted surfaces, furnishing and safety equipment, but also stores, e.g. disposable plastics, cleaning cloths and packaging materials and personal belongings. Whereas primary MPs in many cases are intentionally released directly to water during product use (Boucher and Friot, 2017), secondary MPs mainly concern unintentional losses. These MPs may end up in wastewater, e.g. through wet cleaning, but could also be disposed of in solid waste or transported off the ship via air. As a result, not all MP sources may be equally relevant to wastewater. Laundry is an exception, as most of the microfibers released during laundry would be drained with laundry effluents to the grey water system. Synthetic textiles are considered a major source of MPs in the marine environment (Carney Almroth., et al., 2018). Azizi et al. (2022) have summarized the findings of over 400 studies about MPs evaluation in conventional wastewater treatment plants on land. The authors concluded that, throughout the plants evaluated in these studies, fibers were most commonly found, with an average abundance of 57% fibers throughout the different treatment steps. The high contribution of fibers is commonly attributed to the washing of synthetic textiles (e.g. Browne et al., 2011; Napper and Thompson, 2016; Ziajahromi et al., 2017; Raju et al., 2018). Cruise ships have extensive laundry facilities for the washing, drying and folding of professional textiles and most ships also offer laundry services for guests and have launderettes for crew. On the Oasis of the Seas about 42,000 kg of laundry is processed on embarkation day[10]. This suggests that laundry may be a major source of MPs on board cruise ships, depending on the nature of professional textiles such as sheets, towels and crew uniforms. To which extent these MPs reach the grey water system also depends on the use of laundry filters, which could remove up to 78% of fibers (Napper et al., 2020) and, as such, could substantially lower the concentration of microfibers in grey water. Many cruise lines have a policy in place, or have pledged to do so, to phase out certain single use plastics such as straws, stirrers and cups[11,12,13,14], to reduce their plastic footprint. In line with this

[10]https://www.theshipyardblog.com/single-post/2018/08/28/How-Cruise-Ships-Work-Part-2-Laundry-Housekeeping-and-Kitchens

trend, cruise lines could consider the use of plastic-free or non-synthetic alternatives for the MP sources that are reported in this study. The majority of the primary MP sources relate to "stores", indicating that these products are purchased on a regular basis by the cruise line. The company that was consulted in this study already stopped the sale of products containing microbeads in onboard shops. Such a policy could be further extended to also cover PCCP that are used throughout the ship (e.g. in bathrooms and spas) as well as cleaning & maintenance products, including industrial hand soaps. Secondary sources of MPs are more varied and also include permanent ship and hotel facilities, for which plastic-free alternatives are either unfeasible or excessively expensive. However, considering that laundry potentially is a major source of MPs in wastewater, measures addressing this specific source could be effective in order to minimize the total MP load in untreated wastewaters, for instance through replacing synthetic textiles with natural alternatives or the use of microfiber filters in laundry systems.

4.2 Pathways Through Wastewater

The results demonstrate that the MP sources attributed to the different wastewater streams vary significantly. The main sources related to sewage are pharmaceuticals, detergents and the disposal of larger plastic items in toilets. The sources related to grey water include PCCP, detergents, fibers from synthetic textiles and secondary MPs that are removed by wet cleaning. The sources attributed to oily bilge water mainly relate to engine room operations. The findings for sewage and grey water are in line with the findings of Westhof et al. (2016), who evaluated the presence of different types of micropollutants in various wastewater streams on board a cruise ship. Their findings reveal a predominance of oral pharmaceutical residues in sewage with lower concentrations of other pollutants attributed to human excretion. In grey water the highest concentrations were found for caffeine, attributed to the draining of remaining coffee and residues to the grey water system, and flame retardants, which according to the authors diffused from the host material and were consequently discharged to wastewater via laundry, handwashing, bathing and showers. In addition, significant concentrations of pharmaceuticals, UV filters, fragrances and a plastic softener were found, indicating the relevance of PCCP, skin applied pharmaceuticals (e.g. salves) and laundry detergents for grey water.

This paper focused on MPs in the main wastewater streams on board cruise ships. Miscellaneous wastewater streams include ballast water, wastewater from pools, whirlpools and spas, food pulper effluents, effluents from sinks and drains, deck wash water and runoff, wash water from exhaust gas cleaning systems, cooling water, condensates as well as various types of operational wastewater from different types of equipment and machinery (EPA, 2008; EPA, 2013; MEPC, 2017). These could also act as significant pathways of MPs. Ballast waters, for instance, have been reported to contain very high concentrations of MPs (Matiddi et al., 2017). In order to assess the total contribution of MP pollution from cruise ship wastewater, these pathways should also be considered.

4.3 Wastewater Management

MPs in cruise ship wastewater may be discharged to the ocean through the discharge of both untreated and treated effluents, as well as through the overboard discharge of waste products from wastewater treatment.

The performance of wastewater treatment systems that are in use in the industry is not well documented due to a lack of administrative monitoring (Westhof et al., 2016) and their effectiveness in retaining MPs in particular has not been comprehensively documented. EPA (2008) reports various pollutant concentrations in the effluents from various sampling efforts of AWTS and MSD effluents of cruise ships operating in USA waters between 2003 and 2005, with non-detected values for both settleable and suspended solids in most AWTS effluents. This indicates that the cruise ship AWTS included in the sampling efforts were generally effective in capturing solids. Furthermore, both membrane ultrafiltration, a main component of MBR systems, and DAF, a main component of MBBR systems, are associated with very high MP removal rates in onshore systems. For MBR and membrane ultrafiltration, rates reported in literature (Talvitie et al., 2017a; Lares et al., 2018; Ma et al., 2018; Lv et al., 2019) exceed 99% and this is considered the most effective technology to remove MPs in onshore WWTP (e.g. Sun et al., 2019). For DAF, values between 70% and 96% are reported (Talvitie et al., 2017a; Esfandiari and Mowla, 2021), for different types of flocculants and coagulants that are added during the process. On the other hand, EPA (2008) reports values of suspended solids in the effluents of cruise ship MSD systems which are substantially higher than the USA discharge standards for onshore wastewater treatment systems, indicating that these systems may be less effective in capturing MPs. No data could be retrieved regarding the effectiveness of OWS in capturing particulate matter. Onshore wastewater treatment plants are generally considered important sources of MPs in aquatic environment, despite their effectiveness in removing MPs from influents, due to the large volumes of wastewater that pass these plants [e.g. Talvitie et al. (2017b)]. Considering the volumes of wastewater that are generated on board cruise ships, treated wastewater from cruise ships therefore represents a significant pathway.

The results of this study reveal that, currently, 25% of the world cruise fleet discharges all grey water without treatment to the ocean as these ships do not have AWTS. In addition, AWTS configurations not necessarily cover all grey water sub-streams and as a result, a potentially significant volume of grey water is discharged without treatment from ships with AWTS. Further study of typical configurations is required to assess the volumes and characteristics of such discharges throughout the industry.

Various studies of onshore wastewater treatment plants have investigated the fate of MPs in onshore wastewater treatment plants, demonstrating that the vast majority of MPs in the influent are captured in sludge (Carr et al., 2016; Talvitie et al.,

[11] https://presscenter.rclcorporate.com/press-release/18/royal-caribbean-to-eliminate-plastic-straws-by-end-of-2018/

[12] https://www.maritime-executive.com/article/carnival-targets-single-use-plastics

[13] https://www.cruiseindustrynews.com/cruise-news/24042-msc-cruises-signs-single-use-plastic-charter.html

[14] https://www.ncl.com/travel-blog/norwegian-eliminates-single-use-plastic-bottles

2017b; Gies et al., 2018). Since sewage sludge is commonly recycled as fertilizer in agriculture applications (Nizzetto et al., 2016), this represents a major pathway of MPs to the environment on land, leading to the accumulation of MPs in agricultural soils (Corradini et al., 2019). Similarly, biosludge resulting from AWTS treatment on board cruise ships likely contains high concentrations of MPs, due to the expected effectiveness of MBR and DAF in capturing MPs. The results of this paper indicate that while three options for the disposal for biosludge are used throughout the industry, overboard discharge is the most common method. The overboard discharge of this substance therefore leads to a delayed and concentrated discharge of the MPs in grey water and sewage and this practice should be avoided. The development of adequate PRF for biosludge in cruise regions could be instrumental in reducing the volumes of MPs that are discharged through this pathway, especially in vulnerable areas receiving large numbers of cruise ships. The literature review as well as questionnaire response indicates that overboard discharge of screening solids is not common, however this practice has been reported for one ship by EPA (2006a), indicating that this scenario cannot be ruled out.

In general, the available literature on wastewater treatment systems is restricted to a small number of dated reports (e.g. King County (2007); Huhta et al. (2007); EPA (2008); EPA (2011), most of which were produced by USA government authorities. Furthermore, the available data regarding the practices of discharging untreated grey water and sewage as well as the overboard discharge of biosludge concern a limited number of isolated and largely dated case studies (e.g. EPA, 2006a; EPA, 2006b; EPA, 2006c; EPA, 2006d; Klein, 2009; Kotrikla et al., 2021). In order to address these knowledge gaps, this research collected information on both wastewater treatment systems and wastewater management practices from one large cruise line. The results confirm trends and practices in wastewater management as reported by other studies (see section 3.5.1). However, it should be noted that these efforts either build on voluntary contributions or on cruise operations in the USA, and Alaska in particular; an area that is more strictly regulated and monitored than the mainstream cruise regions in the Caribbean and Mediterranean. Therefore, these results are likely biased and caution should be taken when extrapolating these results to the industry as a whole, in particular in vulnerable areas with little regulation and/or inadequate enforcement. An industry-wide overview of wastewater management systems and practices, ideally linked to regions of operation, would greatly support the understanding of leakages of MPs and other pollutants from cruise ship wastewater.

Finally, as recently raised on one of the leading digital platforms in the maritime industry[15], the improper management of solids in sewage may lead to discharges through other pathways, such as the disposal of any solids remaining in the holding tanks and the use of cutter pumps in the collection and treatment of sewage. These cutter pumps are purposely designed to remove the load on screens by breaking down solids in smaller particles. This is rendering screenings less effective, and even contributing to the formation and release of MPs to the environment. This further emphasizes the need for a holistic approach of wastewater management in order to prevent leakages of MPs.

[15] https://www.maritime-executive.com/editorials/ships-discharge-10-000-cubic-meters-of-plastic-a-year-from-sewage

4.4 Conclusions

This paper for the first time explored the sources and pathways of MPs in cruise ship wastewater, providing insight on the array of sources and pathways, highlighting priority areas for mitigation and identifying additional knowledge gaps. On the level of individual companies or ships, the overview of sources and pathways allows for the identification of mitigating measures from source-to-sea, by identifying the full array of sources and mechanisms that contribute to the release of MPs to wastewater, as well as the connections between sources and the different wastewater streams. As a result, it also provides guidance for purchasing policies by cruise lines and the need for ongoing education of crew and passengers.

In general, it is recommended that cruise lines consider the inclusion of PCCP well as cleaning and maintenance products containing primary MPs in their policies to phase out the use of single-use plastics. In addition, the replacement of professional synthetic textiles with non-synthetic alternatives and the use of laundry filters could be effective in reducing the MP load in wastewaters. Furthermore, adequate wastewater management is key to prevent MP leakages and reduce the MP load in wastewaters that are discharged to the ocean. This is greatly supported by the increased use of AWTS. However, the use of these systems is only a partial solution, which should be part of a holistic management of wastewater streams. Efforts should be made to minimize discharges through waste products, wastewater streams bypassing AWTS as well as wastewater streams other than discussed in this study. Although at the global scale, the quantitative contribution of MPs from cruise ship wastewater is small in comparison to land-based sources, local impacts could still be significant due to the large amounts of wastewater, waste products that are discharged without treatment, the vulnerability of the exposed coastal and marine ecosystems and the concentrated nature of cruise activities. To better place the problem in perspective, identify cost-effective measures and areas at risk, it is required that MP concentrations in different effluents and waste products are quantified through measurements and that contemporary wastewater management systems and practices throughout the industry are better understood.

In conclusion, the approach for this study was successful in exploring the major sources and pathways of MPs within the study scope, and to highlight knowledge gaps and starting points for mitigation. This makes it a valuable tool that could also be applied in other maritime sectors and will support global efforts to identify all sources and pathways of MPs within the context of the UNEA-5.2 resolution.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

The research was performed by MF and she also wrote the paper. CC and AL contributed by guiding the research, discussing ideas and supervising the writing of this paper. All authors contributed to the article and approved the submitted version.

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Current State of Microplastic Pollution Research Data: Trends in Availability and Sources of Open Data

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The rapid growth in microplastic pollution research is influencing funding priorities, environmental policy, and public perceptions of risks to water quality and environmental and human health. Ensuring that environmental microplastics research data are findable, accessible, interoperable, and reusable (FAIR) is essential to inform policy and mitigation strategies. We present a bibliographic analysis of data sharing practices in the environmental microplastics research community, highlighting the state of openness of microplastics data. A stratified (by year) random subset of 785 of 6,608 microplastics articles indexed in Web of Science indicates that, since 2006, less than a third (28.5%) contained a data sharing statement. These statements further show that most often, the data were provided in the articles' supplementary material (38.8%) and only 13.8% via a data repository. Of the 279 microplastics datasets found in online data repositories, 20.4% presented only metadata with access to the data requiring additional approval. Although increasing, the rate of microplastic data sharing still lags behind that of publication of peer-reviewed articles on environmental microplastics. About a quarter of the repository data originated from North America (12.8%) and Europe (13.4%). Marine and estuarine environments are the most frequently sampled systems (26.2%); sediments (18.8%) and water (15.3%) are the predominant media. Of the available datasets accessible, 15.4% and 18.2% do not have adequate metadata to determine the sampling location and media type, respectively. We discuss five recommendations to strengthen data sharing practices in the environmental microplastic research community.

Keywords: microplastics, bibliometric analysis, data repository, data availability statement, data management, data sharing, environmental research, plastic

INTRODUCTION

There is an increasing awareness of microplastics in the environment and their potential negative consequences for water security, biodiversity, ecosystem services, human health and well-being (Bergmann et al., 2015; Barboza et al., 2018; Li et al., 2018; Provencher et al., 2020; Woods et al., 2021; Stokstad, 2022). Along with other novel entities, microplastic pollution is now considered to exceed safe planetary boundaries (Persson et al., 2022). This awareness has spurred a surge in research on microplastics, including their occurrence and environmental distributions, chemical and physical properties, fate and transport (Domercq et al., 2022), impacts on biota and ecosystems (Abeynayaka and Norihiro, 2019; Covernton et al., 2019; Jacques and Prosser, 2021; Tekman et al., 2022) and integration into life cycle inventories and impact assessment (Abeynayaka and Norihiro, 2019; Woods et al., 2021). The increasing interest in microplastics is reflected in the number of published peer-reviewed articles and news articles (Ryan, 2015; Cowger et al., 2020; Can-Güven, 2021). The rapid growth of publications on microplastic pollution since the turn of the century is primarily associated with research on marine environments, freshwater bodies, wastewater, and fate and transport of microplastics, with publications spanning eighty-seven countries across the globe (Can-Güven, 2021). Simultaneously, funding to support microplastics research has increased in the past decade (Maes et al., 2019). For instance, the Government of Canada has made the detection and characterization of microplastics a priority area for research funding to develop the knowledge base and research capacity required to support Canada's Plastics Science Agenda (CaPSA) (Environment and Climate Change Canada, 2019; NSERC, 2020). In the United States, the National Oceanic and Atmospheric Administration (NOAA) Marine Debris and NOAA Sea Grant programmes offer research funding that focuses on plastic pollution. Many state sea grant programs also now include plastic pollution as a priority area (Sea Grant, 2018; NOAA and NECEI, 2022).

Researchers are developing new approaches to isolate, count, and measure microplastics in different environmental settings to characterise the global distribution of microplastic (*e.g.,* see Arctic Monitoring and Assessment Programme (AMAP) report; Wayne State's Smart Management of Microplastic Pollution), which is critical to guide the state of our knowledge on sources, fate, and effect of microplastics, and to facilitate and assess effective policy decision-making. For example, the United Nations passed a major global resolution on plastic pollution in March 2022 (Stokstad, 2022), while the State of California adopted a state-wide microplastics strategy in February 2022 (State of California, 2022). To ensure good decision-making and to enforce these policies, microplastic data must be made FAIR (Findable, Accessible, Interoperable, and Reusable), as they are key to the process. To advance research, protect funder investments in data collection, enable policy development, and support public interest into the human and environmental health impacts of microplastics (Koelmans et al., 2019; Cowger et al., 2020; Igalavithana et al., 2022), research data must be properly curated, deposited and preserved in adherence with the FAIR guiding principles (Wilkinson et al., 2016).

As more and more data on microplastics are acquired and as policies begin to emerge around the world (e.g. Stokstad, 2022), it is important that scientists are able to conduct meta-analyses, confirm reproducibility, and meaningfully compare data from different studies (Cowger et al., 2020; Provencher et al., 2020; Brandes et al., 2021). The international workshop on microplastic particles organised by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) articulated the fundamental gaps in microplastics standardisation must be filled in order to enable the comparison and merging of data from researchers from across various geographic regions (GESAMP, 2019). These fundamental gaps include data capture standards, quality control practices, data storage and sharing, as well as reporting and dissemination. A decade later, microplastics datasets are generated rapidly and stored in a variety of formats, from open source to proprietary, and data range in size, from kilobytes to terabytes and many of these datasets are still not finable (Brandes et al., 2021). Nonetheless, subsequent international activities have been initiated to address some of these gaps, including coordination of global and regional efforts to characterize plastics pollution by generating guidelines for sampling and reporting that will minimize the duplication of work (e.g., Global Partnership on Marine Litter, Japan's Ministry of Environment, OSPAR Commission, NOAA's NCEI Microplastics). In addition, recently a number of microplastics-focused data repositories have also been created in an effort to homogenise subsets of data (Morgan Stanley, National Geographic, University of Georgia, and National Oceanic Atmospheric Administration, 2010; Tekman et al., 2020; NOAA NCEI Microplastics n. d; EU EMODNET, 2017), but how much and what types of microplastics data are generated by, and readily available for the academic research community remains unclear. An assessment of the state of microplastics research data accessibility would assist researchers and stakeholders in identifying best data management practices in the field.

In this study, we explore the extent to which the data that underpin environmental microplastics research articles are openly shared. Two strategies were adopted to identify and locate open datasets: 1) we reviewed the data sharing statements in a representative subset of peer-reviewed publications on environmental microplastics, and 2) we undertook a comprehensive search of relevant online data repositories. Based on our findings, we highlighted five practices that researchers in the microplastics community can readily implement to advance data sharing in this emerging field.

DATA SOURCES AND METHODOLOGY

Analysis 1: Publications on Microplastics With Open Data

The methods outlined by Read et al. (2021) and Roche et al. (2022b) were adapted to determine if authors of microplastic research articles shared the underlying data. A Web of Science database search was performed using the Boolean phrase (microplastic OR microplastics)

TABLE 1 | The selection criteria and number of peer-reviewed journal articles that were identified and used for data analysis in this study.

Selection Criteria	Number of articles
Web of Science search (microplastic OR microplastics) between 1964 and 2021	6608
Stratified random sampling of up to 100 articles per year between 1964 and 2021	1045
Abstract only—removed from sample set	2
Articles not related to environmental microplastics—removed from sample set	256
Articles that could not be accessed—removed from sample set	1
Articles that were retracted—removed from sample set	1
Total articles removed from sample set between 1964 and 2021	260
Percentage of articles removed from sample set	24.9%
Number of articles included in the final sample set between 2006 and 2021	785

together with the "All Fields" option. Only English-language articles published between 1964 and 2021 were included, which yielded 6,608 articles. A stratified random sample selection of these articles was conducted in *R* (version 4.0.3) using the dplyr package (version 1.0.2) to select 100 studies per year. For years in which fewer than 100 studies were published, all articles were considered. The resulting subset consisted of 1,045 articles (15.8%). A number of articles were removed from this subset after manual inspection (n = 260; 24.9%) as these articles dealt with unrelated topics (for example "microplastic deformation" of metal alloys), or with topics not directly dealing with environmental samples (such as microbial colonisation of microplastics), or they were perspective-style or review papers. Additionally, articles that were inaccessible, retracted, or consisted solely of an abstract were removed. After the manual inspection and removal of articles that were not environmental microplastics related, a total of 785 peer-reviewed publications (11.9%) were included in the final assessment (**Table 1**).

Each of the selected articles was examined to determine 1) whether a data sharing statement was included in the article and, if so, 2) what the nature of the data sharing statement was. The nature of data sharing statements was categorised as: (i) available upon request via the author(s), (ii) available in a data repository, (iii) available in the supplementary files, (iv) no data were used, (v) data will be made available at a future date, (vi) no evidence of data sharing, (vii) data are considered sensitive, or (viii) data are available in the article.

We ensured that our metrics for percent of studies with data statements would be reproducible using simulations to determine the number of studies we needed to assess. A thousand simulations of subsampling from a two-class set (article does/does not have a data statement) with uniform probability distributions were measured by calculating the high mean absolute errors of the class percentages at the 95% quantile of the simulation distribution. Imposing the minimum number of studies to review at 100 per year yielded a maximum mean absolute error in class size of ±9%. Thus, if data accessibility changed by at least 18% over our period of study or in future studies we would be highly likely to identify the temporal change.

Analysis 2: Microplastics Datasets in Data Repositories

To assess the availability of data, the google dataset, DataONE Data One (2015) and OpenAIRE (2013) discovery portals were searched in October 2021 using the same search terms as used in Analysis 1 (i.e., microplastic OR microplastics) to identify available microplastics datasets. This search generated 10 repositories (**Table 2**). This was followed by a site-specific search of the 10 repositories to assess data access and metadata. The search engine of each repository was queried using the term "microplastic*" or the Boolean phrase "microplastic OR microplastics" when the search interface did not support the use of a wildcard. The search was not restricted to any specific time period and duplicated datasets (n = *21*) were removed from the final sample of datasets. For each dataset, we recorded the following attributes: repository, year of publication, DOI, study site, environmental media type, keywords, and whether the dataset was linked to a journal article (yes/no). For each repository, we noted the disciplinary data it accepts (**Table 2**) and whether the repository was CoreTrustSeal, 2022 (CTS) certified as a trustworthy data repository as of February 2022 (**Table 2**), according to the CTS Certified Repository website. All metadata were recorded in a Microsoft Excel spreadsheet and OriginPro 2020 software was used to visualise the findings.

RESULTS

Microplastics Data Sharing Trends in Peer-Reviewed Articles

Of the final 785 articles analysed, 224 (28.5%) contained data sharing statements in the body of the article (**Figure 1A**). Prior to 2013, only eight out of 31 articles included data sharing statements. Since then, the numbers have steadily increased, with approximately half of all articles published after 2019 containing a data statement (**Figure 1B**). However, the proportion of articles with data sharing statements did not increase further between 2019 and 2021.

Further evaluation of the 224 data sharing statements (**Figure 2**) showed that authors most frequently shared the data associated with their article in the form of supplementary materials (n = 87; 38.8%) or stated that they had included all their data in the main body of the article (n = 60; 26.8%). In a small number of cases, the data underlying the publication were classified as sensitive and could not be shared (n = 2; 0.9%). A similar small number of statements indicated that the data would be made available in the future (n = 2; 0.9%). Others referred the reader to the corresponding author to

TABLE 2 | Selected research data repositories used in this study.

Repository name	Acronym	Discipline(s)	CTS Certified	No. of Datasets (*n*)
Dryad	Dryad	General, ecology and evolutionary biology	Not listed	21
Environmental Data Initiative Portal	EDI Portal	Environmental and ecological data	Not listed	14
Environmental Information Data Centre	EIDC	Datasets related to terrestrial and freshwater sciences	16 August 2019	4
Figshare	Figshare	General	Not listed	29
Harvard Dataverse	Harvard DV	General, social sciences	Not listed	5
Mendeley Data	Mendeley	General	Yes (expired)	75
Pangaea	Pangaea	Earth system science	17 June 2019	90
Polar Data Catalogue	PDC	Focus on cold and high latitude regions	16 February 2021	15
SEA scieNtific Open data Edition	SEANOE	Marine sciences	Not listed	4
Zenodo	Zenodo	General	Not listed	22

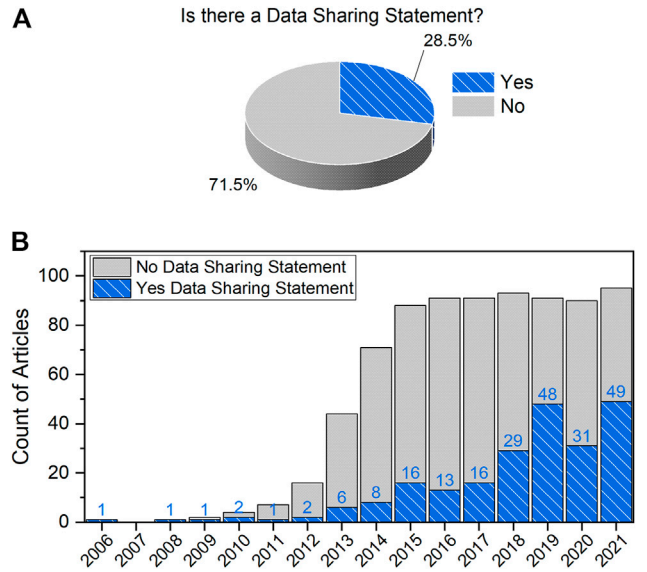

FIGURE 1 | Proportion of data sharing statements **(A)** overall and **(B)** annually between 2006 and 2021 in the peer-reviewed publications. Numbers displayed on the bars in panel b refer to the yearly number of data sharing statements.

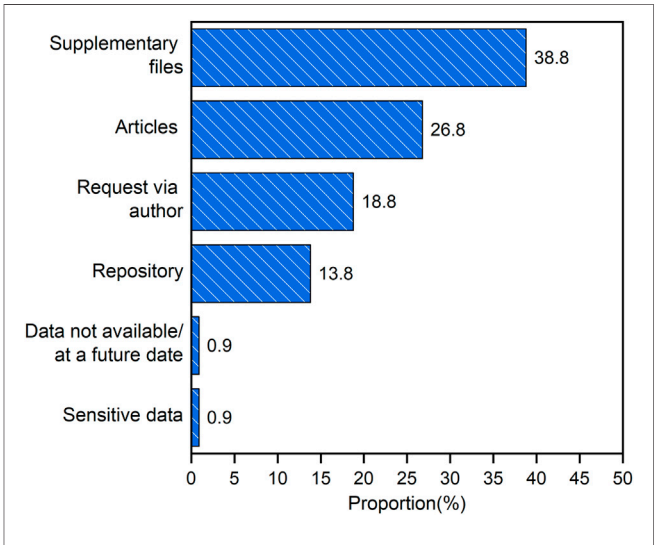

FIGURE 2 | Data dissemination methods indicated in the data availability statements of the articles analysed in this study.

request the data ($n = 42$; 18.8%). Data were explicitly shared via a data repository in only 31 (13.8%) articles.

Microplastics Data Sharing Trends in Data Repositories

In our sample set derived from Web of Science, the earliest microplastic article was published in 2006 while our data repository search yielded the first dataset in 2013 hence in this section our analysis is focused from 2013 to 2021 (**Figure 3**). Searches in the google dataset, dataONE Data One (2015) and OpenAIRE discovery portals returned 72 datasets on microplastics. Further site-specific searches of 10 data repositories increased the number of datasets to 279 (**Table 2**; **Figure 4**). Of these 279 datasets, 222 (79.6%) had data files that were directly accessible, while for 57 datasets (20.4%) the files were not accessible (*i.e.,* only metadata were provided) or further approval was required to access and download the data files. Search results were not limited by year, but the first datasets in the sample were published in the year 2013, with evidence of data sharing in data repositories trending up thereafter (**Figure 3A**).

In addition to the datasets in repositories, 6,363 microplastics articles were published between the years 2013 and 2021 (**Figure 3B**). During this time, the number of articles increased exponentially from 50 to 2,295, while the number of datasets from those studies provided within the repositories also increased rapidly from 1 to 112 (**Figure 4**). Of the 10 repositories queried, the CoreTrustSeal certified repositories Pangaea and Mendeley published the majority of microplastics datasets, with approximately 32 and 27%, respectively.

Geographic Distribution of Data Site Locations

The metadata collected highlights the unequal geographic distribution of the provenance of the microplastics samples (**Figure 5**). Of the 279 repository datasets, the majority of data were sampled in Europe (13.4%), North America (12.8%), and in

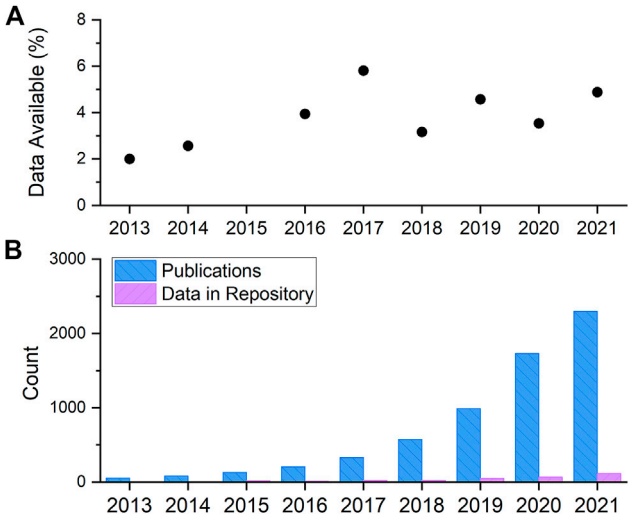

FIGURE 3 | (A) Proportion of microplastic publications found in Web of Science between 2013 and 2021 that have a dataset in an open access repository; **(B)** Number of data sets in open access repositories during the same period along with the annual number of peer-reviewed microplastics articles published.

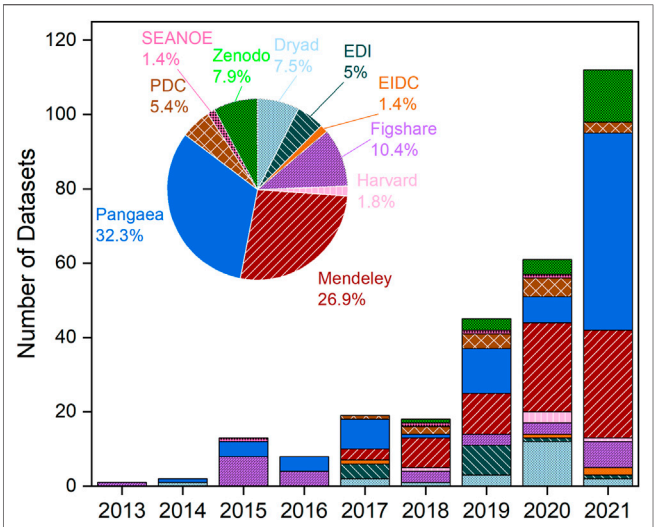

FIGURE 4 | Yearly (bar graph) and cumulative (pie chart) distributions of microplastics datasets uploaded to open access repositories between 2013 and 2021.

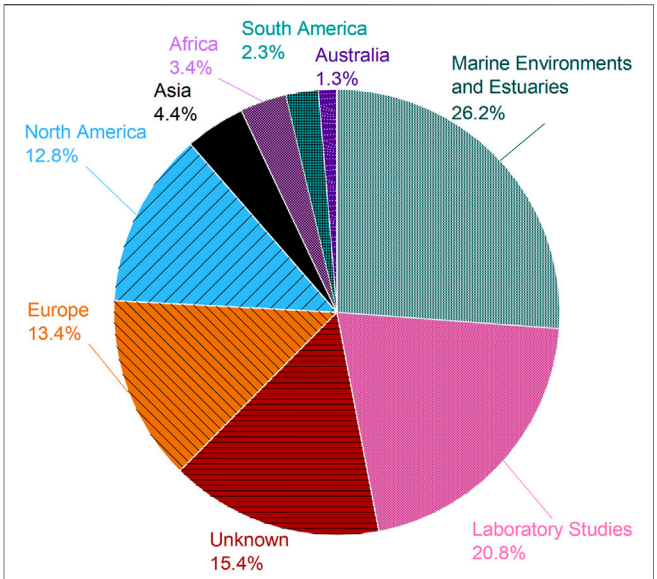

FIGURE 5 | Geographical distribution of microplastics datasets according to sample provenance. Note that the marine environments and estuaries section includes beaches, coastal areas, seas, and trenches. Unknown means that there were not sufficient metadata in the dataset to determine the location.

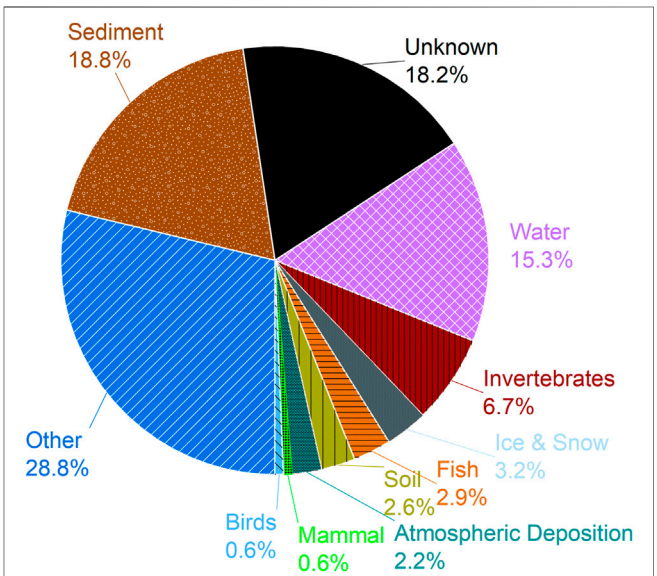

FIGURE 6 | Distribution of open datasets by media type. The "other" section includes effect and fate studies, as well as methods papers and modelling experiments. Unknown means that there were not sufficient metadata in the dataset to determine the media type.

marine environments and estuaries (26.2%). Approximately 11% of the data originated from Australia, South America, Asia, plus Africa. About a fifth of the datasets were generated in controlled laboratory studies. The latter include, for example, studies looking at uptake of microplastic particles by biological organisms or microplastic particle transport in porous media (20.8%), while 46 studies (15.4%) did not report any location information as part of the repository dataset.

Environmental Media Type Reported With Dataset

The largest fraction of datasets (28.8%) contained data from studies that focused on purchased plastics, uptake of microplastics in organisms, and modelling experiments. The

most reported media types included sediments (18.8%), water (15.3%), and invertebrates (6.7%), with other types of studies accounting for the remaining 12.1%. The least studied media were atmospheric deposition, mammals, the cryosphere (ice and snow), fish, and birds. An unexpected 18.2% of datasets did not provide sufficient information to identify the media type (**Figure 6**).

DISCUSSION

Although the number of new datasets made available publicly are increasing annually, the numbers continue to be very low relative to the rapid growth in articles about environmental microplastics (**Figures 1**, **3**, **4**). The increase in data sharing, especially since 2016, are likely because publishers have implemented data policies which state that authors are expected to include an explicit data sharing statement within their manuscript at the time of submission (e.g., Piwowar and Vision, 2013; Science, 2019; Colavizza et al., 2020; AGU, 2021; Elsevier, 2022; Springer, 2022). Less than 30% of microplastic research articles assessed in this study included any form of data sharing statement, with only 13.8% of articles explicitly sharing their data in a repository. Pangaea and Mendeley were among the most commonly used repositories, perhaps because they are free and easy to use (**Figure 4**). It is not entirely known why these were most used, but the earth science Pangaea community has a long history of depositing and archiving data. In addition, numerous microplastic researchers are based at one of the host institutes of PANGAEA (AWI), which encouraged sustainable data archiving early on and often curated data produced in large European Union projects where it was a partner. Mendeley Data was purchased in 2013 by Elsevier and researchers publishing in Elsevier journals are encouraged to deposit their data in Mendeley Data (Dumon, 2013). It is possible that many of these researchers are unaware of other repository options and, hence, may gravitate towards using the publisher's controlled repositories.

The challenge of finding and accessing research data is not unique to the microplastics research community. Similar patterns in data sharing practices are common in well-established disciplines such as social sciences, water resources, low-temperature geochemistry, ecology, and health sciences (Stagge et al., 2019; Brantley et al., 2021; Tedersoo et al., 2021; Roche et al., 2022b). For example, Stagge et al. (2019) assessed data availability and research reproducibility in hydrology and water resources across several journals and found that, while approximately 70% of the sampled articles stated some materials were available, only around 48% of the materials could be accessed online. In experimental biology, only one in five papers (21.5%) included a data sharing statement or associated open data (Roche et al., 2022b). An overview of published research funded by the Canadian Institutes of Health Research (CIHR) (Government of Canada, 2021), a federal funding agency which has an explicit data sharing expectation, showed that for a subset of CIHR funded projects, only 45.2% of studies had readily accessible data (Read et al., 2021). The challenges outlined by Brantley et al. (2021) for the field of Earth surface geochemistry similarly resonated with our assessment of the emerging field of environmental microplastics. Perhaps the biggest challenge faced in both cases is the diverse nature of the data due to the environmental media involved, QA/QC issues, data structure, diversity in analytical techniques used, and multiple other factors, which makes it challenging to develop standardised reporting structures (Cowger et al., 2020; Provencher et al., 2020; Brandes et al., 2021; Brantley et al., 2021). A promising trend in all the disciplines mentioned, microplastics included, is that more and more researchers are making their data available. In addition to publishers' data policies, this increase may be attributed to the generational shift with the research community as younger researchers are getting more access to technology and databases, and early exposure to the data management concepts and practices; they are integrating all of these as part of their daily research workflow.

Microplastics are a relatively young field, and thus it can be expected that it will lag behind more established disciplines with respect to data sharing, especially given its multi-disciplinary nature. In this regard, researchers are likely to be influenced by their home discipline which may slow consensus on the discipline-specific metadata and data sharing standards, guidance, and education. However, the microplastics data sharing practices observed in this study showed that the microplastics field is on par with well-established disciplines such as water resources and ecology (Stagge et al., 2019; Roche et al., 2022a). Efforts to develop microplastic metadata sharing practices, which will increase the findability and interoperability of microplastics data, are currently underway (Cowger et al., 2020; Cowger et al., 2020; AMAP, 2021; Jenkins et al., 2021). Such methods or other regulatory measures and incentives are urgently needed because the progress in data sharing over the past 3 years appears to have stabilised instead of continuing on an upward trajectory. Given the early stages of this area of research, these valuable data are not easily discoverable via peer reviewed literature and data repositories. However, they often constitute vitally important baselines needed for future monitoring purposes. As the data collection efforts expand to include indigenous lands in North America, data management should additionally adhere to guiding principles for data collected on indigenous lands such as the Collective Benefit, Authority to Control, and Ethics (CARE; Carroll et al., 2020; Carroll et al., 2021) and the Ownership, Control, Access and Possession (OCAP®) (FNIGC, 2020).

The microplastic research community can learn from, and lean on work in other disciplines to promote good practices for data sharing. There are a growing number of research data management best practice guidance papers available (Michener, 2015; Wilkinson et al., 2016; Briney et al., 2020; Persaud et al., 2021, Contaxis et al., 2022, among others). As emphasised by Brantley et al. (2021), targeted education and awareness are still needed across scientific disciplines in order to implement and sustain best data management practices. Given the rapid growth of microplastic research papers, the microplastic research community, the target audience of this paper, is unlikely to have the time to thoroughly review existing papers that have been published about research data management (RDM)

standards and best practices for other fields that are transferable to the field of environmental microplastics. Five simple strategies for advancing good data management and data sharing practices in microplastics research are therefore provided in the next section. We hope that these will help to maximise the positive impacts of microplastic research and improve the FAIRness of microplastic research data.

Strategies for Advancing Good Research Data Management Practices in Microplastics Research

1) Use Available Standards/Practices to Describe Data

A major challenge in translating the rapidly increasing body of new scientific knowledge and data into actionable policy is the lack of standardised procedures for microplastics RDM practices. There are currently no international or national data governance standards for environmental microplastics, including metadata standards, database structures, and RDM best practices, which limits the effective sharing and comparison of data on the abundance, size distribution, shape, surface roughness and chemical (polymer) composition of microplastics. This, in turn, hampers efforts to harmonise, and eventually standardise, the evaluation and validation of sampling and analytical methodologies and protocols that are needed across the research community. This study acknowledges there are many challenges that still need to be addressed to standardise data reporting for microplastics research, however, resources such as the AMAP report (2021), GESAMP (2019), Cowger et al. (2020), Michida et al. (2020), Jenkins et al. (2021) and Miller et al. (2021) provide guidelines that will help ensure data collection and reporting are robust. Existing metadata standards, such as the United States EPA Water Quality Exchange, the Dublin Core™ Metadata Element Set, and European monitoring under the Marine Strategy Framework Directive (EMODnet) should be integrated to ensure data are described consistently across the microplastics community.

2) Share Raw Data - Or as Close to Raw as Possible

It may be necessary to perform QA/QC, or to transform data from a format that is ideal for analysis into a format that is ideal for accessibility (*e.g.,* CSV, mzML, JCAMP-DX, JSON, cif, TIF), but the goal should always be to share data that are as close to raw as possible. The dataset should include a README with information on how, when, and where data were collected and any pre- and post-processing steps, which travel alongside the data and provide necessary context and contact information. If any data were provided by a third-party source, or derived from data provided by a third-party, that information should be documented with the dataset.

3) Use a Trusted Digital Repository

Whenever possible, data should be shared in a trusted digital repository that will steward data in the long term. Ideally, the repository will provide DOIs or another unique and persistent identifier that can be used to reference individual datasets. Embargoes may be used to temporarily protect data from downloads, especially if analyses are still ongoing. Some repositories can also restrict access to data in the longer term if they are sensitive. Disciplinary repositories, such as NOAA's NCEI Microplastics database, accept marine microplastics data from all researchers across the globe (NCEI Microplastics nd), while specifically within the European Union member states as a requirement for EMODNet. Otherwise, researchers are encouraged to use general-purpose or institutional repositories, such as Pangaea and the Federated Research Data Repository (FRDR), which offer curation services to deposited data.

4) Link Dataset to Publications

Many journals provide either supplemental information or data availability/open access statements, where the repository name and the dataset DOI (or other identifiers) should be included so readers can find the supporting data. Data obtained from a third-party source should also be included and cited in the references section. If the data cannot be shared, or if restricted access is required, this should be explicitly stated and the steps required to obtain access outlined. Likewise, the DOI of any publications that is associated with the dataset can be added to the data repository metadata record which provides context for the data and positions it as an important part of the scholarly record.

5) Plan to Share Data From the Onset of a Study

Data management and data sharing should be considered as early as possible. A data management plan can be completed at any point in the research process to document what types of data are generated, their format, and the metadata standards that are used to describe them, as well as short and long-term storage requirements, and the costs associated with data collection and data management. Planning early on helps ensure no data are lost and that the resulting dataset relies on existing practices to ensure a measure of consistency and interoperability, and, when necessary, that permission to share data has been sought and provided (*e.g.,* for data that were provided by a third-party, or data that were collected on Indigenous lands or with Indigenous partners).

CONCLUSION

In this bibliometric study, the extent to which environmental microplastic research data are openly shared were assessed. This work showed that between 2013 and 2021, microplastics dataset sharing has increased, but much more slowly than the number of peer-reviewed publications. The large amounts of data being produced, often supported by public funding, are simply not accessible or have insufficient metadata for others to do quality assurance, to assess the quality of the data and to ultimately reuse the data. Data sharing has stabilised in recent years which suggests that there are obstacles to data sharing that will need

to be addressed to ensure the long-term availability and accessibility of data which can serve as vital baseline data for future monitoring. For example, many institutions need to access microplastics data to help guide regulatory frameworks such as safe drinking water levels (California Senate Bill 1422, 2018), and the European Marine Strategy Framework Directive (European European Union, 2008). These findings highlight the need for the environmental microplastics community to focus on not only advancing the science of environmental microplastics research but also on simultaneously embedding data management into their daily research workflow through education and best practices. Efforts should be made by researchers to also make use of data management resources including sharing data on discipline-specific repositories that are available to ingest microplastics data. However, standardised (meta)data reporting templates that implement established microplastic (meta)data reporting standards in a reproducible and usable way for microplastics data are still needed.

The increasing trend of open microplastic data shared in repositories and linked to peer-reviewed publications is promising. Data sharing practices will help increase the reproducibility and comparability of data. The more comprehensive our collective data sharing practices are, the better the microplastics decisions and policies that affect society as a whole. Moreover, it is incumbent upon the microplastics research community to ensure that FAIR data are consistently made available. These types of activities will not only strengthen the data sharing practices in this field but will also support continued advances in understanding the occurrence of microplastics in the environment, which is highly important in terms of pollution monitoring efforts.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed in this study can be found in the Federated Research Data Repository at https://doi.org/10.20383/102.0476.

AUTHOR CONTRIBUTIONS

All co authors were involved in conceptualization of study, data collection, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Plastic-Associated Microbial Communities in Aquaculture Areas

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Microorganisms colonize plastics in the aquatic environment but their composition on plastics used in aquaculture remains poorly studied. Microorganisms play a significant role in aquaculture in terms of water quality and the health of cultivated species. In the current study, we explored the composition of microorganisms on floating plastics and their surrounding water collected from ponds and open aquaculture areas. Using scanning electron microscopy, the diversity of microbial communities, primarily diatoms, and bacteria were identified on the plastic surfaces. Additionally, epifluorescence microscopy revealed that prokaryotes were colonized on all plastic samples from 0.1 to 29.27×10^3 cells/cm^2, with a high abundance found in open aquaculture areas compared to ponds. Bacterial communities were characterized by 16S rRNA sequencing which showed that bacterial communities on plastics were dominated by Proteobacteria, Cyanobacteria, Bacteroidetes, and Actinobacteria. The level of these microbial communities on the plastics differed from those found in the surrounding seawater samples and the abundance of potentially pathogenic bacteria was higher in plastics than in seawater samples. Moreover, hydrocarbon-degrading bacteria were more abundant in the investigated plastic samples than in the water samples. This study contributes to the knowledge regarding the plastisphere community in aquaculture.

Keywords: plastisphere, aquaculture plastic, microbial community, pathogens, plastic polymers

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INTRODUCTION

Plastics are semisynthetic or synthetic materials made of natural products, such as crude oil, natural gas, cellulose, and coal. Because of their flexibility, plastics have been used in many applications, especially packaging (Lambert and Wagner, 2018). Plastic production has grown rapidly since the 1950s compared to other human-made materials and has even replaced metals and wood (Geyer et al., 2017). Thus, plastic production has increased 189 times to reach 330 million metric tons in 2016 (Lebreton and Andrady, 2019). Nevertheless, plastic production may reach 20% of the petroleum used

worldwide and 15% of the annual carbon emission budget (Lebreton and Andrady, 2019). With inadequate waste management, human activities have led to the accumulation of a considerable volume of plastics in the marine environment. In 2010, it was estimated that between 4.8 and 12.7 million tons of plastic litter entered the ocean from 192 coastal cities, accounting for 1.8–4.7 % of the plastic generated globally that year (Jambeck et al., 2015) and this number is predicted to increase in the following decade (Wu et al., 2017).

Plastics can last for a long time in the marine environment and thus present an artificial substrate for microbial colonialization. Microorganisms, such as bacteria, algae, and fungi aggregate on plastics through producing polymeric substances to adhere to each other and the surface forming a biofilm, known as the plastisphere (Amaral-Zettler et al., 2020). It was reported that groups of *Rhodobacteraceae Flavobacteriaceae*, *Alteromonadaceae*, and *Cyclobacteriaceae* are highly abundant on plastics collected from the marine ecosystem (Zettler et al., 2013; De Tender et al., 2015; Oberbeckmann et al., 2016; Xu et al., 2019; Vaksmaa et al., 2021). Microorganisms grown on plastics vary from those in the surrounding water, sediment, and organic particles (Zettler et al., 2013; De Tender et al., 2015). Nevertheless, microorganisms grown on plastics are influenced by different geographic areas (Oberbeckmann et al., 2016) and, to some extent, by polymer type (Basili et al., 2020). Furthermore, plastics in the ocean carry harmful microorganisms, including members of the genus *Vibrio* and other potentially pathogenic microorganisms such as members of *Camplylobacteraceae*, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Shewanellaceae* (Zettler et al., 2013; Amaral-Zettler et al., 2020; Zhang et al., 2021). However, the majority of research on the plastisphere that has employed high-throughput DNA sequencing has focused on plastic samples from Europe, with a few focusing on samples from Asia and Africa (Amaral-Zettler et al., 2020) as well as aquaculture (Wen et al., 2020).

Aquaculture, which is one of the fastest expanding segments of the food industry (FAO, 2020), makes extensive use of plastics due to their positive application in management and packaging (Mahapatra et al., 2011). Aquaculture systems vary greatly worldwide depending on species and region. In mariculture systems, plastics are used to keep the structures floating and are fixed in a place using ropes. For cages, plastics are used from small to high-scale facilities for ropes, nets, and buoys. In ponds, plastics are used in pond linings, ropes, floats, and fish feeders. Furthermore, plastics are generally used in the aquaculture process for packaging, feed, transportation, and in the daily life of farmers, such as cups, bags, and bottles (Lusher et al., 2017). Plastic materials from aquaculture facilities may be discarded, lost, washed ashore, or accumulated on the seafloor posing hazards for animals, fishers, and boat traffic (Andréfouët et al., 2014; Bendell, 2015). Additionally, the breakdown of these materials can lead to the formation of microplastics, which could have a further impact on the marine ecosystem. Furthermore, these plastics present a substrate for microorganism colonization as a result of nutrient accumulation and waste (Cole et al., 2009; He et al., 2022), which may increase their longevity (Carson et al., 2013; Viršek et al., 2017), affect their buoyancy (Lobelle and Cunliffe, 2011), potentially degrade them (Oberbeckmann et al., 2016), and host potential pathogens (Radisic et al., 2020; Bhagwat et al., 2021). For instance, fish pathogens (*Aeromonas salmonicida*) were found to be attached in higher numbers to plastics than to stainless steel used in aquaculture (Carballo et al., 2000). Additionally, nylon and copper nets employed in aquaculture contain potential pathogens belonging to the *Winogradskyella* and *Tenacibaculum* taxa (Canada et al., 2020). Conversely, some bacteria found in aquaculture facilities' biofilms play a critical role in the elimination of toxic metabolic wastes (Moriarty, 1997; King et al., 2004). Therefore, given the extensive use of plastics in aquaculture that can host microbial communities, which could play a critical role in aquaculture ecosystem, the plastisphere in aquaculture needs extensive investigation to determine their ecological effect on cultured species and ecosystem.

In the current study, we compared the microbial communities grown on plastics in two mariculture systems, ponds and marine ranching, in order to examine potential factors affecting the growth of the bacterial community in aquaculture, compare the levels of bacterial diversity, and identify potential pathogens and plastic-degrading bacteria. We employed high throughput 16S rRNA sequencing to identify bacterial populations growing on several kinds of plastic obtained from aquaculture systems.

MATERIALS AND METHODS

Sampling Locations

Samples were collected during July 2021 from four aquaculture sites surrounding Shandong Province in the Yellow Sea and Bohai Sea of China (**Figure S1**). We sampled plastics from two aquaculture systems (ponds and marine ranching) located in different locations. Sites located in Laizhou (S2) and Weihai (S3) were closed aquaculture ponds, whereas sites located in Qingdao (S1) and Haiyang (S4) were open mariculture areas. These sites were used for farming sea cucumbers, mussels, and seaweed. Plastics associated with the aquaculture processes (i.e., ropes, raft balls, bottles, and bags) and surrounding water were collected from each site. At every site, six plastic items that differed in texture and color were collected. Plastics were floated in the water and exposed to sun. Using sterilized scissors, blades, and tweezers, submerged plastic parts were cut into small pieces (approximately 5–10 cm), washed with sterilized seawater, placed in 50 mL sterilized tubes, and preserved in an ice box containing dry ice (approximately -87°C) until reaching to the laboratory. Thereafter, plastics were preserved at -20°C and the water samples were preserved at 4°C until analysis.

Water Nutrients

Seawater samples were collected from each site for analysis. Salinity and temperature were measured using an YSI instrument. Additionally, nitrate (NO_3-N), phosphate (PO_4-P), nitrogen dioxide (NO_2-N), and ammonia (NH_3-N) were measured through colorimetric analysis using a QuaAAtro autoanalyzer (Seal Analytical, Norderstedt, Germany). Furthermore, nutrients of potassium (k), calcium (Ca), sodium (Na), magnesium (Mg), and strontium (Sr) were determined in the water using inductively coupled plasma–mass spectrometry (ICP–MS).

FT-IR Spectroscopy

Every plastic particle was analyzed using Fourier transform infrared (FT-IR) spectroscopy (Nicolet iS50 FT-IR). To provide knowledge on the chemical structure of the samples, the spectrum was compared with several libraries in the OMNIC software (Thermo Fisher Scientific, USA).

Scanning Electron Microscopy

Each plastic sample was immediately placed in an electron microscopy fixative solution and preserved at 4°C until analysis via scanning electron microscopy (SEM). The fixed samples were washed three times for a total of 15 minutes each time with 0.1 M phosphate buffer (pH 7.4). Thereafter, postfixation with 0.1 M (pH: 7.4) phosphate buffer (1% osmium acid) at room temperature was followed by three 15-minute rinses with 0.1 M phosphate buffer. Following that, the samples were dehydrated in a graded sequence of ethanol concentrations of 30%–50%–70%–80%–90%–95%–100%–100% for 15 minutes each time, and isoamyl acetate for 15 minutes. A critical point dryer was used to dry the samples after they were collected. Finally, SEM images were captured after samples were adhered to metallic stubs using carbon stickers and sputter-coated with gold for 30 s.

Procaryotic Cell Abundance on Plastics

To quantify the total prokaryotic abundance on plastic fragments by epifluorescence microscopy, we applied the acridine orange staining protocol as described previously, with a few adaptations (Luna et al., 2002). Acridine orange is a cell-permeable and cationic dye that intercalates with nucleic acids through electrostatic interactions. Each fragment was placed into a sterile 50 mL conical centrifuge tube and covered with 30 mL of filtered (0.2-μm pore size) 2% formalin solution buffered at a pH of 8.5 with a borate buffer and immediately fixed overnight at 4°C. Then, the samples were sonicated three times for 2 min each to release the bacterial cells from plastic samples. The suspension of the plastic fragments was diluted 100 times in prefiltered seawater. Thereafter, each sample was supplemented with acridine stock solution at a final concentration of 0.01% and incubated in the dark for 30 minutes at room temperature. The stained solution was washed 3 times with PBS buffer (pH 7.4) to remove the excess dye. Aliquots were filtered onto a black nucleopore polycarbonate (0.2 μm-pore-size). Finally, 10 μL of each filter was added to microscope slides and examined by epifluorescence microscopy. For each slide, at least 5 randomly selected microscope fields were examined, and the bacterial cells were enumerated and calculated as the mean value of cells abundance per each field.

High-Throughput Sequencing for Biodiversity and Community Composition

DNA Extraction and Illumina Sequencing

Following the manufacturer's instructions, HiPure Soil DNA Kits (Guangzhou, China) were used to extract DNA from the samples. By utilizing the particular primer pairs of 341F (5'-CCT ACGGGNGGCWGCAG-3' and 806R (5'- GGACTACHVG TTTAAT -3'), the V3–V4 region of the 16S rRNA was amplified by PCR, resulting in a product length of ~466. The PCR amplifications were carried out with three replicates of a 50 μL of mixture containing 10 μL of 5 × Q5 reaction Buffer, 1.5 μL of 2.5 mM of dNTPs, 1.5 μL of each primer (10 μM), 0.2 μL of High-Fidelity DNA Polymerase, and 50 ng of DNA template (Biolabs, New England, USA). A two-minute denaturation step at 95°C was followed by 27 cycles of 98°C for 10 s, 62°C for 30 s, and 68°C for 30 s, with an elongation step of 10 min in the final PCR conditions.

An AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, United States) was used to extract and purify the amplified products from agarose gels (2%) and an ABI StepOnePlus Real-Time PCR System was used for quantification (Life Technologies, Foster City, USA). Equimolar purified amplicons were pooled and paired end sequenced (PE250) by Guangzhou Genedenovo Biotechnology Co., Ltd (Guangzhou, China) using the Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA, USA). All of the raw reads were deposited into the NCBI Sequence Read Archive (SRA) database, bioproject PRJNA815345.

Quality Control and Clustering

FASTP (0.18.0) was used to eliminate reads containing more than 10% unknown nucleotides or fewer than 50% of bases with a quality (Q-value) > 20 from the raw data. FLASH (1.2.11) was then used to combine the pair-ended clean reads with a minimum overlap of 10 bp and error rates of 2%. To obtain clean tags of high quality, paired end clean readings were filtered according to the following conditions: 1) when the number of bases in a continuous poor-quality value (the default quality threshold is ≤3) surpasses the specified length (the default length is 3 bp), raw tags were separated from the first low quality base site; and 2) then, tags with a base length of less than 75% of the tag length were excluded. Next, UPARSE pipeline software was used to perform clustering based on the clean tags into operational taxonomic units (OTUs) of 97 % (9.2.64). The UCHIME method was used to eliminate all chimeric tags, resulting in effective tags. Within each cluster, the tag sequence with the greatest abundance was chosen as the representative sequence. A naive Bayesian model was used to classify typical OTU sequences into organisms, using the RDP classifier (version 2.2) and the SILVA database (version 132), with a confidence threshold of 0.8.

Data Analysis

Community distribution and environmental characteristics relationship was studied using R software and the Vegan package version 2.5.3 using redundancy analysis (RDA) (R core Team, 2020). Additionally, Welch's t-test and Wilcoxon rank test were used to compare species between groups in the R project Vegan package (version 2.5.3). Furthermore, biomarker characteristics in each group were screened using LEfSe software (version 1.0) and R software (labdsv package version 2.0.1, pROC package version 1.10.0, and random forest package version 4.6.12). Alpha diversity analysis was conducted through Chao1, Shannon, and Simpson indexes, which were calculated in QIIME version 1.9.1. Alpha index comparisons between groups were

calculated by Welch's t-test and Wilcoxon rank test in the R project Vegan package (version 2.5.3). Analysis of the KEGG pathways of the OTUs was done using PICRUSt (version 2.1.4). BugBase was used to classify bacterial phenotypes in the microbiome. Welch's t-test, Wilcoxon rank test, Kruskal-Wallis H test, and Tukey's HSD were used in R project Vegan package to analyze function differences between groups (version 2.5.3).Where necessary, one-way ANOVA was employed to determine significant differences between samples.

RESULTS

Water Nutrients

The association between the microbial community and environmental conditions was examined using RDA, including salinity; temperature; and the nutrients PO_4-P, NO_3-N, NO_2-N, and NH_3-H. Analysis showed that most phyla were located near salinity and phosphate except of Cyanobacteria, which was located near nitrate and nitrite vectors (**Figure 1**). The results suggest that salinity plays a key role in the abundance of the microbial community rather than other environmental factors. The deviation explained by RDA was 86.02% in composition at the phylum level. Marine ranching sites had significantly higher abundance of K, but ponds had higher concentrations of NO_3 and NO_2. Also, nutrients of Mg, and Na were significantly lower in the pond at S2 than at the other sites (**Table S1**).

Scanning Electron Microscopy

Procaryotic cells were found on plastics mostly with a rod shape. Additionally, pennate diatoms were highly abundant in most samples. Examples of diatom species identified morphologically using SEM include *Amphora* sp., *Nitzschia* sp., *Navicula* sp., *Cocconeis* sp., and *Licmophora* sp. (**Figure 2**). Furthermore, we noticed that the microorganism type on the plastics could be different according to the geographic area. For instance, rod-shapped prokaryotic cells were dominant in plastics collected from S1, whereas diatoms were dominant in S3.

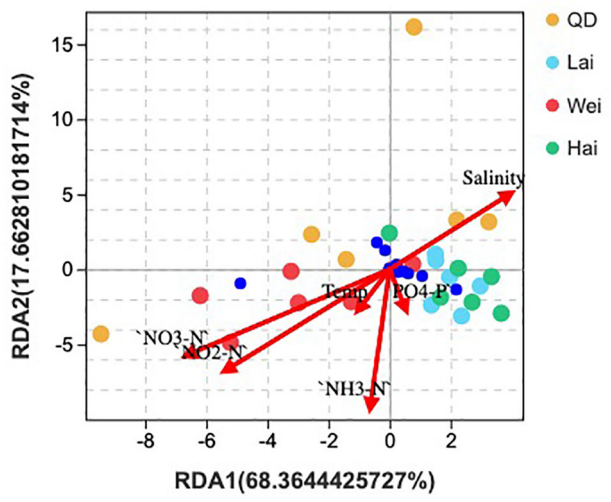

FIGURE 1 | Redundancy analysis on the correlation between relative abundance of microbial community at phylum level and water quality parameters, including phosphate, nitrate, nitrite, ammonia, temperature, and salinity. QD, Qingdao; Lai, Laizhou; Wei, Weihai; Hai, Haiyang.

Procaryotic Abundance on Plastics

To count the net number of the prokaryotes in the collected samples, we applied the acridine fluorescence dye. The numbers of prokaryotic cells were estimated, assuming a range bacterial number of $0.05–5.07\times10^5$ cells/g, with an average of 0.82 ± 1.04 cells/g. When considering the length of the samples, the abundance of the cells was $0.1–29.27\times10^3$ cells/cm^2, with an average of $2.52 \pm 5.37\times10^3$ cells/cm^2 (**Figure 3**). The highest abundance of cells was observed in samples of S1, while the lowest abundance of cells was observed in S3. No significant difference was found in the number of cells between sites ($p > 0.05$). Additionally, similar or different (i.e., PE vs. PVC) polymer type of plastics from different sites showed no significant difference in the number of cells ($p > 0.05$).

Comparing different site characteristics (i.e., ponds vs. marine ranching), we found a significantly higher number of cells in plastics samples collected from marine ranching (S1 and S4) than samples collected from ponds (S2 and S3; $1.35 \pm 1.30\times10^5$ and $0.28 \pm 0.23\times10^5$, respectively; $p < 0.05$), indicating that plastics in open aquaculture sites have higher abundance of procaryotic cells. Furthermore, we noticed high values of prokaryotic cells on plastics with rough surface and grooves, such as ropes and some aquaculture floats, indicating that the physical characteristics of plastic could determine the number of microorganisms.

Microbial Community Composition

16S high throughput sequencing revealed highly diverse of microbial communities. The effective tags reached a ratio of 85.33–91.96%, with an average of 87.61 ± 1.60% (**Figure S2**). The annotated OTUs in each sample were 848–2,584, with an average of 1,811 OTUs. There was no significant difference in OTU numbers among sites ($p > 0.05$). No significant differences in OTU numbers were found between different regions with the same polymer type ($p > 0.05$). Additionally, there were no significant differences in the OTU number of plastics with different characteristics ($p > 0.05$). Furthermore, we compared two polymer types (PE vs. PVC) and there were no significant differences between the two polymers in OTU number regardless of the location or plastic characteristics ($p > 0.05$).

In the water and plastic samples, Proteobacteria had the highest abundance among all samples at the phylum level with relative abundances of 38.29 ± 10.93% and 32.44 ± 33.75%, respectively, which were significantly higher on plastics than seawater (**Figure 4** and **Figure S3**). In the plastic samples, Cyanobacteria, Bacteroidetes, and Actinobacteria exhibited high relative abundances in the descending order of 18.60 ± 17.55%, 17.11 ± 7.14%, and 6.70 ± 5%, respectively, whereas, in water samples Actinobacteria, Cyanobacteria, and Bacteroidetes exhibited relative abundances in the order of 27.01 ± 30.58%, 15.92 ± 20.98%, and 14.31 ± 12.40%, respectively. At the class level, plastic samples had a high abundances of Alphaproteobacteria (31.94 ± 9.88%), Oxyphotobacteria (18.55 ±

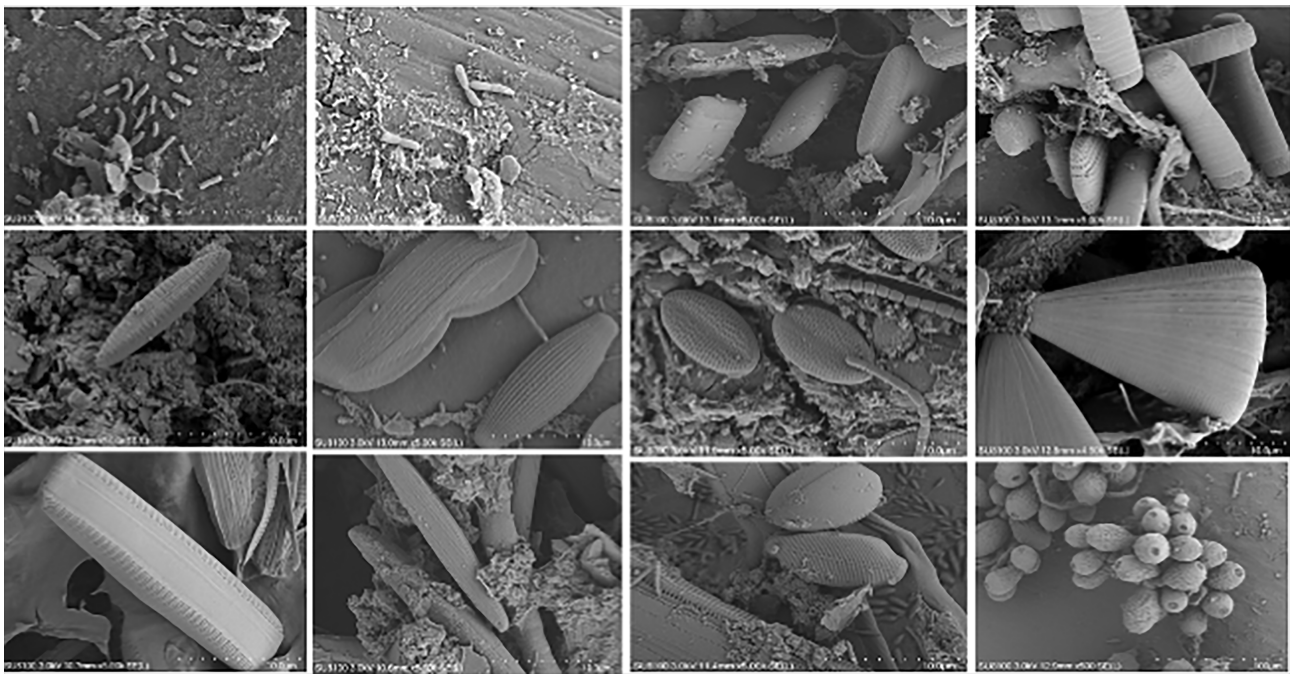

FIGURE 2 | Examples of microbial community on plastics samples using scanning electron microscopy.

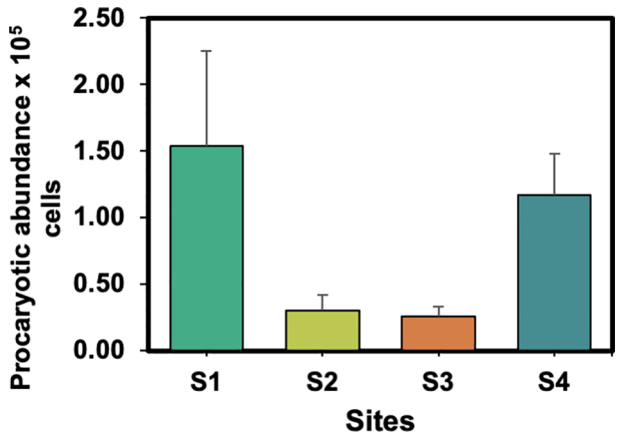

FIGURE 3 | Prokaryotic abundance on plastics sampled from aquaculture areas (mean ± SE).

17.59%), and Bacteroidia (15.87 ± 6.35%), whereas, Actinobacteria (23.33 ± 31.77%), Gammaproteobacteria (21.07 ± 35.13%), Oxyphotobacteria (15.91 ± 20.98%), and Bacteroidia (13.97 ± 12.43%) had the highest abundance in water samples. The most abundant families in plastic samples were Rhodobacteraceae (20.93 ± 9.50), Flavobacteriaceae (10.19 ± 5.50), and Sphingomonadaceae (4.56 ± 4.92), and the most abundant families in water samples were Microbacteriaceae (22.76 ± 31.78), Cyanobiaceae (12.16 ± 22.20), and Flavobacteriaceae (9.01 ± 11.28).

All the samples were identified as plastics, which included polyethylene (PE), polyethylene terephthalate (PET), polyethylene low density LDPE, polyvinyl chloride (PVC), polypropylene (PP), and ethylene vinyl acetate (**Table S2**). When comparing PVC (aquaculture floats) to PE (bags) samples from all sites, we found Cyanobacteria (33.87 ± 29.16%) in the PVC samples at a higher abundance than in the PE samples (7.03 ± 6.74%). Moreover, Bacteroidetes had a higher abundance on PE (20.45 ± 3.88%) than on PVC (8.68 ± 3.26%). Also, Firmicutes had a higher abundance on PE (12.15 ± 23.64%) than PVC (0.90 ± 0.80%) (**Figure S4**). According to the Chao1, Simpson and Shannon indexes, different polymer types (i.e., PE and PVC) had no significant difference in alpha diversity (**Figure S5**).

When considering different sites, 482 OTUs were shared among plastic samples from all sites, which were composed of/or dominated by Proteobacteria and Bacteroidetes phyla (**Figure 5**). However, there was no significant difference in the number of OTUs between the PE and PVC samples, and 1,186 OTUs were shared among them. Furthermore, PCoA analysis showed that S2 and S4 had distinct and overlapping OTU microbial assemblages from S1 or S3 (**Figure 6**).

DNA sequencing showed that there was a difference between the plastisphere community and surrounding water. For instance, a higher number of unique OTUs were found on plastic samples (1,429 OTUs) than in water samples (648 OTUs), suggesting variance in the number of unique species between plastics and

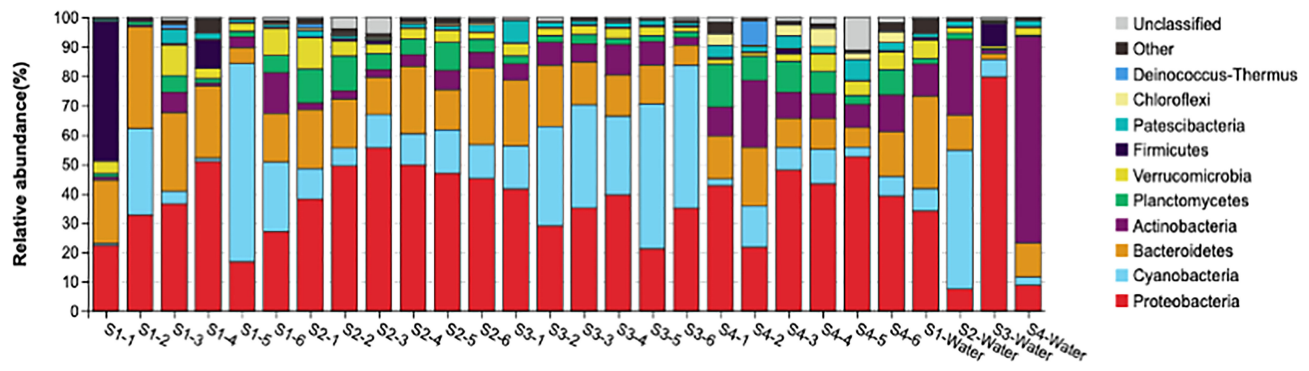

FIGURE 4 | Percentage of prokaryotic community composition of all samples at the Phylum level.

seawater. Additionally, Chao1, Shannon, and Simpson diversity indices showed that higher richness of the microbial community was detected on plastic than in seawater (**Figure S5**). Furthermore, PCoA analysis showed that water samples had distinct microbial OTU assemblages from most plastic samples (**Figure 6**).

Bacterial Community Functions

Similar functional types for bacteria were found between plastics and water samples (**Figure S7**). However, there was a significant difference between all functional composition between plastics and water (Wilcoxon rank test $p < 0.05$). The highest abundance

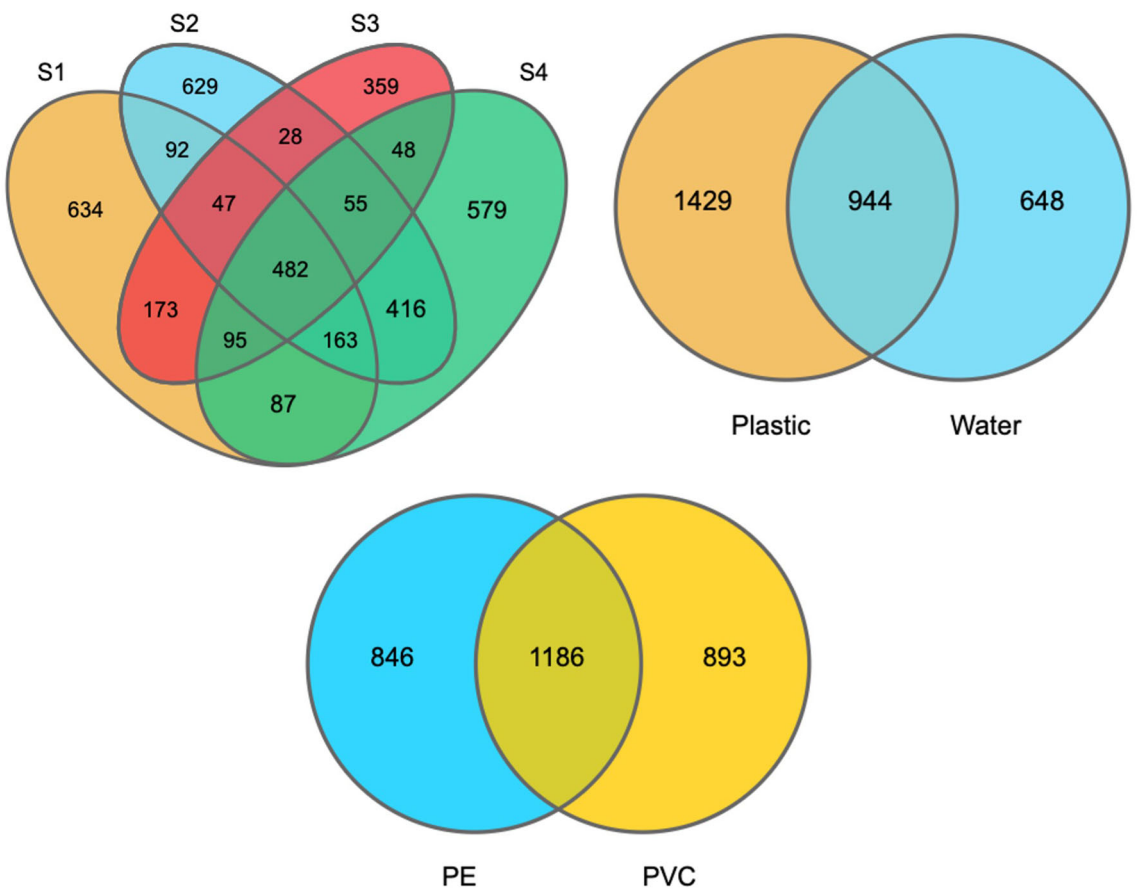

FIGURE 5 | Venn diagram shows the overlapped OTUs between plastic samples from every site (a), plastic vs. water samples, and PE vs. PVC plastic samples.

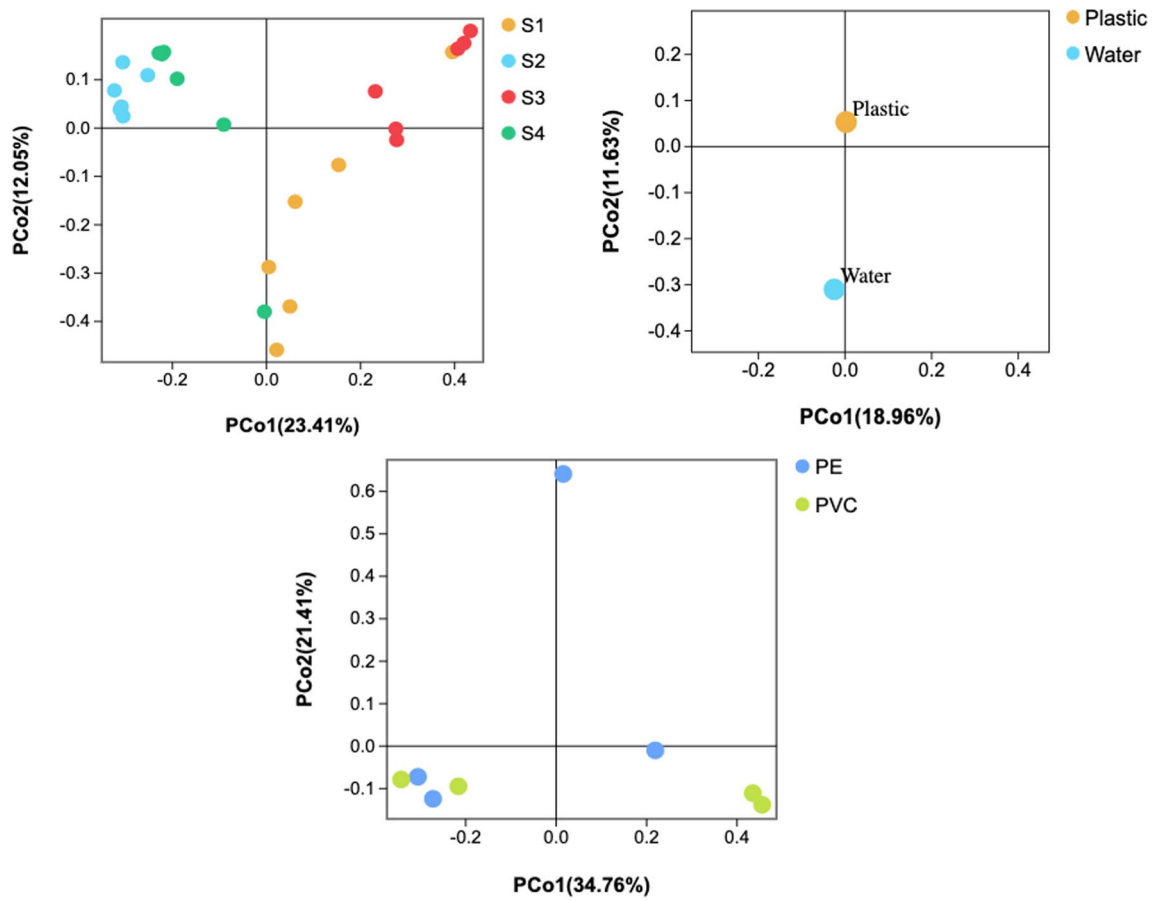

FIGURE 6 | Principal coordinate analysis (PCoA) of microbial communities based on the 16S rRNA sequencing profiles.

function for bacteria was membrane transport function and the lowest abundant function was immune disease.

Potential Pathogens

A total of 6,151 OTUs were matched with potentially pathogenic bacteria, with higher abundance on plastics than water (**Figure S8**). The most abundant phyla on plastics and water were Proteobacteria, Firmicutes, Patescibacteria, Bacteroidetes, and Acidobacteria. For the class of potentially pathogenic bacteria, the most abundant classes in the water were Gammaproteobacteria (21.069 ± 35.13%), Bacilli (1.927 ± 3.62%), Bacteroidia (1.696± 1.32%), and Alphaproteobacteria (1.290 ± 1.18%). In plastic samples, the most abundant classes were Alphaproteobacteria (6.55 ± 4.83%) Gammaproteobacteria (4.66 ± 3.43%), Bacilli (2.14 ± 9.59%), Parcubacteria (1.32 ± 1.77%), and Deltaproteobacteria (1.14 ± 0.76%). The most abundant orders of potentially pathogenic bacteria in the water were Oceanospirillales (14.517 ± 26.96%), Pseudomonadales (4.533 ± 8.78%), and Bacillales (1.927 ± 3.62%). In plastic samples, the abundant orders were Rhodobacterales (3.45 ± 3.56%), Rhizobiales (2.54 ± 1.84%), and Bacillales (2.14 ± 9.58%). Furthermore, the most abundant families in the water samples were *Halomonadaceae* (13.73 ± 27.45%), *Moraxellaceae* (4.52 ± 8.77%), and *Bacillaceae* (1.71 ± 3.39%), whereas, in the plastic samples, the most abundant families were *Rhodobacteraceae* (3.45 ± 3.58%), *Rhizobiaceae* (2.07 ± 1.66%), and *Planococcaceae* (1.95 ± 9.13%). Additionally, the average abundances of *Vibrionaceae*, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Shewanellaceae*, which are regarded as opportunistic pathogen families, were 0.14 ± 0.25%, 0.31 ± 1.07%, 0.01 ± 0.02% in plastic and 0.01 ± 0.03%, and in water 0.07 ± 0.08%, 0.06 ± 0.05%, 0.02 ± 0.02%, and 0.02 ± 0.03%, respectively. The most abundant genera in the water samples were *Cobetia* (13.63 ± 23.61%), *Psychrobacter* (4.42 ± 7.56%), and *Bacillus* (1.66 ± 2.86%). In plastic samples, the most abundant genera were *Ruegeria* (1.13 ± 2.39%), *Pseudahrensia* (0.30 ± 0.30%), *Psychrobacter* (0.26 ± 1%), and *Granulosicoccus* (0.26 ± 0.35%). For species abundance, *Bacillus hwajinpoensis* was the most abundant species in water (1.64 ± 3.26%), while in plastics, *Marichromatium* sp. (0.23 ± 1.07%) and *Marinicella litoralis* (0.15 ± 0.16%) were the most abundant species.

Plastic Degrading Bacteria

Various bacterial families that include members known as hydrocarbon degraders were identified, including *Rhodobacteraceae, Flavobacteriaceae, Saprospiraceae, Alteromonadaceae*, *Sphingomonadaceae, Rhodospirillaceae*, and *Oscillatoriaceae*. The most abundant families that include members known as hydrocarbon degraders were *Rhodobacteraceae* (20.93 ± 9.50%),

Flavobacteriaceae (10.19 ± 5.50%), and *Saprospiraceae* (3.52 ± 4.35%; **Figure 7**). Among them, the relative abundance of *Rhodobacteraceae* was higher in plastics (20.93 ± 9.50%) than in the surrounding seawater (7.57 ± 7.10%; $p < 0.05$; Wilcoxon rank test and LEfSe analysis; **Figure S3**). Different genera that described previously as hydrocarbon degraders were found on plastics, including *Erythrobacter*, *Lewinella*, *Winogradskyella*, *Persicirhabdus*, *Altererythrobacter*, *Alcanivorax*, *Crocinitomix*, *Alteromonas*, *Hyphomonas*, *Oleibacter*, *Dokdonia*, *Tenacibaculum*, *Owenweeksia*, and *Marinobacter* (**Figure 7**). Among these genera, *Erythrobacter* was the most abundant genus on plastics (2.10 ± 3.39%; $p < 0.05$; Wilcoxon rank test), which was higher on plastics than in the surrounding water (0.41 ± 0.32%), constituting 5% of the Proteobacteria phylum, which followed by *Lewinella* (1.68 ± 4.36%), *Winogradskyella* (0.83 ± 1.30), *Persicirhabdus* (0.68 ± 1.13%), and *Altererythrobacter* (0.52 ± 0.43%).

DISCUSSION

Aquaculture extensively utilizes plastics due to their positive application in management and packaging (Mahapatra et al., 2011).

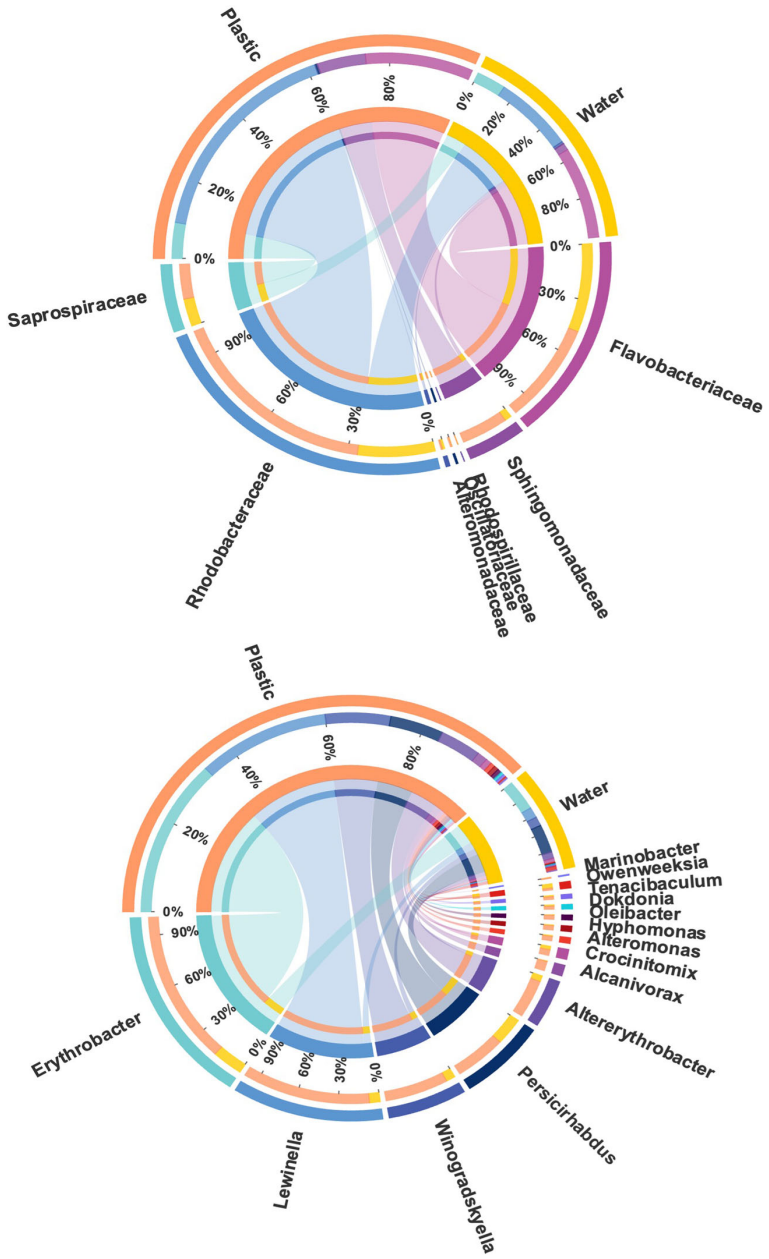

FIGURE 7 | Circos diagram displays the composition relationship between different plastic degrading bacterial families and genera. The lines on both sides indicate the corresponding relationship pair, and the thicker the line, the greater the abundance value.

These plastics present a substrate for microorganisms colonization, some of which are considered harmful or pathogenic (Amaral-Zettler et al., 2020). The abundance of organic matter, as well as phosphorus and nitrogenous metabolites, makes the aquaculture field suitable media for microbial growth (Martínez-Porchas and Vargas-Albores, 2017). These microorganisms play a significant role in aquaculture, maintaining the water quality, and the health of cultured species (Moriarty, 1997; Bentzon-Tilia et al., 2016). Therefore, understanding the role of plastics in microbial colonialization in aquaculture systems is critical for understanding the biological impact of plastics in these systems. Herein, we sampled plastics used in two aquaculture systems, i.e., ponds and marine ranching (i.e., open mariculture areas). The sampled plastics originated from aquaculture floats and nets used in suspended culture, as well as bottles and packaging bags.

The PE polymer was found to be the most prevalent component of the plastics tested. PE is the most common plastic type utilized in daily life and is primarly used in packaging, pipes, and containers. Additionally plastics, such as LDPE and high-density polyethylene (HDPE) are manufactured in vast quantities using PE (Lusher et al., 2017). Furthermore, PE is widely used in aquaculture in ropes and floats due to its low density (Andrady, 2011; Wu et al., 2020). In the current study, all the sampled plastics were floated on the surface of seawater which could explain the high abundance of PE.

SEM analysis showed that procaryotic cells in rod shape and pennate diatoms were dominantly abundant in the plastic samples. Diatoms play a key role in biofilm formation on plastics as they are the first colonizers of the surface (Eich et al., 2015; Zhao et al., 2021). Studies on the biofilm composition of plastics have showed that diatoms, such as coccolithophores and cyanobacteria, were the most abundant microorganisms on the floating plastics (Casabianca et al., 2019). Additionally, SEM analysis showed that diatoms were dominant in S3, whereas rod-shaped bacteria were dominant in S1. This could indicate that the characteristics of the area affect the type of microorganisms grown on plastics in aquaculture areas. Similarly, the microorganism communities differed significantly among locations (Oberbeckmann et al., 2016).

The average abundance of prokaryotic cells on plastics in the aquaculture areas was lower than procaryotic abundance on those collected from coastal areas impacted with anthropogenic pollution (Basili et al., 2020), but higher than that found on plastic particles collected from the Mediterranean Sea (Dussud et al., 2018). Considering different polymer types, there was no significant difference in the prokaryotic abundance between different polymers among sites. Similar results were observed in plastics collected from the Mediterranean Sea (Basili et al., 2020). However, the abundance of prokaryotic cells was significantly higher in marine ranching sites than in ponds. This might be because of nutrient differences between ponds and marine ranching sites. Marine ranching had a significantly higher abundances of K, Na, and Mg than ponds. A nutrient increase positively correlates with the amount of bacteria attached (Cowan et al., 1991; Donlan, 2002). Similarly, nutrients and salinity affect the growth rate of biofilm (Li et al., 2019; He et al., 2022). Furthermore, plastics with special physical characteristics, such as grooves and rough surface, were found to contain a high number of prokaryotic bacteria. Similarly, physical and chemical features of the substrate shape the microorganism community (Kirstein et al., 2018).

In the water samples, the microbial community was dominated by Proteobacteria, which was followed by Actinobacteria, Cyanobacteria, and Bacteroidetes. Similarly, microbial communities in the marine ranching area of Laoshan Bay was dominated by Proteobacteria, which was followed by Cyanobacteria, and Actinobacteriota (Fang et al., 2021). In aquaculture ponds of shrimp *Litopenaeus vannamei* in Zhongshan, China, Proteobacteria was dominant, followed by Tenericutes, and Bacteroidetes (Zhang et al., 2019). In another shrimp pond in Dongying, China, Bacteroidetes were the dominant in the water, followed by Proteobacteria, Actinobacteria, and Cyanobacteria (Huang et al., 2018).

The composition of plastics was dominated by Proteobacteria followed by Cyanobacteria, Bacteroidetes, and Actinobacteria. Similarly, Proteobacteria was the most abundant phylum identified on microplastics placed in aquaculture ponds for shrimp, followed by Bacteroidetes, Planctomycetes, and Cyanobacteria (Deng et al., 2021). Additionally, Proteobacteria was the dominant phylum identified on plastics collected from beaches in the Mediterranean Sea (Basili et al., 2020; Vaksmaa et al., 2021). Furthermore, Bacteroidetes, Actinobacteria, and Cyanobacteria were highly abundant among biofilm communities on plastics collected from beaches in the Mediterranean (Basili et al., 2020). In the Mediterranean Sea, Proteobacteria was followed by Bacteroidetes and Cyanobacteria (Vaksmaa et al., 2021). Moreover, plastic marine debris in the Mediterranean Sea was dominated by Cyanobacteria and Alphaproteobacteria (Dussud et al., 2018).

16S rRNA sequencing showed that there was a difference between the plastisphere community and that in the surrounding water. For instance, a higher number of unique OTUs was found on plastic samples (1,429 OTUs) than in water samples (648 OTUs), suggesting variance in the number of unique species between plastics and seawater (**Figure 5**). Additionally, we observed differences between the microbial community of plastics and surrounding water at the phylum, order, class, family, and genus levels (shown above). Furthermore, the Chao1 and Shannon diversity indexes showed that higher richness of the microbial community on plastic than in seawater (**Figure S5**). Moreover, PCoA analysis showed that water samples had distinct microbial OTU assemblages from most plastic samples (**Figure 6**). This indicates that plastic samples in aquaculture areas have a variable abundance of communities compared to seawater. This observation is in agreement with observations of biofilm on microplastics in aquaculture (Deng et al., 2021) and previous studies in the open ocean (De Tender et al., 2015; Dussud et al., 2018; Vaksmaa et al., 2021).

When comparing different polymer types (i.e., PVC vs. PE), we found Cyanobacteria had a higher abundance on PVC

samples than PE samples, whereas Bacteroidetes and Firmicutes had a higher abundance on PE samples than PVC samples. This difference might not only be because of different polymer types but also because of the surface characteristics, such as polymer type and surface characteristics influence the type of microorganisms that attached (Amaral-Zettler et al., 2020). Alpha diversity indexes showed that there was no significant difference between the two polymer types and both shared high numbers of OTUs. Collectively, polymer type might influence the abundance of microbial groups on plastics (i.e., specific groups of microbes might prefer one polymer over another). This observation is in agreement with previous studies that concluded polymer type affects the bacterial community attached to plastics; there was a significant difference in the microbial community on PE compared to PP or PS (Vaksmaa et al., 2021).

When considering different sites, S2 and S4 had distinct and overlapping OTUs from S1 and S3. Additionally, only 482 OTUs were shared among sites. Furthermore, our SEM observation showed distinct microorganisms on plastics in S1 and S3 from S2 and S4. Collectively, this observation is in agreement with previous studies concluding that the microbial community structure differs according to the geographic area (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2016; Basili et al., 2020). Furthermore, the membrane transport function was highly represented by the biofilm community on plastics compared to other predicted functions. This observation was reported previously from plastics marine debris collected from the western Mediterranean Sea (Dussud et al., 2018) and submerged plastic pellets in Australia (Bhagwat et al., 2021), which is an essential function for biofilm formation (Dussud et al., 2018).

It has been reported that plastic particles can accumulate pathogenic bacteria and harmful microalgae, which indicates that plastic particles may act as carriers of pathogenic bacteria, resulting in the spread of diseases (Amaral-Zettler et al., 2020; Meng et al., 2021). According to the finding of the current study, bacterial families identified include well-known fish and shellfish potential pathogenic strains, which support the potential for plastics to serve as vectors for possible pathogenic microbes. This might pose a threat to aquaculture profitability and cultured species. For instance, Rhodobacteraceae was the most abundant family on plastic samples, and is widely regarded as potential pathogenic bacteria (Meng et al., 2021). Some members of this family may contribute to shrimp, sea cucumber, and coral disease (Soffer et al., 2015; Zhang et al., 2019; Deng et al., 2021). Additionally, bacterial families commonly regarded as potential pathogens, such as *Vibrionaceae, Enterobacteriaceae, Pseudomonadaceae,* and *Shewanellacea* were higher on plastics than in water samples. The abundance of these families was lower than or comparable to those detected on plastics employed in the Yellow Sea (Zhang et al., 2021). Also, the family *Vibrionaceae* has been widely confirmed on marine plastics and is dominated by the *Vibrio* genus and a large number of potential pathogens (Amaral-Zettler et al., 2020). The *Vibrio* genus includes animal and human pathogens that have been responsible for catastrophic pandemics and innumerable epidemics around the world and could result in significant financial loss for aquaculture farms (Laverty et al., 2020). Taken together, our findings support the hypothesis that plastic acts as a vector for potential pathogens.

Conversely, some microbes were reported to have a positive impact on aquaculture management. For instance, some members of the family *Rhodobacteraceae* produce antibacterial compounds that can inhibit fish pathogens (Henriksen et al., 2022). Also, members of the family *Rhizobiaceae* were identified among the denitrifying bacteria in the recirculation aquaculture system (Chen et al., 2021). Additionally, the genus *Ruegeria* had the highest abundance on plastic samples, which is a common bacteria in the aquaculture system and was previously detected on microplastic particles (Zhang et al., 2020). This genus includes members that have probiotic potential due to inhibition of fish pathogens (Sonnenschein et al., 2017). Furthermore, *Bacillus hwajinpoensis*, which was the most abundant species in water samples, has been reported as a dominant species in aquaculture water and is regarded as probiotic to improve the water quality and inhabit pathogenic bacteria (Wei et al., 2021). Additionally, *Marichromatium* sp. was the most abundant species identified on plastics, which was reported to improve water quality (Zhu et al., 2019). We anticipate that the extent of the presence of harmful or beneficial bacteria in the aquaculture system may depend on the effective management of the water quality in the farm.

Microorganisms maybe able to provide solutions for plastic pollution through biodegradation (Amaral-Zettler et al., 2020). The *Rhodobacteraceae* family was found to be the most abundant family on the plastic samples and higher than of seawater. Members of this family are known as hydrocarbon degraders of which *Rhodococcus ruber* has been shown to degrade PE (Gilan et al., 2004; Dubinsky et al., 2013). Several studies have reported a high abundance of the family Rhodobacteraceae on plastic samples (Bryant et al., 2016; Dussud et al., 2018). Furthermore, we found 14 genera that were previously reported to include hydrocarbon degrading bacteria, of which *Erythrobacter* had the highest abundance, and these were previously detected on PE plastic samples (Vaksmaa et al., 2021).

CONCLUSION

In the current study, we investigated the microorganisms associated with plastics collected from aquaculture areas (i.e., ponds and marine ranching). Our findings indicated that the amount of bacterial community associated with plastics was significantly different in open aquaculture areas than in closed ponds, regardless of polymers type. Additionally, 16S rRNA gene sequencing and SEM analysis showed that the type of microbial communities differed among the aquaculture areas. Also, our results showed that plastic samples in aquaculture areas had a distinct abundance of the microbial community from seawater samples. Additionally, different polymers may influence the abundance of specific microbial communities. Furthermore, the abundance of potential pathogenic bacteria was higher on plastics than in seawater. However, the dominance of potential probiotic bacteria

that have the potential to inhabit pathogens might explain the limited abundance of potential pathogens in the samples collected from aquaculture fields. Moreover, the high abundance of genera including hydrocarbon degrading bacteria indicated that these groups might play a role in plastic degradation. Further research could focus on manipulating and managing beneficial microbes on plastics to enhance the aquaculture management.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI [accession: PRJNA815345].

AUTHOR CONTRIBUTIONS

MM and CL study design and samples collection. HH samples analysis. MM and AA-Z manuscript writing and review. MM data analysis. HY fund and supervision. All authors revised and/or edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.895611/full#supplementary-material

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Influence of Microplastics on Microbial Structure, Function, and Mechanical Properties of Stream Periphyton

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Periphyton is a freshwater biofilm composed of prokaryotic and eukaryotic communities that occupy rocks and sediments, forming the base of the food web and playing a key role in nutrient cycling. Given the large surface that periphyton comprises, it may also act as a sink for a diverse range of man-made pollutants, including microplastics (MP). Here we investigated the effect of 1–4 µm and 63–75 µm sized, spherical polyethylene MP with native and ultraviolet (UV)-weathered surface on developing natural stream periphyton communities over 28 days. In order to ensure proper particle exposure, we first tested MP suspension in water or in water containing either Tween 80, extracellular polymeric substances – EPS, fulvic acids, or protein. We found the extract of EPS from natural periphyton to be most suitable to create MP suspensions in preparation of exposure. Upon exposure, all tested types of MP were found to be associated with the periphyton, independent of their size and other properties. While biomass accrual and phenotypic community structure of the photoautotrophs remained unchanged, the prokaryotic and eukaryotic communities experienced a significant change in composition and relative abundances. Moreover, alpha diversity was affected in eukaryotes, but not in prokaryotes. The observed changes were more prominent in periphyton exposed to UV-treated as compared with native surface MP. Mechanical properties, as assessed by compression rheology, showed that MP-exposed periphyton had longer filamentous streamers, higher stiffness, lower force recovery and a higher viscoelasticity than control periphyton. Despite the observed structural and mechanical changes of periphyton, functional parameters (i.e., photosynthetic yield, respiration and nutrient uptake efficiencies) were not altered by MP, indicating the absence of MP toxicity, and suggesting functional redundancy in the communities. Together, our results provide further proof that periphyton is a sink for MP and demonstrate that MP can impact local microbial community composition and mechanical properties of the biofilms. Consequences of these findings might be a change in dislodgement

behavior of periphyton, a propagation through the food chains and impacts on nutrient cycling and energy transfer. Hence, taking the omnipresence, high persistence and material and size diversity of MP in the aquatic environment into account, their ecological consequences need further investigation.

Keywords: particles, community composition, microbial function, ecotoxicity, aquatic biofilms

INTRODUCTION

Microplastics (MP) are solid particles of polymeric matrix in the size range of 1 μm–5 mm. They are either intentionally produced (primary MP) for use, for example, in cosmetics and paints, or are generated as secondary MP from fragmentation of larger plastic debris, for example, by UV-oxidation, mechanical abrasion and/or biodegradation (Verschoor, 2015; Frias and Nash, 2019). Despite a persistent lack of standardized MP identification and quantification methods, which hampers comparability among data sets (Shim et al., 2017; Abdolahpur Monikh et al., 2022), many monitoring studies showed the ubiquitous abundance of MP in different environments. For instance, MP have been detected in freshwater (Li et al., 2018), marine (Galloway et al., 2017), and terrestrial environments (Rillig and Lehmann, 2020) and found to enter even the most remote areas (e.g., Alps, arctic (Bergmann et al., 2019), and deep-sea sediments (Van Cauwenberghe et al., 2013). However, impacts of the presence of MP on environmental and ecological processes remain largely unresolved (Ockenden et al., 2021).

MP reach the aquatic environment *via* aerial deposition, storm water runoff or wastewater treatment plant effluents (Horton and Dixon, 2018). Once in rivers and streams, MP can be retained in the river bed (Hurley et al., 2018) by entering the hyporheic zones (Drummond et al., 2020) and concentrating in stream biofilms (Huang et al., 2021). Such biofilm communities, also known as periphyton, are complex mixtures of prokaryotes and eukaryotes, including microalgae, cyanobacteria, and heterotrophic microorganisms, attached to submerged surfaces. Indeed, Sgier et al. (2016) detected MP in periphyton downstream of a wastewater treatment plant when examining stream biofilm composition using individual cell-based analysis. Yet, little is known about the interaction of MP and periphyton despite its importance as the base of the food web (Guo et al., 2022), nutrient cycling (Battin et al., 2003; Battin et al., 2016), and its role as early warning systems for contamination detection (Montuelle et al., 2010). The only prior study to examine the interactions of MP and periphyton reported negligible effects on certain periphyton parameters in short-term (3 h) exposures: chlorophyll a, photosynthetic yield, extracellular enzymatic activity of ß-glucosidase, leucine aminopeptidase, alkaline phosphatase (Miao et al., 2019). Only plastic particles in the nanosize (NP) in a high concentration (100 nm, 100 mg L^{-1}) led to a decrease of chlorophyll *a* content and the activity of ß-glucosidase and leucine aminopeptidase, and induced oxidative stress (Miao et al., 2019). However, there are no reports on effects of MP on periphyton following long-term exposures, as was highlighted also by a recent review (Kalčíková and Bundschuh, 2021).

Peripyhton organisms form complex three-dimensional structures embedded in a matrix of extracellular polymeric substances (EPS) (Lock et al., 1984). These polymeric substances may aid in suspending MP (Balakrishnan et al., 2019). Next, MP can interact with periphyton organisms. Thus far, effects on periphyton communities are unknown; however, results from studies with microalgae showed lower growth rate (Yang et al., 2020) and photosynthetic efficiency (Mao et al., 2018) with decreasing MP size suggesting MP size as an important parameter ruling MP-cell interactions. Moreover, considering that the surface of MP can serve as a substrate or attachment of microorganisms (Zettler et al., 2013; McCormick et al., 2014), MP can be hypothesized to affect periphyton community composition by selecting for specific taxa. MP surface characteristics, for example, weathering state and with this hydrophobicity and roughness, have been also shown to impact the attachment of microorganisms (Kelly et al., 2020; Rummel et al., 2021). Different surface properties may therefore influence community composition differently. Finally, it can be envisioned that MP, when incorporated into periphyton, affect the mechanical properties of the biofilm, such as its stiffness and viscoelasticity. Both these properties are related to biofilm architecture. Architecture is defined by a base (substrate attached) and a streamer (floating in the water column) zone (Besemer et al., 2009), both of which comprise important factors for how periphyton breaks up and disperses under perturbations (Lopez-Sanchez et al., 2014; Battin et al., 2016).

In the present study, we set out to examine the interaction of MP with periphyton growing over 28 days from microorganisms stemming from a natural stream. Maturing periphyton was exposed to spherical polyethylene (PE) MP of two different size ranges (1–4 μm and 63–75 μm in diameter), in native and UV-weathered (aged) forms. Dispersion experiments identified EPS to be the most suitable to maintain a relatively homogeneous particle suspension for subsequent exposure experiments of the biofilm. At three time points, peripyhton structural (community composition and abundance of its members), functional (photosynthetic yield, respiration, nitrogen uptake rates), and mechanical (stiffness, viscoelasticity) properties were measured. This is the first long-term periphyton-MP interaction study revealing a variety of structural and mechanical changes in the periphyton while functional properties were found to be maintained.

MATERIALS AND METHODS

Materials

Three spherical MP particle types were purchased from Cospheric (United States) as dry powder with characteristics and terminology as shown in **Table 1**. MP size ranges were

TABLE 1 | Physical and chemical properties of the microplastic particles (MP) used in this study.

Experiment[a]	Cospheric product ID	Material	Color	Size (μm)	Density (g mL^{-1})	Abs.[c] (nm)	UV-treated	Code
1	CPMS-0.96 1-4um	PE[b]	Trans-parent	1-4	0.96	No	No	sMP
1	CPMS-0.96 1-4um	PE	Trans-parent	1-4	0.96	No	Yes	sMP*aged*
2	WPMS-1.25 63-75um	PE	white	63-75	1.25	No	No	bMP
2	WPMS-1.25 63-75um	PE	white	63-75	1.25	No	Yes	bMP*aged*
3	FMR	Thermoset amino formaldehyde polymer	red	1-5	1.3	607	No	rMP

[a]*Experiment number in which MP type was used (see* ***Figure 1****).*
[b]*PE, polyethylene.*
[c]*Abs, absorbance.*

selected to be either similar/smaller (sMP, rMP) or bigger (bMP) than periphyton organisms (**Supplementary Figure S1**). Fulvic acids (Suwannee River I standard) were purchased at the International Humic Substances Society (United States). All other chemicals were purchased from Sigma-Aldrich (Buchs SG, Switzerland) unless indicated otherwise.

MP Aging, Dispersion, and Characterization

The surface of both smaller (sMP) and bigger (bMP) was altered by UV irradiation in closed glass Petri dishes placed in a weathering chamber (Q-Sun XE-3 Xenon Test Chamber, Q-Lab, Germany). The weathering chamber was equipped with a Xenon light source with a power of 0.55 W m^{-2} and a wavelength of 340 nm. UV irradiation was stopped after considerable surface changes were observable by electron microscopy (1248 h of UV irradiation). UV exposure lasted 1248 h at 65 ± 3°C (black standard temperature for polymers). To maintain humidity in the dishes, MP were moistened once a week with deionized water. In the following, sMP*aged* and bMP*aged* refers to the UV-treated sMP and bMP (**Table 1**).

The effect of UV exposure on sMP and bMP surface structure was characterized by scanning electron microscopy (SEM) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) measurements. The SEM images were taken using an ULTRA 55 (Carl Zeiss SMT) microscope. An accelerating voltage of 2 kV was applied. The ATR-FTIR measurements were performed using a Cary 640 FTIR spectrometer (Agilent) with a diamond ATR accessory type IIa synthetic diamond crystal (penetration depth of ~2 μm). The spectra were recorded in a frequency range of 4000–600 cm^{-1} with a spectral resolution of 4 cm^{-1}. A total of 128 scans were co-added for every spectrum. The background was measured with the same settings against air. The spectrometer was controlled by Agilent Resolutions Pro software 5.2.0.

With the objective to disperse MP in aqueous solution, different suspension agents were tested for their ability to suspend and stabilize the particles in water: Tween 80 (0.01% w/w), bovine serum albumin (BSA, 5 mg mL^{-1}), starch (5 mg mL^{-1}), standard fulvic acids (FA, 40 mg mL^{-1}), and an extract of periphyton extracellular polymeric substances (EPS, for isolation see section on Periphyton colonization and EPS extraction below) (**Supplementary Figure S2**). Briefly, 18 mg of bMP, and 10 mg of sMP and rMP were weighted into a 20 mL glass vial, and 10 mL of the respective medium was added to bMP and sMP. For rMP, only EPS extract was tested. The respective vials were sonicated for 30 s in an ultrasonic bath (45 kHz, 60 W, VWR Ultrasonic Cleaner) and vortexed. The vials were then sampled (20 μL) in reposing state at a fixed height after 10, 30, 60, and 300 s. To determine the MP behavior after a longer period, rMP concentrations in the EPS-derived suspensions were additionally measured after 15 and 80 min. To quantify the particle concentrations in the subsamples, two different instruments were used to account for the size restrictions of the respective hardware: a CASY cell counter Model TT (Roche 468 Innovatis AG) was used for sMP and rMP (smaller size range) while a Multisizer II (Beckmann Coulter, Fullerton, CA, United States) was used for bMP (larger size range). Since the resulting suspensions contained visible aggregates sticking to the glass walls of the vials, we calculated MP dispersion as the percentage of the initially weighted number of MP. Results showed that in all suspensions, no more than 12% of sMP and rMP and 36% of bMP of the initially weighted particles were dispersed (**Supplementary Figure S2**). Overall, EPS yielded the best dispersion in terms of % MP over time and was therefore used for all exposure experiments.

Periphyton Colonization and EPS Extraction

For the 1) inoculation of the MP exposure experiments and 2) the extraction of EPS as medium for MP suspensions, periphyton was grown from a natural stream water as described previously by Gil-Allué et al. (2015). Briefly, water from the Chriesbach in Dübendorf, Switzerland, was pumped through indoor, flow-through channels that held 80 microscope slides (76 × 26 mm) in vertical direction aligned with the water flow. After 21 days, the colonized slides were sampled and periphyton was scraped off the slides and transferred into a glass beaker (250 mL).

To create the stock suspension for the inoculation of the microcosms (see section on Design of exposure experiments below), periphyton was suspended in synthetic freshwater medium that was designed to closely mimic creek water elemental composition (PERIQUIL, **Supplementary Table S1**; Stewart et al., 2013). Periphyton concentration was adjusted to an optical density (OD) of 4 at a wavelength of λ = 645 nm (Cary 100

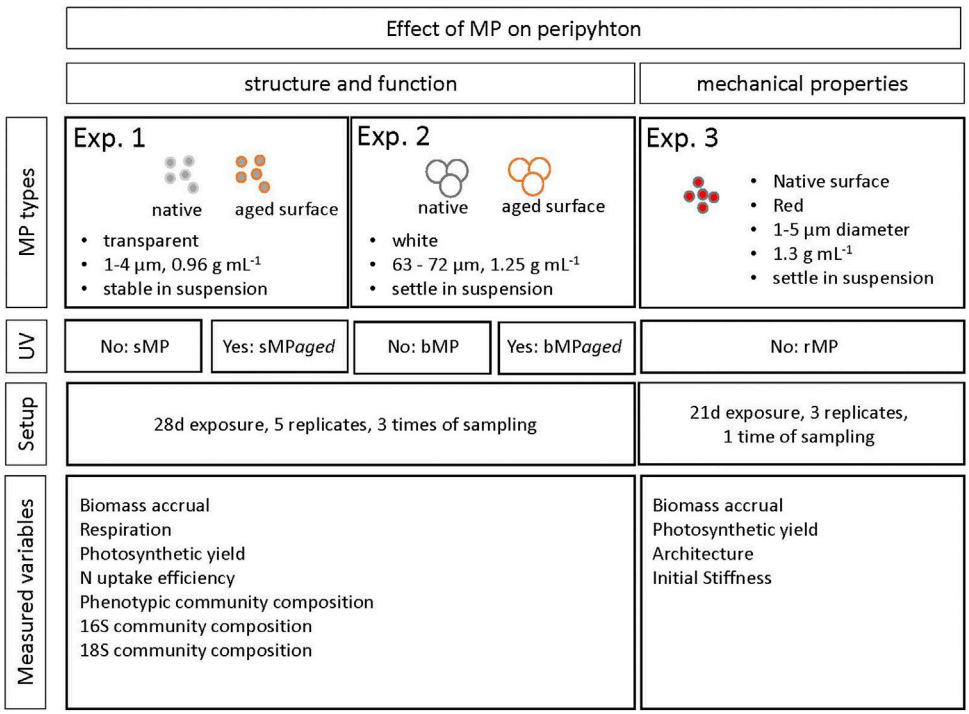

FIGURE 1 | Overview of the study design, including indication on MP characteristics used in each experiment and measured variables.

Thermo Fisher spectrophotometer, Kontron Instruments, Basel, Switzerland).

For the extraction of EPS, periphyton of three glass slides was dispersed in 3 mL of 2 mM sodium hydrogen carbonate, sonicated for 30 s in an ultrasonic bath, before adjusting the OD to four. Subsequently, this periphyton suspension was centrifuged at 1880 x g for 10 min and the supernatant filtered (0.2 µm pore size, Whatmann) into a sterile Falcon tube as previously described (Stewart et al., 2013). The remaining periphyton pellet was again suspended in 3 mL of 2 mM sodium hydrogen carbonate, sonicated, and centrifuged as described above. The supernatant of the second extraction step was filtered *via* 0.2 µm filters into a sterile Falcon tube. This suspension is subsequently referred to as "EPS". The periphyton stock suspensions as well as the EPS were prepared freshly from the same periphyton source just before use in the exposure experiments (see below: Design of exposure experiments).

Design of Exposure Experiments

In three independent experiments, we grew periphyton in microcosms in the presence of sMP/sMP*aged* (Experiment 1), bMP/bMP*aged* (Experiment 2), and rMP (Experiment 3, **Figure 1**). Experiments 1, 2 aimed to characterize and quantify the effects of MP with native and UV-treated surface on the developing periphyton, and hence periphyton community structure (phenotypic composition, 16S and 18S community composition) and function (photosynthetic yield, respiration, nutrient uptake rates) were measured. Experiment 3 aimed to evaluate the effect of MP on the mechanical properties of the mature periphyton. All exposure experiments were carried out in glass microcosms (210 × 150 × 70 mm) that were filled with 500 mL PERIQUIL. For Experiments 1, 2, each microcosm was loaded with 12 clean glass slides (76 × 26 mm, Schott). Each treatment was replicated five times. For Experiment 3, microcosms were loaded with three glass slides and each treatment replicated three times. To start the experiments, microcosms were inoculated with periphyton stock suspension and the respective MP (see below). The MP concentrations were chosen so as to provide comparable plastic surface areas in each experiment.

Microcosms of Experiment 1 were inoculated with 10 mL of a freshly prepared periphyton stock suspension (obtained after colonization in the flow-through channels as described above) and 1 mL of either sMP or sMP*aged* stock suspension (1 mg mL^{-1}) in EPS, resulting in 2 µg mL^{-1} MP in the microcosms. This concentration leads to a total MP surface of 8.0×10^6 μm^2 within each microcosm, assuming smooth particle surface. Those values reflect the nominal concentrations, and exposure concentrations are expected to be lower due to the findings from the dispersion experiments (see: M&M, MP aging, dispersion, and characterization), which indicated lower concentrations of MP in dispersion than nominal concentrations because of agglomeration. Control microcosms received 1 mL EPS alone.

In Experiment 2, bMP or bMP*aged* were weighted into Erlenmeyer flasks and were dispersed in peripyhton stock suspension to a concentration of 1.4 mg mL^{-1}. Then, the microcosms were inoculated with 10 mL periphyton-bMP and

periphyton-bMP*aged* suspension resulting in 28 μg mL^{-1} exposure concentration in the microcosms. This concentration leads to a total MP surface of 3.0×10^6 μm^2 within each microcosm, assuming smooth particle surface. Control microcosms were inoculated with 10 mL peripyhton stock suspension without bMP.

In Experiment 3, microcosms were inoculated with 10 mL periphyton and 1 mL of rMP stock suspension (1 mg mL^{-1}) in EPS. Control microcosms received 1 mL EPS alone.

In each experiment, all microcosms were placed on a two-dimensional shaker at 18°C; a 12 h dark/light photoperiod was applied (LED light source). The experiments were run for 28 (Experiments 1, 2) and 21 (Experiment 3) days. After a 7 day period to allow periphyton to attach, medium was changed for the first time and then every 3 days in order to maintain nutrient levels (Gil-Allué et al., 2018). For this, the medium was taken out carefully using a syringe (50 mL), aiming to not disturb periphyton growth and to minimize the MP removal during water change. In order to compensate for the loss of dispersed sMP and sMP*aged* in Experiment 1, both were re-spiked to the microcosms after each medium change with concentrations similar as in the initial spiking. Re-spiking was not necessary in Experiments 2, 3 since the MP sedimented within 30 s (**Supplementary Figure S2**).

In Experiments 1, 2, periphyton was sampled for MP quantification and assessment of functional and structural parameters (see below) at day 7, 21, and 28 of exposure by randomly collecting three slides per microcosm. These sampling days were selected to cover microbial succession based on previous studies using the same experimental set up (Tlili et al., 2011b; Sgier et al., 2016). Periphyton was scraped off from each glass slide using a clean glass slide and samples from one microcosm were pooled and suspended in 13 mL PERIQUIL. After sampling the periphyton, the medium was collected from each microcosm and replaced by fresh medium to maintain nutrient levels. The collected medium was immediately filtered through a glass fiber filter (FVF, 0.7 μm nominal pore size, Whatmann) and stored at 4°C for the quantification of ammonium ($N\text{-}NH_4$), nitrite ($N\text{-}NO_2$), and nitrate ($N\text{-}NO_3$) concentrations.

In Experiment 3, periphyton was sampled after 21 days. Periphyton of one of the three slides was scraped off and dispersed in 13 mL PERIQUIL as described above. From this suspension, rMP concentrations, periphyton dry weight, and photosynthetic yield were determined. The remaining two slides were used for rheological measurements.

MP Quantification

The number of sMP, sMP*aged*, and rMP in the periphyton were quantified using flow cytometry (FC, Beckman Coulter Gallios; capillary size: < 50 μm) combined with single-cell visualization by viSNE (visual stochastic network embedding) (Amir et al., 2013). ViSNE is a tool for nonlinear dimension reduction and visualization of high-dimensional data (e.g., FC data) and has been used earlier to determine the phenotypic community composition of phototrophs in periphyton (Sgier et al., 2016; Sgier et al., 2018b). The fluorescence and scattering properties of each cell and particle in the sample were measured individually and clustered into subpopulations following their similarities as described in Sgier et al. (2016). For these measurements, 3 mL of the periphyton suspensions from the exposure experiments were transferred into a Falcon tube (15 mL) and were sonicated for 1 min to break up the colonies. After filtration through 50 μm filters (Partec), samples were fixed (0.01% paraformaldehyde and 0.1% glutaraldehyde (w/v) in tap water) and left at 4°C overnight.

To identify the sMP, sMP*aged* and rMP particular fluorescence and scattering properties, suspensions (0.001 mg mL^{-1} in EPS) of each particle type were prepared. All samples were measured using the same laser settings as described by Sgier et al. (2016). The resulting FC data were then analyzed using the bh-SNE version of SNE (Amir et al., 2013; Van Der Maaten 2014), implemented as viSNE in cyt software (http://www.c2b2.columbia.edu/danapeerlab/html/cyt-download.html, downloaded in January 2015). The output is a 2D scatter plot (viSNE map) representing cells with similar fluorescence and scattering properties close to each other forming clusters, which are interpreted as subpopulations (Sgier et al., 2016; Sgier et al., 2018b). These allow to deduce, on the one hand, a phenotypic community composition (see section on Structural endpoints below). On the other hand, they allow to detect MP: the sMP/sMP*aged* exhibit a low fluorescence intensity at 695 nm, while rMP have high fluorescence intensity at 575 nm. These wavelengths were used to identify the sMP, sMP*aged*, and rMP in the viSNE maps resulting from the periphyton samples (**Supplementary Figure S3**). Respective clusters were quantified using Mat lab as previously described (Sgier et al., 2016). The method results in a relative abundance of MP compared with the total number of measured cells. The analysis of the data showed that with the applied laser settings, up to 0.2% of the measured cells fall into the MP cluster "false-positive" (**Supplementary Figure S4**) in the control treatment. This suggests a sensitivity for the detection of MP with the periphyton suspension of 80%. The 20% false-positive events have previously been determined as decaying, non-organic and not-assigned particles (see Supplementary Figure S23 in Sgier et al., 2016).

Inasmuch as bMP/bMP*aged* were too large (i.e., >50 μm) to be analyzed with FC and viSNE, they were counted visually, using a Neubaur chamber. Briefly, 10 μL of periphyton samples were pipetted onto the chamber, closed with a glass lid and examined using a microscope (DMI 6000 B, Leica). Results are represented as bMP and bMP*aged* per m^2 glass slide surface.

To be able to compare exposure concentrations despite differences in MP quantification methods due to substantial size differences, we estimated the relative volume of sMP/sMPaged, bMP/bMPaged, and rMP in relation of periphyton volume (%). Equations are detailed in **Supplementary Text S1**.

Biomass Accrual and Microbial Structural Characteristics of Periphyton Upon MP Exposure

Periphyton biomass accrual (dry weight, DW) was determined by filtering 2 mL of periphyton suspension through pre-weighed glass fiber filters (GF/F, 25 mm diameter 0.7 μm average pore size; Whatman Ltd., Maidstone, United Kingdom). Filters were dried

for 48 h at 60°C and weighted again to the nearest 0.01 mg. Dry weight was estimated as the mass difference between empty filters and dry filters and was reported per unit of surface area.

Phenotypic community composition of photoautotrophic organisms of Experiments 1, 2 was determined by analyzing the viSNE maps also used for sMP and sMP*aged* quantification (see above). In the viSNE maps, the subpopulations of interest were identified based on the visual separation between regions of the map and the distribution of the flow cytometry markers as previously explained (Sgier et al., 2018b). For quantification, number of the particles/cells that belonged to each subpopulation in each of the samples was counted.

Community structure (i.e., composition and relative abundance) of periphyton was also determined for prokaryotes (i.e., heterotrophic bacteria and phototrophic cyanobacteria) and microeukaryotes (i.e., green algae and diatoms) via amplification and sequencing of the16S and 18S rRNA genes, respectively. This was done in samples from Experiments 1, 2 at day 7 of exposure, considering that microbial composition and sensitivity to MP might be affected more strongly at early stages of colonization and development (Guo et al., 2015; Rummel et al., 2021). We applied amplicon sequencing at the MiSeq platform using a 2 x 300 bp kit. To do so, 1.5 mL periphyton suspension was transferred to 1.5 mL Eppendorf tubes and centrifuged at 13000 rpm for 10 min. The supernatant was discarded and the pellet stored at −80°C. DNA was extracted using MOBIO Biofilm DNA extraction kit (Quiagen) following the manufacturer's instructions. Concentration and quality of the resulting DNA was checked with a Nanodrop 8000 Spectrophotometer. DNA extract was used for MiSeq sequencing when DNA:RNA ratio was ~1.8.

The 16S and 18S regions were amplified using frameshift primers that resulted in 463bp and 558bp fragments, respectively (**Supplementary Table S2**). For the polymerase chain reaction (PCR), the appropriate annealing temperature and cycle number for each sample were previously determined using a gradient PCR and quantitative PCR approach. The final thermocycling conditions consisted of an initial denaturation of the DNA at 98°C for 3 min which was followed by 25 cycles of 98°C for 20 s, 55°C for 15 s, and 72°C for 15 s for the 16S primer set. For the 18S primers, amplification started with an initial denaturation of the DNA at 98°C for 3 min followed by 24 cycles of 98°C for 20 s, 56°C for 15 s, and 72°C for 15 s. Both programs were finalized with an elongation step at 72°C for 5 min. All reactions were performed in three technical replicates using KAPA HiFi HotStart ReadyMix, which includes the buffer, dNTPs, and the enzyme for the PCR reactions and 0.3 μM of each primer. PCR products were cleaned using AMPure beads (Beckmann Coulter). For MiSeq sequencing, the PCR products were indexed using Nextera XT index adaptors following manufacturer's instructions. The resulting libraries were cleaned up and quantified using AMPure beads and the Spark fluorimeter following manufacturer's instructions. The libraries were pooled to a final concentration of 4 nM using a pipette robot (Liquid Handling Station, Brand). After denaturation (0.2 N NaOH), the library was sequenced on an Illumina® MiSeq machine in a single flow cell in paired-end mode with 300bp-read-lengths. The sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive database under BioProject ID PRJNA744749 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA744749).

The raw data were clustered into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). The taxonomic affiliations of the ASVs were assigned using SILVA database release 138 (Quast et al., 2012). Subsequent analyses were conducted using *R* version 4.0.3 (R language core team 2021). Alpha diversities at each sampling site were estimated by Chao1 and Shannon indices using function estimate richness implemented in phyloseq 1.34.0 (McMurdie and Holmes, 2013). The variation of the p16S and 18S community composition in response to different treatments was analyzed by redundancy analysis (RDA) using the function capscale embedded in vegan 2.5-7 (Oksanen et al., 2013). The 16S and 18S ASV counts represented response variables while treatments were used as explanatory variables. The R code used for the analyses is available on Github at https://github.com/manutamminen/periphyton_microplastic_interactions.

Functional Characteristics of Periphyton on MP Exposure

$N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$ concentrations in the medium were analyzed following standard colorimetric methods (APHA 2005) using a spectrophotometer (Cary 60, Alient) to derive the uptake rates (k) of ammonium ($N\text{-}NH_4$), nitrite ($N\text{-}NO_2$), and nitrate ($N\text{-}NO_3$). These were calculated as the difference of nutrient concentration in the water column over the exposure time and expressed in mg day^{-1} using the following equation:

$$k = \frac{c_t - c_0}{t} * 0.5\, l,$$

where t is time (in days), and c_0 and c_t are the concentrations of the nutrient at time zero and at the respective time point (i.e., time between medium changes). The 0.5 L represent the volume of the medium in the microcosms. Positive values indicate a net consumption and negative values indicate a net production of the respective nutrient.

Photosynthetic yield was assessed by measuring the quantum yield of photosystem II (ϕ') with a a pulse-amplitude-modulated fluorimeter (PHYTO–PAM; Heinz Walz GmbH, Effeltrich, Germany) (Gil-Allué et al., 2015). Periphyton was acclimated for 15 min to ambient light conditions and measured subsequently. Quantum yield was calculated as follows:

$$\phi' = \left(F'_m - F'_t | F'_m\right),$$

with $F`_m$ being the background fluorescence and $F`_t$ the instantaneous fluorescence.

Respiration measurements were conducted using the MicroResp system according to Tlili et al. (2011a), which is based on a colorimetric method where color changes of a pH indicator dye are related to the release of CO_2(g). A 500 μL periphyton suspension was used for the measurements. Incubations were carried out in triplicate in the dark to allow

for the measurement of heterotrophic respiration. Absorbance of the detection gel was measured on a microplate reader (Tecan Trading AG, Männedorf, Switzerland). The average change in absorbance was normalized to the periphyton dry weight (DW) and expressed as mg CO_2 per DW per day.

Mechanical Properties of the Periphyton Matrix

Compression rheology was used to measure mechanical properties and potential differences in architecture of periphyton matrix upon MP exposure in samples from Experiment 3 (rMP) (Lopez-Sanchez et al., 2014; Rühs et al., 2020). To meet the technical requirements of the rheometer (i.e., periphyton height > MP size), the measurements were exclusively carried out with periphyton containing MP in the smaller size range (1–4 μm, rMP, **Table 1**). The used glass slides were covered with intact, 21 days old periphyton. These were placed onto the rheometer (MCR502, Anton Paar, Austria), which was equipped with a sand blasted plate geometry to account for potential slip and increase attachment of the biofilm to the geometry. Axial compression measurements (using three technical replicates) with constant speed of 1 μm s^{-1} were carried out and stopped when a previously defined normal force (F_N, expressed in N) compression of either 0.15 N or 1.5 N was reached. During this compression step (i.e., stress relaxation), an amplitude sweep was performed to measure the elastic (G′) and viscous (G″) moduli at a frequency of 1 rad s^{-1} and at strains of 0.01–100 % at a pre-determined compression (F_N = 0.16 N).

The resulting compression curves were interpreted as follows: the top of the sample was reached as soon as the F_N decreased below zero. When $F_N < 0$ occurs, capillary forces predominate and pull the plate geometry of the rheometer toward the periphyton. When $F_N > 0$ occurs, the geometry exhibits purely compression on the periphyton matrix. Under this compression regime, the water flows perpendicular to the direction of the applied compressive load across the periphyton matrix. The stiffness indicates the resistance that the water encounters by crossing the periphyton matrix and was calculated from the slope of the compression curves. The stress relaxation (force recovery) is the observed decrease of FN under fixed compression (i.e. 0,15 N or 1.5 N) and was calculated from the applied maximal F_N divided by the F_N after compression and expressed as (%). The dynamic yield point represents the strain needed to induce movement and potential displacement within the periphyton matrix and was determined as the crossover point between G′ and G″ from the strain-controlled amplitude sweeps measurements. In all measurements, the fluid movement was not constrained during compression and thus water could freely flow out of the sample. All measurements were done under ambient conditions.

Statistical Analysis

Differences between MP abundance in the periphyton over time were determined using one-way ANOVA. Differences between treatments over time for dry weight, respiration, photosynthetic activity, $N\text{-}NH_4$, $N\text{-}NO_3$ uptake rate and $N\text{-}NO_2$ production rate were tested with mixed effect models with time and treatment as fixed value. Differences in algal community composition at phenotypic level (as measured by FC and viSNE data analysis) between treatments and time were analyzed using Adonis permutational ANOVA. The significance of the treatment effect on the 16S and 18S community composition was tested using permutational ANOVA. Differences among the treatments between relative abundances of the gene encoding for 16S and 18S ASV as measure of taxonomic community composition at day 7 were tested using a two-tailed t-test. All calculations were carried out in R (Version 3.6.1). *p*-values of ≤0.05 were considered significant.

RESULTS

MP Surface Characteristics After Aging

As evidenced by electron microscopy, MP exposure to UV radiation induced visible surface alterations with cracks as well as grained surface structures on both, sMP and bMP (**Supplementary Figure S5**). In addition, in the FTIR spectra of UV-treated MP, an absorption band at 1712 and 1715 cm^{-1} appeared. These wavenumbers are assigned as carbonyl stretching mode of ketone functional groups (Gulmine et al., 2003), suggesting an increase in their abundance upon UV exposure. However, the breadth of the adsorption band suggests that other carbonyl-containing groups might be also present. Other FTIR active modes remained unchanged (**Supplementary Figure S6**).

Experiment 1. Effects Of Smaller MP (1–4 μm) with Aged and Native Surface On Structure and Function Of Developing Periphyton.

sMP and sMP*aged* represented 0.002% of the total volume of the periphyton at day 28 in each microcosm (for calculations, see **Supplementary Text S2**). The sMP abundance was measured by FC & viSNE data analysis. At all time points, the sMP to cell ratio was constant with an average of 0.86% and 0.70% of the cells counted by FC identified as sMP and sMP*aged*, respectively (**Table 2**). Considering the biomass accrual at the same time points (**Figure 2A**), this indicates a continuous incorporation of the small particles in the biofilm proportional to biomass increase. This was also highlighted by the fact that sMP and sMP*aged* relative abundance was not correlated with periphyton growth rate at different time points (**Supplementary Figure S7**).

Periphyton grown in the presence of sMP and sMP*aged* developed constantly over the study period up to 14.16 g m^{-2} dry weight (**Figure 2A**), with time playing a significant role while sMP and sMP*aged* treatments having no significant effect (**Table 3**). Photosynthetic yield ranged from 0.40 to 0.49 from day 7–28 with lowest levels at day 21 (**Figure 2B**). Respiration increased significantly by a factor of 4 and 3.25, respectively, from day 7 to day 28 (**Figure 2C**). $N\text{-}NH_4$ uptake rate decreased from 7 to 21 days and increased at day 28 (**Figure 2D**). $N\text{-}NO_2$ uptake

TABLE 2 | Relative abundance of sMP/sMP*aged* in periphyton over time, measured by FC and viSNE.

Days	Control		sMP		sMP*aged*	
	%	SE	%	SE	%	se
7	0.3	0.1	0.8	0.3	0.6	0.3
14	0.2	0.2	1.0	0.4	0.7	0.1
28	0.1	0.1	0.8	0.2	0.8	0.3

*The sMP/sMP*aged *cluster in the periphyton sample was identified based on its characteristic fluorescence properties. Values indicate the percentage of events in a total of 10,000 analyzed cells identified as MP. Represented are mean values of five biological replicates and the standard error (SE) of the mean. Values in the control treatment are due to detection of false positive events (e.g., decaying, non-organic and not-assigned particles (see Material and Methods).*

increased by an order of magnitude from day 7 to day 28 (**Figure 2E**). In contrast, N-NO_3 uptake rate was negative and decreased by factor 5 suggesting increasing N-NO_3 demand with increasing biomass (**Figure 2F**).

Based on the FC & viSNE data analysis, 23 different phenotypic groups were identified (CL1-23, **Figure 3**). In each sample, representatives of all cluster were found. The viSNE maps of each time point are provided in **Supplementary Figure S8**, optical scatter and fluorescence intensities at specific wavelengths in **Supplementary Figure S9**, and a heat map of the differences in scatter and fluorescence between the different clusters in **Supplementary Figure S10**. Statistical testing of the number of cells per phenotypic group yielded significant differences between phenotypic community composition of photoautotrophic organisms over time, but insignificant differences between treatments (**Figure 3**). A detailed description of the results concerning the influence of time is available in **Supplementary Text S3**.

The 16S and 18S community composition was analyzed *via* MiSeq amplicon sequencing on day 7 of exposure. Growth of periphyton in the presence of sMP and sMP*aged* resulted in significantly different microbial communities as compared with the control (**Figure 4**). Redundancy analysis resulted in principle components 1 and 2 explaining 9.83% of the differences in 16S data and 28.95% in 18S data (**Figure 4**).

With respect to alpha diversity, no significant differences were detected in the richness (Chao1 index) and diversity (Shannon index, H') calculated for 16S communities ($p \leq 0.05$) (**Supplementary Figure S11**). However, while alpha diversity was as well similar between control and sMP communities, significant differences were found between the Chao and Shannon indices of control and sMP*aged* 18S communities. In addition, sMP*aged* and sMP 18S communities differed

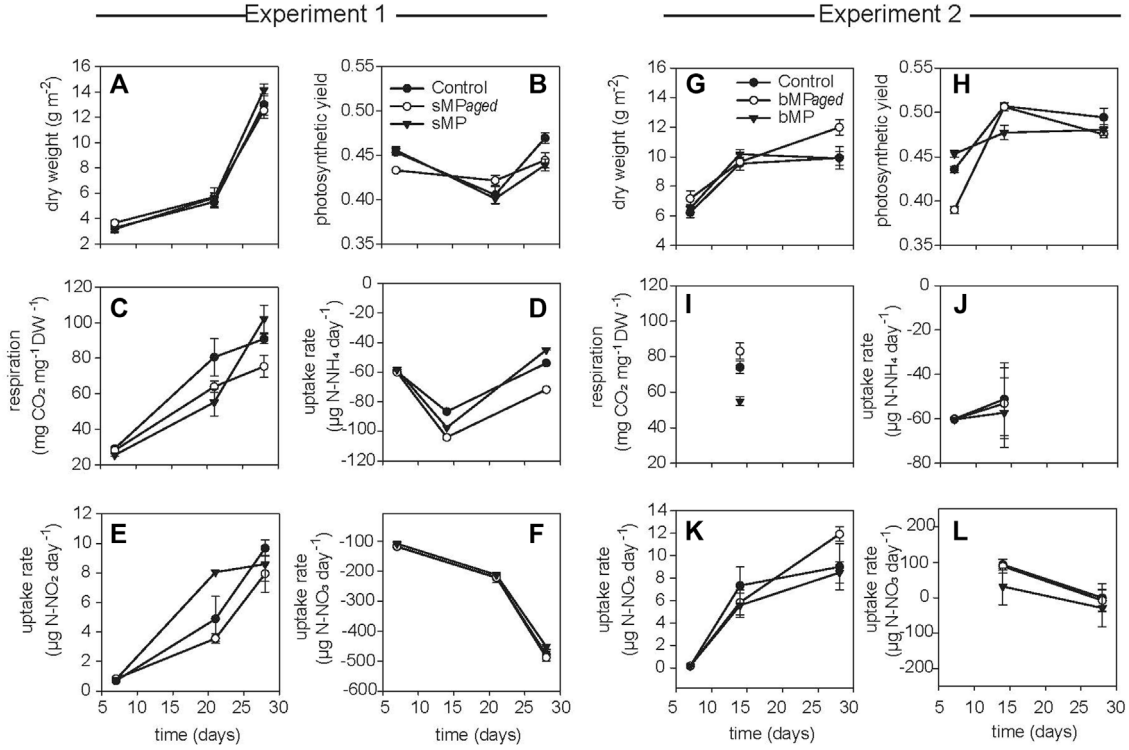

FIGURE 2 | Periphyton descriptors resulting from experiment 1 (left panels, sMP/sMP*aged*) and 2 (right panels, bMP/bMPaged). Represented are dry weight **(A,G)**, photosynthetic yield **(B,H)**, respiration **(C,I)**, and N-NH_4^+ **(D,J)**, N-NO_2^- **(E,K)** and N-NO_3^- **(F,L)** uptake rates. The treatments are represented as black dots for control, white dots for UV-treated MP and black triangle for MP with native surface. Shown are the mean of five biological replicates; whiskers indicate the standard error of the mean.

TABLE 3 | Statistical results from mixed effects analysis with dry weight, respiration, photosynthetic yield, and nutrient uptake rates as dependent variable and experimental time and treatment as fixed variables. *p* values <0.05 in bold type indicate significant differences over time or among treatments. Calculations were carried out using the nlme package in R version 3.6.1.

	Response variable	Time		Treatment	
		F	*p*	F	*P*
sMP	Dry weight	330.74	<**0.0001**	0.47	0.6626
	Respiration	32.95	<**0.0001**	0.99	0.3969
	Photosynthetic yield	6.93	**0.0036**	0.94	0.418
	N-NH_4 uptake rate	18.96	<**0.0001**	1.66	0.23
	N-NO_3 uptake rate	312.06	<**0.0001**	0.88	0.4390
	N-NO_2 uptake rate	82.13	<**0.0001**	3.02	0.0867
bMP	Dry weight	67.4557	<**0.0001**	2.67	0.1097
	Respiration	na		**7.39**	**0.0081**
	Photosynthetic yield	55.367	<**0.0001**	**5.89**	**0.0156**
	N-NH_4 uptake rate	18.95	<**0.0001**	1.660	0.2309
	N-NO_3 uptake rate	312.06	<**0.0001**	0.88	0.4390
	N-NO_2 uptake rate	82.13	<**0.0001**	3.02	0.0867

significantly in their respective Shannon index (**Supplementary Figure S11**). When significantly different, indices were systematically lower in sMP*aged* communities than in control and sMP communities.

With regard to 16S ASV, 704 ASV were identified with 382 above the threshold of 3 (relative abundance of ASV >1% listed in **Supplementary Table S4**, families/genera summarized in **Supplementary Table S5**). Overall, two-tailed t-test of relative abundances suggested significant differences ($p \leq 0.05$) in 5% and 4.2% ASV between control and sMP or sMP*aged* communities, respectively, and in 6% ASV between sMP and sMP*aged* communities (**Supplementary Table S3**). The highest abundance was detected for cyanobacterium genus *Chamaesiphon* (ASV9) in control communities (3.1%) and was statistically significantly reduced in sMP (1.9%) and sMP*aged* (1.6%) communities. A statistically significant increase in abundance in sMP*aged* compared with the controls was detected in the genera *Phreatobacter* (ASV59), *Flavobacterium* (ASV117), *Hyphomonas* (ASV131), *Elstera* (ASV132),

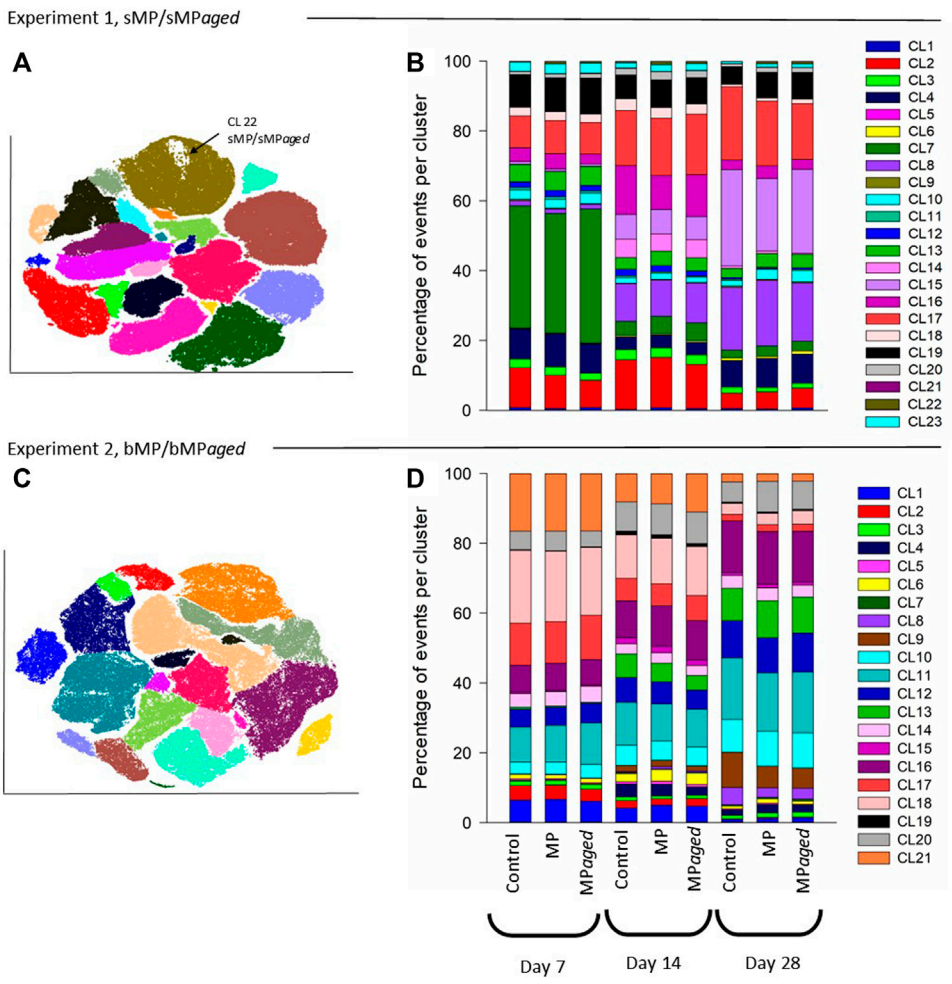

FIGURE 3 | Effects of sMP/sMP*aged* **(A,B)** *and* bMP/bMP*aged* **(C,D)** on the photoautotrophic community composition at day 7, 14, and 21 days of exposure. Control indicates periphyton grown in the absence of MP. Differences in community composition between treatments and time were analyzed using Adonis permutational ANOVA in R. Results indicated no significant differences between treatments, while differences over time were significant in both experiments (experiment 1: $p < 0.001$, $R^2 = 0.63$ F = 36 and for experiment 2: $p = 0.001$, $R^2 = 0.92$, F = 282.3 in experiment 1 and 2, respectively).

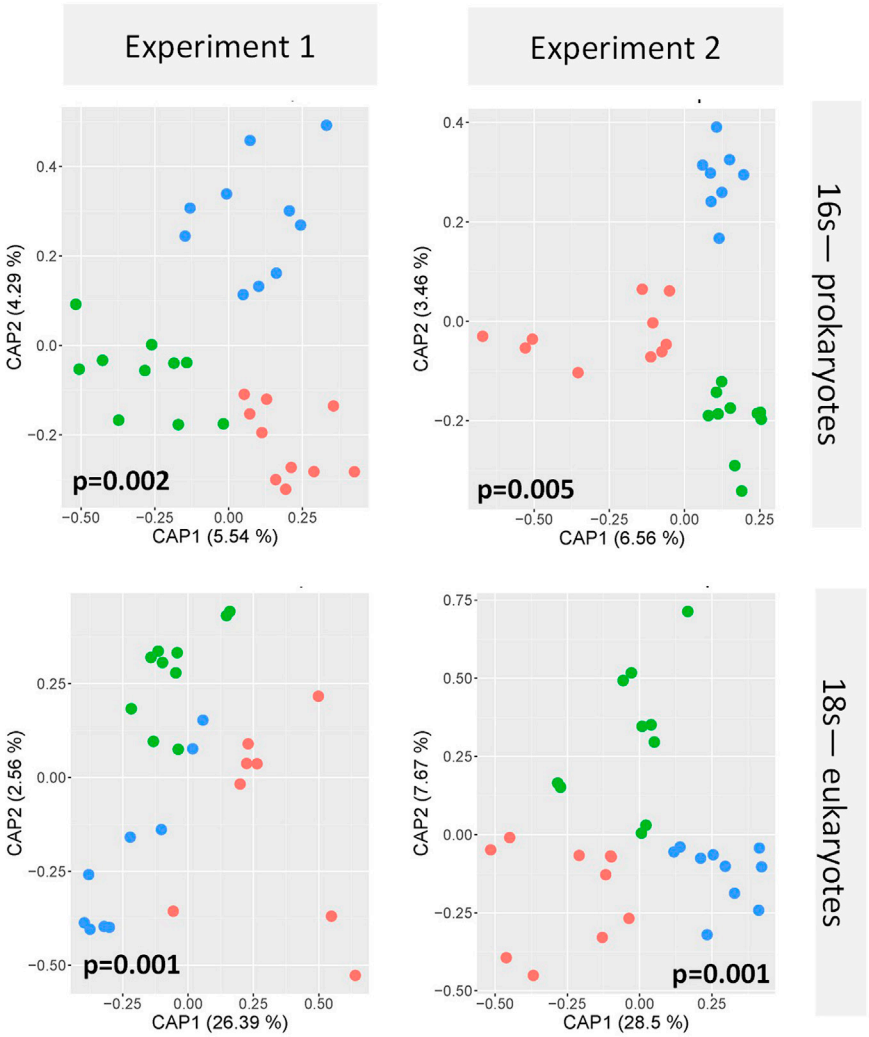

FIGURE 4 | Microbial community composition of periphyton resulting from Experiment 1 (left column) and Experiment 2 (right column) based on 16S (upper panels) and 18S amplification (lower panels) at day 7 of the exposure. Green, red, and blue dots represent control, aged and native MP exposures, respectively. Five independent biological replicates and two technical replicates per sample were included in the redundancy analysis, which reveals a significant effect of the exposure on the community composition ($p < 0.05$; permutational ANOVA).

Haliscomenobacter (ASV152), while a decrease was detected in *Methylotenera* (ASV98) and another member of *Haliscomenobacter* (ASV213). Examples of increase in sMP communities as compared with the controls are in members of the genus *Flavobacterium* (ASV110), and the order *Sphingobacteriales* (ASV134) and of a decrease in a member of the genus *Emticicia* (ASV184).

With regard to 18S communities, 281 ASV were identified with 251 above the threshold of 3 (relative abundance of ASV >1% listed in **Supplementary Table S6**, families/genera summarized in **Supplementary Table S7**). Approximately, 40 of these were only identified as eukaryotic without further phylogenetic information. Some 234 of these were accounted for less than 1% of the total number of ASV. Two-tailed t-test of relative abundances suggested statistically significant differences ($p \leq 0.05$) in 11% and 22% ASV between control and sMP or sMP*aged* communities, respectively, and in 18% ASV between sMP and sMP*aged* communities (**Supplementary Table S3**). The detected ASV were dominated by members of the diatom genera *Achnanthidium* and *Gomphona* (ASV1-3, 5) and by members of the phylum *Cryptomycota* (ASV4), which represented about 50% of all ASV. *Cryptomycota* accounted for 4.65% of all ASV in control communities, and were significantly increased in sMP treatments (6.37%) but significantly decreased in sMP*aged* treatments (2.58%). At the same time, the sum of the strongly represented diatom ASV showed the opposite distribution in sMP (40.88%), sMP*aged* (48.91%), and control communities (42.65%). Part of the low abundance ASV (< 1%) was significantly reduced in sMP and sMP*aged* treatments vs. controls. Examples are members of the diatom genus *Melosira* (ASV24, decreased in sMP*aged*), *Achnanthidium* (ASV28, decreased in sMP and sMP*aged*), *Planothidium* (ASV48, decreased in sMP and sMP*aged*) and the ciliate genus *Telotrochidium* (ASV53,

TABLE 4 | Absolute abundance of bMP/bMP*aged* in peripyhton over time (MP m^{-2}).

Days	bMP		bMP*aged*	
	MP m^{-2}		MP m^{-2}	
	Avg	SE	Avg	SE
7	2.0E+05	1.6×10^4	2.3E+05	1.6E+04
14	2.0E+05	2.4×10^4	3.3E+05	2.3E+04
28	1.8E+05	1.6×10^4	2.1E+05	1.6E+04

*bMP/bMP*aged *were quantified by counting using light microscopy. Represented are mean values of five biological replicates and the standard error of the mean (SE). Statistical differences between absolute abundances among time points were tested using ANOVA in R.*

decreased in sMP and sMP*aged*). Overall, 13 of the 251 ASV were not identified in the sMP*aged* communities as opposed to 4 in the sMP communities. The frequency of detection of 72% of all ASV was reduced in sMP*aged* samples and of 42% of all ASV in sMP samples.

Experiment 2. Effects Of Bigger MP (65–75 μm) with Aged and Native Surface On Structure and Function Of Developing Periphyton.

bMP and bMP*aged* represented 0.0025% of the total volume of the periphyton at day 28 in each microcosm (for calculations see **Supplementary Text S2**). These (big) particles were added only once at the beginning of the experiment because they were found to settle on the biofilm (**Supplementary Figure S2**) and hence were assumed to not be affected by the medium changes. Indeed, total abundance of bMP and bMP*aged* per m^2 glass slide was constant over the study period and no statistical differences between bMP and bMP*aged* per m^2 were detected at different time points ($p > 0.05$, ANOVA, **Table 4**). Considering the biomass accrual at the same time points (**Figure 2G**), the constant abundance of bMP and bMP*aged* indicates a dilution of the big particles in the biofilm proportional to biomass increase, and hence, a decrease of the exposure conditions. This is also highlighted by the observed significant decrease of the ratio of bMP:biomass and bMP*aged*:biomass over time (mixed effects analysis, $p < 0.05$, **Supplementary Figure S12**).

Periphyton grown in the presence of bMP and bMP*aged* developed constantly over the study period up to 10.6 g m^{-2} dry weight (**Figure 2G**) with time playing a significant role while bMP and bMP*aged* treatments having no significant effect (**Table 3**). The photosynthetic yield ranged from 0.39 to 0.51 with significantly highest values measured at day 14 in bMP*aged* and control treatment (**Figure 2H**).

Respiration was only measured on day 14 where it was lowest in periphyton grown in the presence of bMP (**Figure 2I**). The N-NH_4 uptake rate was negative, indicating N-NH_4 uptake, and increased slightly from day 7 to 21(**Figure 2J**). Similar to Experiment 1, N-NO_2 uptake rate was positive, indicating N-NO_2 production, and increased by one order of magnitude from day 7 to day 28 (**Figure 2K**). N-NO_3 uptake rate was positive on day 14 and decreased to close to 0 on day 28 (**Figure 2L**). This indicates a change from N-NO_3 production to N-NO_3 assimilation between day 14 and 21.

Based on FC & viSNE data analysis, 21 different phenotypic groups were identified (CL1-21, **Figures 3C,D**). In each sample, representatives of all clusters were found. The viSNE maps of each time point are shown in **Supplementary Figure S13**, optical scatter and fluorescence intensities at specific wavelengths in **Supplementary Figure S14**, and heat map of the differences in scatter and fluorescence between the different clusters in **Supplementary Figure S15**. Statistical testing of the number of cells per phenotypic group yielded significant differences between phenotypic community composition of photoautotrophic organisms over time, but no significant differences were observed between treatments (**Figure 3**). A more detailed description is available in **Supplementary Text S4**.

The 16S and 18S community composition was analyzed *via* MiSeq amplicon sequencing on day 7 of exposure. Growth of biofilms in the presence of bMP and bMP*aged* resulted in significantly different microbial communities as compared with the control (**Figure 4**). Redundancy analysis resulted in principle components CPA 1 and 2 explaining 10.02% of the differences in 16S data and 36.17% in 18S data (**Figure 4**). No significant differences were detected in the richness (Chao1 index) and diversity (Shannon index, H') calculated for 16S communities ($p \leq 0.05$) (**Supplementary Figure S11**). Significant differences were found in the Shannon indices of bMP and bMP*aged* communities with lower values determined for MP*aged* communities (**Supplementary Figure S11**).

With regard to 16S communities, 1170 ASV were identified with 875 above the frequency threshold of 3 (relative abundance of ASV > 1% listed in **Supplementary Table S8**, families/genera summarized in **Supplementary Table S10**). Two-tailed t-test of relative abundances suggested significant differences ($p \leq 0.05$) in 3.4% and 5.5% ASV between control and bMP or bMP*aged* communities, respectively, and in 4.3% ASV between bMP and bMP*aged* communities (**Supplementary Table S3**). The highest abundance was detected for ASV1 (genus *Inhella*, around 1.5% in all communities) followed by the *Chitinophagaceae* genus *Terrimonas* (ASV2, around 1% in all communities). Cyanobacterial ASV 50 and 148 accounted for 0.7% on average.

Examples of decrease in abundance in bMP*aged* communities are ASV 39 (*Terrimonas*), 47 (*Sphingobacteriales*), 135 (*Solitalea*, *Sphingobacteriales*), and 265 (*Sphingopyxis*), while an increase was observed in ASV 113 (*Microscillaceae*), 203 (*Lacihabitans*), 279 (*Methylotenera*), 354 (*Flavobacterium*), and 356 (*Methylophilaceae*). In the bMP communities, different ASV were decreased or increased in abundance, but were partially associated with the same phyla. Examples for decreased abundance are ASV 184 (*Flavobacterium*), 250 (*Lacihabitans*), 273 (*Flavobacterium*), 428 (*Haliscomenobacter*, *Chitinophagales*), while increased abundances were observed in ASV 267 (*Flavobacterium*) and 465 (*Flavobacterium*).

With regard to 18S and 16S communities, 306 ASV were identified with all above the frequency threshold of 3 (relative abundance of ASV >1% listed in **Supplementary Table S10**, families/genera summarized in **Supplementary Table S11**). Of these, 28 were only identified as eukaryotic without further phylogenetic information. Two-tailed t-test of relative abundances suggested significant differences ($p \leq 0.05$) in

13.7% and 10.5% ASV between control and sMP or sMP*aged* communities, respectively, and in 20% ASV between bMP and bMP*aged* communities (**Supplementary Table S3**). As opposed to the communities exposed in Experiment 1, the most abundant ASV were less dominant. The 16 most abundant ASV represented about 50% of all ASV. These were associated with members of the diatom genera *Achnanthidium, Gomphona, Melosira, Ulnaria, Nitzschia,* and *Encynoma* (ASV1, 3, 4, 7, 15, 16), ciliate family *Oligohymenophorea* (ASV 6, 9, 14), the environmental clone BOLA868 (*Amoebozoa*, *Tubulinea*, ASV10), class *Thecofilosea* (Cercozoa, ASV12), and four eukaryotic ASV (ASV2, 5, 8, 11) not further identified. *Cryptomycota* accounted for 0.3–0.4% of all ASV (ASV74, 170, 258; ASV74 being identical to ASV4) without significant differences between the treatments. As observed in Experiment 1, the sum of the strongly represented diatom ASV showed a distinct distribution in bMP (21.12%), bMP*aged* (28.63%) and control communities (27.85). Due to a larger heterogeneity of individual samples in this experiment, differences in the low abundance ASV were less often statistically significant. One exception is the higher abundance of *Telotrochidium* (ciliate, ASV22) in bMP communities (1.7 % vs 0.67% in bMPaged and 0.56% in control communities). Overall, 7 of the 306 ASV were not identified in the bMP*aged* communities and 8 in the bMP communities. The frequency of detection of 32% of all ASV was reduced in bMP*aged* samples and of 44% of all ASV in bMP samples.

Experiment 3. Effects Of Smaller MP (1–4 μm) On Mechanical Properties Of Periphyton.

rMP represented 0.002% of the total volume of the periphyton at day 21 in each microcosm (for calculations see **Supplementary Text S2**). Relative abundance of rMP in the periphyton was quantified using FC & viSNE. This analysis showed that 1.62% of the cells were identified as rMP. Dry weight and photosynthetic yield at day 21 of exposure were similar in periphyton grown in the presence and absence of rMP ($p > 0.05$, Student's T-test), amounting to 9.79 ± 0.33 mg m^{-2} and 0.33 ± 0.01, respectively.

To determine the mechanical differences between periphyton grown in the presence or absence of rMP, axial compression tests with oscillatory rheology were performed (as illustrated in **Figure 5A**). Axial compressions indicated that the height of control and rMP-exposed periphyton were at 1.5 and 1.4 mm, respectively (**Figure 5B**). Yet, the architecture and mechanical behavior upon compression differed. The architectural differences in the periphyton were derived from the shape of the compression curves. Here, the negative F_N zone ($N < 0$) was larger for periphyton-rMP (0.62 ± 0.17 mm) than for control periphyton (0.32 ± 0.06 mm, **Figure 5B**). The mechanical properties, that is, stiffness and stress relaxation, were measured upon axial compression. Results showed higher initial stiffness for periphyton exposed to rMP than for control periphyton (**Figure 5C**). In contrast, the force recovery after compression was higher for control periphyton (**Figure 5D**). Here, the energy dissipation of the applied F_N of 0.15 reached 30% for rMP-exposed periphyton, whereas control periphyton reached values of 40% of the applied F_N (**Figure 5D**). This energy dissipation is a direct measure of the load-bearing capacity of the periphyton systems, revealing that both types of periphyton are able to store elastic energy.

Both, control- and rMP-exposed periphyton were of viscoelastic character with an elastic modulus G′ and a viscous modulus G″. However, rMP-exposed periphyton had a higher viscoelasticity. This was indicated by a higher elastic modulus (G′, **Figures 5E,F**). At low strains (0.1–10%), both control periphyton and rMP-exposed periphyton behaved like a solid-like material (G′>G″). At strains > 10%, the periphyton became liquid-like (G″<G′). At higher compressions (0.3N), the elastic and viscous moduli increased in both periphyton types, due to a squeezing of the biofilm resulting in less water in the sample, while, at the same time, increasing the amount of connection points between the biofilm components.

DISCUSSION

In this study, three independent microcosm exposure experiments shed light on the effect of MP on periphyton, an important component in freshwater ecosystems. In particular, exposures to small MP (1–4 μm, 0.96 g mL^{-1}, Experiment 1) and big MP (63–75 μm, 1.25 g mL^{-1}, Experiment 2) with aged and native surface aimed to test MP effect on periphyton biomass accrual, microbial community structure and function. Exposures to red fluorescent small MP (1–5 μm, 1.3 g mL^{-1}, Experiment 3) aimed to test the effects on periphyton mechanical properties. The periphyton inoculum for each experiment was collected freshly, resulting in natural variability in the community composition of the respective starting inoculum. Hence, the results obtained in the three experiments cannot directly be compared. However, within each experiment, the results are discussed with respect to the unexposed control.

Periphyton Incorporates all MP Types

The quantification of MP either in relative abundances (sMP/sMP*aged*) or in absolute counts (bMP/bMP*aged*) was used as an indication for the MP incorporation into the periphyton. Especially, the finding that the relative abundance of sMP/sMP*aged*, being added after each medium change, remained constant over the study period, supports the notion of a continuous incorporation of the MP with increasing periphyton biomass. The bMP/bMP*aged* were added only once at the beginning of the experiment. The fact that their absolute counts remained constant over the study period and that they were not washed out during medium change again indicates a high affinity of the MP to periphyton, which was additionally supported by the immediate settlement of the bMP/bMP*aged*. These results suggest that both MP types were incorporated into the periphyton already at an early stage of biofilm development, that is, in less than 7 days according to our experimental design.

The MP settlement depends on their specific material properties, especially size and density. In experiments to identify the best MP suspension procedure, we found that bMP accumulated on the bottom of the glass vials within 30 s, whereas sMP did not settle within the 5 min observation period. Therefore, the bMP/bMP*aged* settlement in the periphyton was

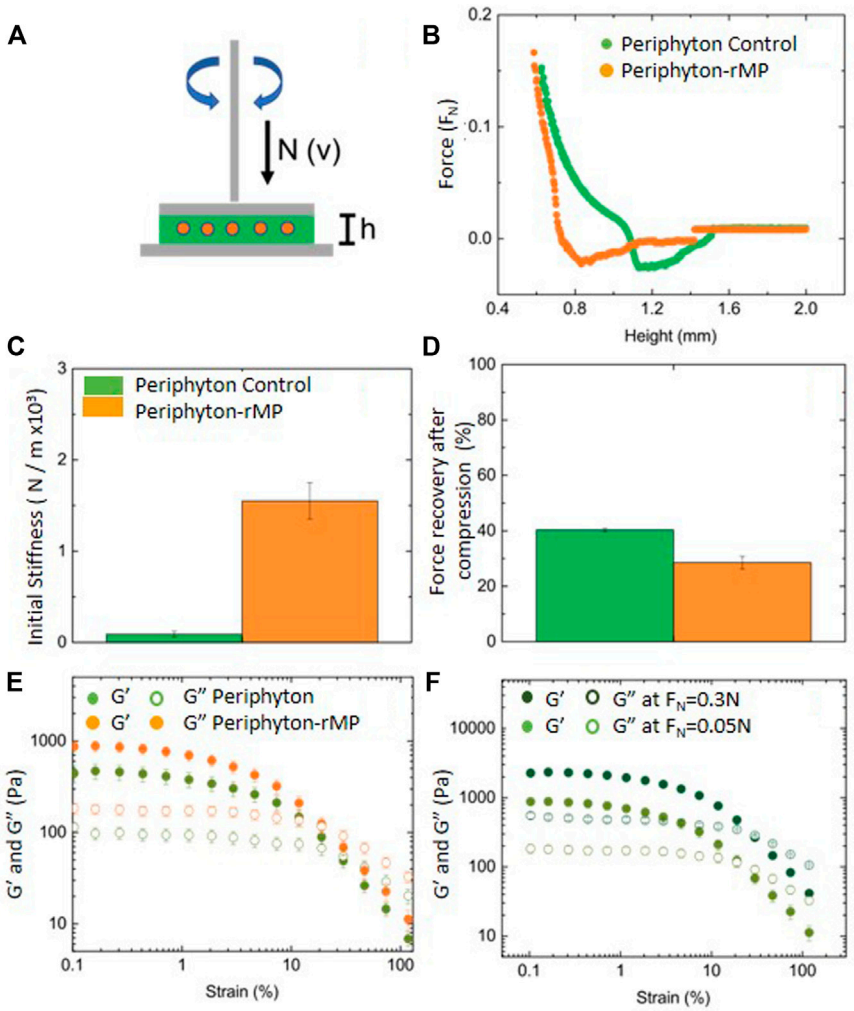

FIGURE 5 | Mechanical properties of periphyton growing with MP (periphyton-rMP, orange) and without (periphyton-control, green). Represented is a sketch of the rheometer indicating the plate, gap, sample, and sample holder **(A)**, the representative normal force (F_N) dependence on the gap **(B)**, initial stiffness **(C)**, stress relaxation **(D)** and viscoelastic properties of the matrix of periphyton-rMP (orange) and (green) measured using amplitude sweeps with fixed F_N **(E)** and for periphyton-rMP at two different F_N **(F)**.

likely driven, at least initially, by the high material density of the particles themselves. However, other factors may have contributed to the settlement of both the bMP and the sMP. For instance, interactions with microorganisms and EPS either in the water column or on the periphyton surface might have favored MP incorporation. Such a phenomenon has been observed in streams were MP settlement was correlated with periphyton standing stock (Roche et al., 2017). In the case of the sMP, this process may have been driven specifically by the high surface area compared with the bMP. Importantly, UV treatment to age the particles did not influence incorporation of the MP with the biofilms.

MP Changes Periphyton Microbial Community Structure

The MP in periphyton interact with organic compounds of the matrix and the microbial cells. These latter direct interactions can provide advantages for certain types of cells, such as facilitated attachment and reproduction, while bringing disadvantages, such as stress and negative selection, for others. In the present study, however, not all periphyton cells will have directly interacted with MP because exposure concentrations resulted in a sMP/sMP*aged* and bMP/bMP*aged* volume of only 0.0002% and 0.0025% of the total periphyton volume, respectively. This indicates that the MP:cell interactions must have been far less occurring than the cell:cell interactions. Despite this presumably low MP:cell ratio, the presence of MP during periphyton development leads to a different genetic community structure, that is, composition and relative abundance, of the prokaryotic and eukaryotic communities after 7 days of exposure. This might be due to the enrichment of certain cell types on the MP and in their proximity, thereby shaping community composition (Datta et al., 2016).

These community shifts upon MP exposures were independent of MP size but dependent on the surface

characteristics, which were altered by UV radiation-induced aging. The surfaces of both the sMP*aged* and bMP*aged* particles were cracked and keton-groups were more abundant on aged than on native (no UV treatment) surfaces. This is in line with the previous findings showing that UV radiation provokes chain scission, cross-linking, cracking and an increase of polar groups (Feldman 2002; Gulmine et al., 2003). These alterations must have led to a greater surface availability and possibly changes toward a more hydrophilic character of the surface of aged MP. Such changes in surface characteristics of MP have been shown to be a strong selective factor of organic compound adsorption (Rummel et al., 2021) and can partly drive direct MP:cell interaction *via* facilitating microbial attachment (Erni-Cassola et al., 2019).

The surface-dependence of community composition was also reflected in taxonomic alpha diversity. In particular, the alpha diversity of eukaryotic but not prokaryotic communities resulted to be less diverse upon exposures to aged but not to native MP. That biofilms developing on weathered MP in suspension had lower diversity than biofilms developing on natural particle substrates has been observed previously for both prokaryotic (McCormick et al., 2014; Miao et al., 2019) and eukaryotic (Kettner et al., 2019) communities. However, mechanisms (i.e., processes and pathways) leading to such a reduced diversity following MP:cell interactions are poorly understood.

Although alpha diversity was reduced in the presence of weathered MP, the relative abundance of some genera increased. Examples are *Phreatobacter* and *Hyphomonas* (both phylum Alphaproteobacteria), *Inhella* (phylum Betaproteobacteria) as well as *Flavobacterium* (phylum Bacterioidetes), all of which have also been found to enrich on suspended MP in the marine environment (Oberbeckmann and Labrenz, 2020), in rivers (McCormick et al., 2014; Kelly et al., 2020) and the Baltic sea (Ogonowski et al., 2018). Specific physiological traits, such as the ability of *Hyphomonas* to adhere firmly to surfaces by forming polysaccharides holdfast (Oberbeckmann and Labrenz, 2020), and the potential of Burkholderiales (*Inhella* sp.) to metabolize and use polycyclic hydrocarbons and other petroleate derivatives (Juhasz et al., 1997), have been suggested as factors favoring the enrichment of those species on plastic surfaces. Whether these factors also contribute to their abundance increase in the periphyton remains to be determined.

Aside from genera previously shown to thrive in the presence of MP, we found representatives of other genera to decrease in abundance instead. For instance, the cyanobacterium genus *Chamaesiphon* decreased in sMP/sMP*aged* treatments compared with control. This is surprising, since this genus occurs in a broad range of environmental conditions (Gutowski et al., 2015; Kurmayer et al., 2018). This might point toward an indirect MP effect on community composition. It is possible that material properties drive initial community composition within the first hours or days (Rummel et al., 2021) but that at later stages of biofilm formation, the community composition is rather shaped by cell:cell interactions *via*, e.g., nutrient competition (Datta et al., 2016).

Similarly, relative abundances of representatives of eukaryotes were affected upon MP exposure. For instance, our results indicated that while diatoms increased and decreased in abundance in aged and native sMP-exposed biofilms, respectively, *Cryptomycota* showed the reversed pattern. Both phyla have been shown to enrich on floating MP in the ocean (Kettner et al., 2017; Kettner et al., 2019). The particular cell shape of diatoms could favor their attachment (Sullivan, 2019) and the organic substances that are adsorbed to the MP surfaces in aquatic environments could attract *Cryptomycota,* as a heterotrophic decomposer (Grossart and Rojas-Jimenez, 2016). However, the very low MP:cell ratio in our experiments is rather indicative of indirect MP effects on cell:cell interactions.

The MP-induced shift of the phylogenetic community composition was not reflected at the phenotypic community level. This indicates that the phenotypes among the species that changed in abundance were redundant with respect to phenotypic properties. There was also no MP-induced increase in the fraction of decaying cells as observed upon periphyton exposure to, for example, the herbicide Diuron (Sgier et al., 2018a). This suggests that MP do not seem to have a specific mode of action for microorganisms toxic to cells leading to cell death, which is the case for micropollutants (e.g., pesticides). However, the phenotypic community profile showed time-dependent dynamics (**Supplementary Text S5**).

MP Do Not Affect Functional Parameters

Along with the consistency of community composition as judged from phenotype, all functional parameters assessed, that is, photosynthetic activity, microbial respiration as well as $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, and $N\text{-}NO_3^-$ uptake rates were not altered in periphyton upon MP exposure. We take this as an indication for functional redundancy, a common feature of microbial communities (Louca et al., 2018). Particularly, natural periphyton has been shown to change over space and time while maintaining ecosystem functions (Besemer et al., 2013; Wilhelm et al., 2015). However, functional effects may depend on the availability of MP surfaces for cell attachment. Previous studies have evidenced functional changes (e.g., alteration of N, carbon, and phosphate cycling) in periphyton grown on different plastic surfaces (polypropylene, polyvinyl chloride, and polyethylene terephthalate) for 25 days compared with natural substrates (Chen et al., 2020). Similarly, a different metabolic function (i.e., carbon cycling) was observed in mature biofilms (44 days old) growing on MP compared with glass, suggesting that plastic surfaces can provoke a shift in function (Miao et al., 2021). In our study, MP were present in periphyton in low concentrations. Possibly, higher MP concentrations, providing more colonizable plastic surface, could result in a shift of functional traits.

Small MP Containing Periphyton Differ in Mechanical Properties

Rheological measurements showed that periphyton containing small MP (rMP) gained similar height like control periphyton but differed in architecture and mechanical properties. In particular, a larger streamer zone was observed in MP-containing periphyton compared with control. This is interesting since the formation of streamers has only been linked with increased shear stress, for example, high water flow velocity and turbulences so far (Besemer et al., 2007). Our data suggest that MP have a similar

effect on periphyton architecture. Streamer formation provides several advantages for the periphyton, such as increased oscillation, which can enhance the transfer of solutes into the base part of the biofilm (Battin et al., 2016). Moreover, streamers represent a microniche for photoautotrophs with higher light and nutrient availability than in the base part (Besemer et al., 2009).

Besides architectural differences, we observed higher stiffness, lower stress relaxation and higher viscoelasticity in small MP exposed periphyton compared with control. This suggests that MP containing periphyton has higher resistance to external forces, for example, hydraulic pressure. These mechanical properties result from the interactions between cells, organic substances, and MP within the matrix. Inasmuch as MP:MP interactions are negligible because rMP abundance was very low, being 2 out of 100 cells, the mechanical changes must rather result from how EPS components of the periphyton interact in terms of adhesion and cohesion than by the presence of the MP. The EPS has been suggested to protect from shear stress (Gloag et al., 2020) and several unique compounds were identified to increase elasticity, ductility, and malleability of *Pseudomonas aeruginosa* biofilms (Chew et al., 2014). In this study, we did not analyze the composition of the EPS. However, we observed a significant change of prokaryotic and eukaryotic community composition of the periphyton upon MP exposure. This might have also induced a shift in the EPS composition, both in quantity and quality. Together, our study is first to measure the mechanical properties of natural stream biofilms and the results point toward a stronger periphyton matrix when MP are present. This could lead to less dislodgment and clearance of MP-containing periphyton under a flood event. However, there is a need for more detailed insight on the effects of MP on mechanical properties, for example, the critical MP concentration and MP size. This could be tested by measuring the hydraulic resistance of model hydrogel systems containing different concentrations and sizes of MP.

CONCLUSION

This study showed that both smaller (1–4 μm) and bigger (~ 63–75 μm) MP were incorporated into the periphyton matrix. Thus, with periphyton being a sink for MP but simultaneously serving as food source, MP might be transferred along the food chain with periphyton being the starting point. Whether MP in periphyton diminishes its nutritional value, or otherwise impacts on higher trophic levels, requires further investigation. The MP exposure also altered local community structure with as of yet unknown consequences on, for example, interspecific competition, flux of energy and nutrients or population recovery from other disturbances, especially upon long-term exposures to MP. Finally, exposure to small MP influenced the mechanical properties, leading to greater mechanical strength and increased periphyton stability, which may in turn reduce dislodgement and thereby affect biofilm renewal. These results point to the importance of further identifying the mechanisms underlying MP–periphyton interactions and exploring in how far they are transferable to the natural environment where periphyton grows on rocks and sediment material.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

SM, AT, KS, and RB contributed to conception and design of the study. SM and BW carried out the experiments. MT performed bioinformatics, PR carried out the rheological measurements, OS carried out the FTIR measurements, BA carried out the UV treatment and REM imaging. AK analyzed data from the community structure. SM wrote first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.928247/full#supplementary-material

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Temporal trends in anthropogenic marine macro-debris and micro-debris accumulation on the California Channel Islands

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Accumulation of anthropogenic marine debris on shorelines is an issue of global concern, even impacting areas that are remote, uninhabited, or have protected area status. On islands in Southern California, USA, within the boundaries of a National Park and National Marine Sanctuary, we collected macro-debris on beaches and assessed micro-debris in beach sediment seasonally between 2016-2020. Macro-debris (>5mm) was collected from seven beaches on two California Channel Islands and two sites on the mainland. We assessed both the number of items collected and total mass of debris. Composition of macro-debris items was dominated by plastics, particularly fragmented hard and foamed plastics and food packaging. A substantial quantity of lost or discarded fishing gear was collected, with the most fishery-related debris found at sites with historically highest spiny lobster fishing effort. The initial density of debris items ranged from 0.01-0.13 items m^{-2} and the initial density of debris mass ranged from 0.01-0.02 kg m^{-2}. Mean accumulation rates of debris were strongly site-dependent and ranged from 0.03-0.34 items m^{-2} yr^{-1} and 0.01-0.05 kg m^{-2} yr^{-1}, and tended to be highest in the fall and winter months. Anthropogenic micro-debris (<5mm) was found in beach sediment at all sites. Micro-debris had no statistically significant relationship with accumulation rates of total macro-debris items, or plastic macro-debris items. There were, however, statistically significant relationships between accumulation rates of total macro-debris mass and plastic macro-debris mass. We compared the rate of accumulation of fishing debris items and mass during the lobster season (October-March) for the years 2017 to 2020. The accumulation of fishery-related debris differed significantly among sites, with apparent declines over time, likely reflecting declining effort in the fishery and trap-limit regulations implemented in the 2017-2018 season. Our assessment of marine debris accumulation on California Channel Island beaches has provided detailed information on the types of debris and patterns of accumulation. Unfortunately, remoteness from direct human impact and protected-area status does not protect these habitats against the

onslaught of marine litter. Assessments of marine debris are critical to identify sources, to inform policy and to support efforts to reduce the impact of marine litter on vital coastal ecosystems.

KEYWORDS

anthropogenic marine debris, marine litter, macro-debris, micro-debris, spiny lobster fishery, fishery-related debris, ALDFG

Introduction

Anthropogenic marine debris (AMD) in the ocean, and on shorelines, is a developing issue of global concern (Derraik, 2002; Barnes et al., 2009; Ribic et al., 2010; Galgani et al., 2015; Madricardo et al., 2020), even impacting areas that are remote, uninhabited, or have protected area status (Lavers and Bond, 2017; Whitmire et al., 2017; Uhrin et al., 2020). Marine debris consists of any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment (Marine Debris Research, Prevention, and Reduction Act, 2009; UNEP, 2009). Plastic litter is ubiquitous, entering the ocean as macro- (>5mm) and micro-debris (<5mm) (Serra-Gonçalves et al., 2019; Madricardo et al., 2020), and frequently comprises the majority of AMD found on shorelines (Galgani et al., 2015; Agamuthu et al., 2019). Plastics are of particular concern, due to their persistence in the environment and their potential to degrade into microplastic pollution (Andrady, 2011; Gall and Thompson, 2015; Andrady et al., 2022). As plastics degrade into smaller fragments, *via* photo-degradation and physical abrasion, they become increasingly bioavailable, *via* ingestion, to a wide range of marine organisms from megafauna to zooplankton (Barnes et al., 2009; Besseling et al., 2015; Botterell et al., 2019).

Commercial fishing activity contributes a significant quantity of gear-related debris, including plastics, such as positively buoyant lines and buoys (polyethylene (PE), polypropylene (PP), expanded polystyrene (PS), polyurethane (PU) and negatively buoyant nets (polyamide (PA), pots and traps (Andrady, 2022). Fishery gear losses can be significant, with estimated percentages of line loss of 29%, average proportion of net loss of 5.7%, and loss percentages for pots and traps of 19% (Richardson et al., 2019). AMD can impact the economic success of fisheries and tourism, and the biodiversity and ecological function of marine ecosystems, (Chen and Liu, 2013; Gall and Thompson, 2015; Lucrezi et al., 2016). There are economic impacts of gear loss as fishers bear the cost of replacing lost traps and harvestable organisms are lost to derelict fishing gear (Arthur et al., 2014). Abandoned, lost or otherwise discarded fishing gear (ALDFG) is a component of AMD that can particularly impact marine wildlife *via* 'ghostfishing', entanglement, or ingestion (Macfadyen et al., 2009; Ryan, 2018; Richardson et al., 2019). The accumulation of marine debris along shorelines can negatively impact marine wildlife and the biodiversity of coastal ecosystems (Uneputty and Evans, 1997; Gall and Thompson, 2015; Kühn et al., 2015), and can increase the threat of entanglement for coastal-nesting seabirds and other organisms (Votier et al., 2011; Lavers et al., 2013). This is especially important in the context of remote islands that support significant endemism and species richness (Kier et al., 2009), where large volumes of debris are known to accumulate, and where mitigation, prevention, and debris removal efforts are expensive and challenging (Edyvane et al., 2004; Eriksson et al., 2013; Lavers and Bond, 2017).

Patterns of transport and the fate of AMD can be difficult to determine because of complex topographical and oceanographic forces acting upon debris (Barnes et al., 2009; Eriksson et al., 2013), the physical characteristics of individual items, e.g., density, surface area, and size (Schwarz et al., 2019), and the degradation, bio-fouling, and changes in buoyancy of the items over time (Kaiser et al., 2017; Madricardo et al., 2020). Larger fishery-related debris, e.g. traps and pots, may be moved along the benthos, particularly during extreme weather events, and may be ensnared by rugose benthic structure (such as coral reefs in the Florida spiny lobster fishery) (Uhrin et al., 2014; Renchen et al., 2021). The highest densities, however, of accumulated AMD are frequently observed on shorelines closest to the main sources (Ribic et al., 2010; Ribic et al., 2012; Thiel et al., 2013). Benthic sediments and sedimentary shores are often considered to be sinks of AMD, as items may sink or become trapped in the sand after stranding (Kusui and Noda, 2003; Thompson et al., 2004; UNEP, 2005; Woodall et al., 2014).

In coastal Southern California (Figure 1), marine macro- and micro-debris is pervasive in coastal watersheds and epi-benthic environments throughout the Southern California Bight (SCB) (McLaughlin et al., 2022), as well as on the more remote shorelines of the California Channel Islands, (Cole, 1998, Whitmire et al., 2017, Miller et al., 2018). In this region there are various harbors, storm drains, watersheds, vessels, dense urban populations, commercial and recreational fisheries, and

FIGURE 1
Location of marine debris monitoring and removal sites on two islands within the Channel Islands National Park and on the Ventura County Mainland, California, USA. Santa Rosa Island sites were Sandy Point (SAN), Tecolote Canyon (TEC), Soledad Canyon (SOL) and Skunk Point (SKP). Santa Cruz Island sites were Forney's Point (FOR), Christy's Beach (CHB) and Sauces (SAU). Two sites on the Ventura County mainland were Oxnard Shores (OXN) and Ormond Beach (ORM).

international shipping lanes which all can contribute to marine debris entering the SCB and potentially depositing on island shorelines (Moore et al., 2011; Ribic et al., 2012; McLaughlin et al., 2022). The Santa Barbara Channel, at the northern end of the SCB, is bounded to the north by the southern central California coast and to the south by four of the California Channel Islands (San Miguel, Santa Rosa, Santa Cruz and Anacapa) (Harms and Winant, 1998). Winds influencing the region are predominantly from the northwest for most of the year (Breaker et al., 2003). The SCB is oceanographically complex, with currents influenced by seasonally variable patterns in the equatorward California Current from the north Pacific, modest poleward flow on the continental shelf, and recirculation patterns including the counterclockwise Southern California eddy and a counterclockwise cyclonic circulation of variable intensity in the western half of the Santa Barbara Channel (Harms and Winant, 1998; Bray et al., 1999; Chen and Wang 2000).

The highly populated mainland coast, with 17.8 million inhabitants in 5 coastal counties (U.S. Census Bureau, 2020), and the vast stretches of isolated beaches in the Channel Islands National Park (population <10, U.S. Census Bureau, 2020) have variable types and abundances of marine debris on shorelines (Miller et al., 2018). Despite the proximity of the northern Channel Islands to urban centers on the California mainland, there are no permanent island residents, and these seldom-visited beaches are not easily accessible by vehicle or boat. As such, they are important habitats for a variety of species including pinnipeds, birds, and the Channel Island-endemic island fox. Beaches on the Channel Islands are vulnerable to accumulation of marine litter from nearby mainland sources and debris from fishing activities nearby, particularly lobster-fishing industry activities concentrated in nearshore habitats (Guenther et al., 2015). A previous study of four beaches on Santa Rosa Island (Arlington Canyon, Cluster Point Sandy Point, Skunk Point), one of the five islands in the Channel Islands National Park, found that the relative proportion of ALDFG had significantly increased on all beaches between surveys conducted 1989–1993 and 2015–2016 (Miller et al., 2018).

The commercial fishery for the California spiny lobster is active in the waters of the Southern California Bight, from Point Conception, south to the U.S. border with Mexico. Commercial fishery activities are generally concentrated in the fishing blocks around the offshore islands and in the nearshore waters of the mainland (California Department of Fish and Wildlife, 2019). The number of active participants in this limited-entry fishery declined from a high of 352 active permits in 1994 and has remained relatively consistent, between 135 to 166 participants since 2000; however, the number of annual trap pulls increased in the late 1990s, increased again in 2011-2014, and has since declined (California Department of Fish and Wildlife, 2019) (Figure S1). Commercial landings increased slowly from a low of

69 metric tons during the 1974-1975 fishing season until the 2000-2001 fishing season, when 319 metric tons were landed. Landings have remained fairly stable since then, exceeding 300 metric tons each season. The fishing season operates from early October to mid-March each year, with 80% of the total landings generally occuring before the end of January (NOAA Fisheries, 2022). Commercial fishers use wire box traps deployed from boats, usually positioned at a depth of less than 31m, and pulled at least every 96 hours. A change in the spiny lobster fishery regulation was implemented in the 2017-2018 season restricting fishermen to 300 traps per permit, with the ability to hold a maximum of two permits (14 C.C.R. §122). As in other crustacean fisheries (Richardson et al., 2019), a proportion of traps are lost each season due to line/buoy loss and winter storm events. The reported number of traps lost for the 2019-2020 season was 2,431 and for the 2020-2021 season was 3,311 (Hofmeister, J. *pers. comm*).

Understanding patterns in AMD abundance and identifying areas where debris accumulation is highest can assist in identifying regional factors, locating hotspots and streamlining removal efforts (McLaughlin et al., 2022). This is critical to effectively removing AMD from the Channel Islands where resources are limited, logistics are challenging, and most beaches are hard to access. Our goal was to assess the amount and types of AMD present and obtain a clearer understanding of the extent of this issue on California Channel Island beaches through seasonal monitoring and removal efforts. Regular monitoring of marine debris can also glean information that can be used to inform management decisions and measure the success of implemented policies that directly or indirectly target debris reduction (McLaughlin et al., 2022). We assessed if recent trends in lobster fishing effort and fisheries regulation changes impacted the accumulation of fishery-related debris on beaches in the region.

The objectives of this study were to (1) describe the composition of anthropogenic macro-debris found on beaches in the study area, (2) assess the initial density of macro-debris on beaches prior to removal efforts (3) calculate seasonal accumulation rates of macro-debris (4) determine if the abundance of micro-debris in beach sediment was predicted by accumulation rates of macro-debris and (5) assess if recent trends in lobster fishing effort and fisheries regulation changes impacted the accumulation of fishery-related debris on beaches in the region. To address these objectives we focused on the following research questions: (i) Does the composition of macro-debris differ among sites? (ii) does the initial density of macro-debris differ among sites? (iii) do rates of accumulation differ among sites, seasons or years?, (iv) does the rate of accumulation of macro-debris predict the abundance of micro-debris in beach sediment, and (v) does the rate of accumulation of fishery-related debris change following the lobster fishery regulation change beginning in the 2017-2018 season?

Methods

Overview

On sparsely-inhabited islands in coastal California, within the boundaries of a National Park and National Marine Sanctuary, we quantitatively surveyed and collected marine macro-debris on beaches, and assessed micro-debris in beach sediment, seasonally, between 2016 - 2020. We began initial collections of marine debris in 2016, and there were no prior debris collections for at least a year, or perhaps much longer, on island beaches. We surveyed two mainland beaches in Ventura County to enable comparison of debris composition and accumulation. Debris was collected and cataloged using NOAA's Marine Debris Program (NOAA MDP) accumulation protocol (Lippiatt et al., 2013). Many studies report only the numerical abundance of marine debris items in the environment (Galgani et al., 2015); however, mass is an equally important metric for monitoring marine debris (Ryan et al., 2020) as it is important to managing the logistics of debris removal efforts. We used both metrics, numerical abundance and mass, and measured the area surveyed to estimate initial density and rates of marine debris accumulation. We also assessed the abundance of micro-debris in beach sediments using density extraction and visual microscopy.

Study area

The California Channel Islands are an eight-island archipelago located within the Southern California Bight off the coast of Southern California (Figure 1). Santa Rosa Island (SRI) and Santa Cruz Island (SCI) are two of the five northern islands that comprise Channel Islands National Park (CINP) within the Channel Islands National Marine Sanctuary (CINMS). These islands are virtually uninhabited (limited numbers of hikers, campers, rangers, and researchers) in comparison to the neighboring highly populated mainland coast. The more remote island beaches are not accessed regularly by visitors.

Macro-debris surveys were conducted at nine sites in total: four on Santa Rosa Island, three on the west end of Santa Cruz Island, and two on the mainland in the city of Oxnard (Ventura County, California, USA) (Figure 1). Sites were chosen based on the following criteria: minimum beach length of 500 m, sandy beach habitat, and safe access (considering sensitive cultural and natural resources). Mainland sites in Oxnard (Ormond Beach and Oxnard Shores) were primarily chosen as outreach and volunteer engagement sites, and data from these sites provided information on the major differences between island and mainland debris.

Quantifying initial density of macro-debris

We collected and cataloged marine macro-debris using a modified National Oceanic and Atmospheric Administration Marine Debris Program (NOAA MDP) accumulation protocol (Lippiatt et al., 2013). At each of nine sites, we established three 100m fixed transects to account for variability in debris deposition within sites (Lippiatt et al., 2013). The initial collections were performed between Fall 2016 – Spring 2017 (Table S1). At each site, three 100m long transects, parallel to the shoreline, were measured using a fiberglass measuring tape. Unlike the NOAA MDP protocol, the entirety of the 100 m transect was surveyed in our study. The width of each transect was determined by the water's edge and the back of the beach, (defined as the location of the first barrier or primary substrate change (Lippiatt et al., 2013). The start and end points for each 100m transect were recorded, and the perimeter of the transect was mapped using GPS to calculate the area of each transect. All AMD greater than 5mm in diameter was collected and categorized according to the methods of Lippiatt et al. (2013) except that we created subcategories within the broad 'plastics' category to enable comparison with a historic data set from the CINP (Cole, 1998; Miller et al., 2018), and added a category for ALDFG (Table 1). Lippiatt et al. (2013) has one category of plastics, while the historic CINP database split plastic into three types: miscellaneous plastics, plastic packaging, and personal effects. Collected AMD items were transported to the laboratory, where they were cleaned of sand, dried, categorized and weighed. Debris that was buried, stranded, or too large to remove was recorded, tagged to indicate it had been recorded, and left in place. The initial collection at each site was used to calculate the 'initial density' of macro-debris as number of items (items m^{-2}) and mass of debris (kg m^{-2}). Removal of debris on the initial sampling occasion at each site allowed us to calculate accumulation rates in subsequent sampling occasions. The remote nature of the island beaches meant that there was no known prior removal of macro-debris for at least one year, and perhaps many years preceding our study. Debris was removed from transects but not from the entire beach due to its sheer volume and the challenging logistics required to remove marine debris from the remote island locations.

Assessing accumulation rates of macro-debris

Using the same method as the initial collection, we recorded and collected debris from the three fixed 100m transects at each site to assess accumulation rates. Similarly, the start and end points for each 100m transect were recorded, and during each survey occasion the perimeter of the transect was mapped using GPS to calculate the area of each transect. Surveys were performed seasonally (during astronomical seasons commencing on the equinoxes and solstices) (Table S1). Since tides and seasons determine the overall width of the beach, the area of each transect was recorded each time a survey was conducted. A number of logistical difficulties hindered collection, particularly during Winter seasons, where heavy rain and erosion closed island roads and boat access to the islands was limited. Additional challenges including the closure of beaches due to pinniped presence and limited access to the CINP in Winter and Spring 2020, due to the COVID-19 global pandemic. Because of this inconsistency in sampling frequency, we calculated the number of days since previous survey for each site to enable us to standardize accumulation rates by area (m^2) and time (year). Excluding the initial debris collections, we calculated the accumulation rate of macro-debris. Annual accumulation rate at each site were calculated as ((annual item accumulation rates = number of items per m^2/days since last survey)* 365)), and [(annual mass accumulation rate = mass of items per m^2/days since last survey) * 365)].

Collecting and quantifying anthropogenic micro-debris in beach sediment

In order to examine if the rate of macro-debris accumulation predicted the number of micro-debris particles in beach sediment, we collected sand samples at the sites of macro-debris collections. Prior to the survey of macro-debris at each beach, two (~250 mL) sand samples were collected from the top 5 cm of sand – one from the swash zone and one from the high-tide line. A total of eighty pairs of these samples were collected between Fall 2016 and Summer 2019 (Table S1). Samples were collected in polyethylene zip top bags and transported to the laboratory where they were dried in a drying oven at 20°C. We used a density separation method (Thompson et al., 2004) to separate anthropogenic micro-debris from the beach sediment. To minimize contamination, cotton clothing and lab coats were worn during sample processing. Each piece of glassware was rinsed three times with filtered deionized water and covered before use. A hyper-saline (sodium chloride, NaCl) solution (1.2 g cm^{-3}) was prepared and double-filtered (Whatman GF-A, nominal pore size 1.6 µm). Sand samples were measured to 100 mL from the collected sample using a pre-rinsed beaker, then placed into a pre-rinsed 1L glass jar with 400mL of hyper-saline solution. The contents of the jar were swirled vigorously for one minute, then allowed to settle for at least one minute or until the mineral matrix had settled. The supernatant was filtered onto glass fiber filters (Whatman GF-A), each filter was examined for anthropogenic microparticles using stereomicroscopy [Olympus Binocular Stereoscope (6.7x-45x) Zoom Body Trinocular Tube

TABLE 1 Major categories and sub-categories used to categorize collected macro-debris adapted from Lippiatt et al. (2013), with the inclusion of subcategories Miscellaneous Plastics, Plastic Packaging and Personal Effects within the major category Plastic, and with the addition of a major category for Abandoned, Lost or Discarded Fishing Gear.

Major Category	Subcategory	Items
PLASTIC (PLA)	Miscellaneous Plastics	Hard fragment
	Miscellaneous Plastics	Foam Fragment
	Miscellaneous Plastics	Film fragments
	Miscellaneous Plastics	Food Wrappers
	Miscellaneous Plastics	Other miscellaneous plastic
	Plastic Packaging	Bottle Cap
	Plastic Packaging	Drinking straw
	Plastic Packaging	Beverage bottle
	Plastic Packaging	Food Wrapper
	Plastic Packaging	Food Container
	Plastic Packaging	Other plastic packaging
	Plastic Packaging	Cup
	Plastic Packaging	Eating utensil
	Plastic Packaging	Plastic Bag
	Plastic Packaging	Oil containers
	Plastic Packaging	6 pack rings
	Personal Effects	Balloon
	Personal Effects	Toy
	Personal Effects	Cigarette butt
	Personal Effects	Other personal effects
	Personal Effects	Lighter
	Personal Effects	Pen
	Personal Effects	Footwear
	Personal Effects	Combs/brush/glasses
	Personal Effects	Feminine Products
	Personal Effects	Hats/helmets
	Personal Effects	Gloves
OTHER (OTH)	Metal	Metal Fragment
	Metal	Aluminum/tin cans
	Metal	Other metal
	Metal	Aerosol cans
OTHER (OTH)	Glass	Glass fragment
	Glass	Beverage bottle
	Glass	Other glass
	Glass	Jars
RUBBER (RUB)	Rubber	Rubber fragment
	Rubber	Other rubber
	Rubber	Rubber ball
	Rubber	Flip-flop
	Rubber	Tire
	Rubber	Rubber glove
OTHER (OTH)	Processed Lumber	Paper and cardboard
	Processed Lumber	Lumber/building materials
	Processed Lumber	Other lumber
	Processed Lumber	Cardboard cartons
	Processed Lumber	Paper bag

(Continued)

TABLE 1 Continued

Major Category	Subcategory	Items
OTHER (OTH)	Cloth/Fabric	Clothing/shoes
	Cloth/Fabric	Fabric fragment
	Cloth/Fabric	Other clothing
	Cloth/Fabric	Towels/rags
	Cloth/Fabric	Fabric glove
	Miscellaneous	Miscellaneous
ABANDONED, LOST OR DISCARDED FISHING GEAR (DFG)	Discarded Fishing Gear	Plastic rope fragment
	Discarded Fishing Gear	Float
	Discarded Fishing Gear	Buoy
	Discarded Fishing Gear	Lobster trap
	Discarded Fishing Gear	Other
	Discarded Fishing Gear	Non plastic rope fragment
	Discarded Fishing Gear	Lure
	Discarded Fishing Gear	Bait container
	Discarded Fishing Gear	Open strap
	Discarded Fishing Gear	Net fragment
	Discarded Fishing Gear	Fishing line
	Discarded Fishing Gear	Closed strap

Model (SZ61TR)], and items were counted and categorized by color and type. Anthropogenic microparticles, "microlitter", or micro-debris are usually defined as man-made particles less than 5 mm in size (Andrady, 2011), and fragments, fibers, and pellets are commonly found in beach sediments (Hidalgo-Ruz et al., 2012) and in other diverse ecosystems around the world (Rochman and Hoellein, 2020). Visual microscopy has been frequently used in studies to quantify and characterize larger microplastics (≥500 μm) (e.g., Lusher et al., 2017), and some studies have effectively confirmed these microparticles as plastic (Lusher et al., 2020). We defined anthropogenic micro-debris as particles that were smaller than 5 mm in their longest axis and having characteristics that indicated that they were manufactured rather than natural (Lusher et al., 2020). Density separation using saturated salt (sodium chloride, NaCl) solution (1.2 g cm^{-3}) will suspend polypropylene, polyethene and ethyl vinyl acetate, polystyrene, acrylics, polyamides and polymethylmethacrylate (Lusher et al., 2020). We categorized and enumerated items based on their morphology (fibers, particles), color (homogenous, clear) and behavior (yielding, not easily broken with minimal force) (Hidalgo-Ruz et al., 2012; Lusher et al., 2020). We excluded items of abiogenic (e.g., quartz, mica) or biogenic sediment (e.g., shell, urchin spines) or those that might be mistaken for biological components (e.g., dark or light brown fibers or particles), or the filter (white fibers) (Hidalgo-Ruz et al., 2012). To minimize misidentification of natural articles as anthropogenic micro-debris, we counted only items that were visually characterized as synthetic or semi-synthetic (Kroon et al., 2018).

Statistical analysis

To assess if there were significant differences in the initial density of macro-debris items or mass among beaches we used nested ANOVA, with transect (random) nested within site (random) and *Post-hoc* Tukey's HSD test. Marine debris items (items m^{-2}) [ln(χ +0.0001)] and mass (kg m^{-2}) [ln(χ+0.0001)] were log transformed to improve adherence to assumptions of normality and homoscedasticity. The same transformations were used in all other analyses. Adherence to the assumptions were evaluated by visual inspection of Q-Q plots and plots of residuals.

To test if site, year, or season influenced the rate of accumulation of macro-debris items or mass, we used a mixed-model ANOVA with year and season as fixed factors and site and transect nested within site as random factors.

On sampling occasions when both macro-debris was surveyed and sediment samples were collected (which averaged nine occasions per site) (Table S1) we calculated the mean accumulation rate (items and mass) of macro-debris across each site's transects on each sampling occasion, then calculated the grand mean by site. We then calculated the mean number of micro-debris items per 100 mL sediment from two samples (swash zone and high-tide line) at each site, then calculated the grand mean by site across all sampling occasions. We used a linear regression to test if the number of micro-debris items per 100 mL of beach sediment was predicted by the density of macro-debris items (items m^{-2} $year^{-1}$), or by macro-debris mass (kg m^{-2} $year^{-1}$).

Because our assessment of anthropogenic microparticles in beach sediment was most likely to identify buoyant *plastic* particles rather than other types of anthropogenic micro-debris, we assessed if there was a relationship between micro-debris in sediment and plastic macro-debris items and mass. We calculated the mean accumulation rate (items and mass) of plastic macro-debris across each site's transects on each sampling occasion, then calculated the grand mean by site. We calculated the mean number of micro-debris items per 100 mL sediment from two samples (swash zone and high-tide line) at each site, then calculated the grand mean by site across all sampling occasions. We used a linear regression to test if the number of micro-debris items per 100 mL of beach sediment was predicted by the density of plastic macro-debris items (items m^{-2} $year^{-1}$), or by plastic macro-debris mass (kg m^{-2} $year^{-1}$). Plastic macro-debris comprised items categorized as 'Miscellaneous Plastics, Plastic Packaging and Personal Effects' (Table 1).

We assessed if recent declining trends in the number of lobster traps pulled and the implementation of lobster fisheries regulation changes beginning in the 2017-2018 season affected the rate of accumulation of fishing debris items and mass. The initial survey at each site, representing an initial density of debris accumulated over an unknown time frame was excluded from the analysis. Data were not collected from Santa Cruz Island during the 2016-2017 lobster season, so we excluded that year from the analysis. We compared the rate of fishing debris item (mean number of items m^{-2} yr^{-1}) and mass accumulation (mass m^{-2} yr^{-1}) during the lobster season (October-March) for the season years 2017-2018 to 2020-2021. We used a mixed-model ANOVA with Lobster Season Year as a fixed factor and site and transect nested within site as random factors. Statistical analyses were performed using IBM SPSS Statistics Version 28.0.0.0.

Results

Macro-debris composition

We collected and cataloged 28,263 items of macro-debris over the course of this study (Table S2). There were substantial differences in the types and amounts of marine debris removed across all island and mainland sites. Plastics were the predominant type of macro-debris found across all surveyed beaches comprising about 87% of items collected. Hard and foam plastic fragments were the types of plastic most collected (Table S2). Single-use items such as beverage bottles, bottle caps and food wrappers were also very common. Of non-plastic items removed from beaches, metal fragments, rubber fragments, paper, cardboard and lumber were often found. Glass and cloth/fabric articles were generally found relatively infrequently. The mainland sites (Ormond Beach and Oxnard Shores) tended to have the highest densities of glass and metal fragments, paper/cardboard and processed lumber, as compared to the island sites. Cigarette butts, an item commonly found in beach cleanups, were an order of magnitude more common on the mainland than on the islands. Items categorized as ALDFG were much more common on Santa Rosa Island than at other sites. More than a third of ALDFG items were plastic rope fragments, about 30% were floats and buoys, and about 10%

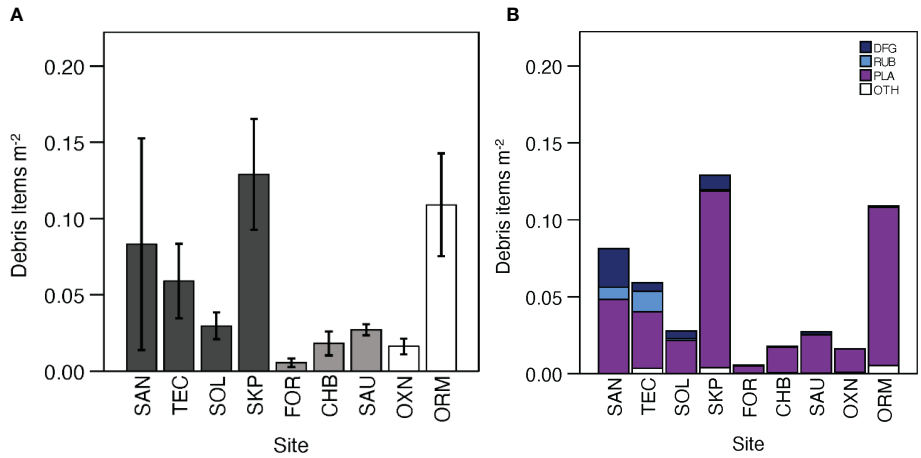

FIGURE 2
The initial density of marine debris **(A)**, represented as the number of debris items per m^2 (mean ± S.E.) collected on three transects per site during the initial collection at Santa Rosa Island (SAN, TEC, SOL, SKP - dark gray), Santa Cruz Island (FOR, CHB, SAU - light gray), and Ventura County mainland sites (OXN, ORM - white). Composition **(B)** of initial density of marine debris items represented as the mean number of debris items per m^2 collected during the initial collection at each site. Items were classified as Discarded Fishing Gear (DFG), Rubber (RUB), Other (OTH), and Plastics (PLA).

were lobster traps or lobster trap fragments (Table S2). Conversely, the greatest contribution of debris by mass was from the ALDFG category. Less commonly found ALDFG items were lures, bait containers, net fragments and monofilament. The items of AMD collected from beaches in the study averaged 0.039 ± 0.009 items per m^2 (Mean ± S.E.) and ranged from 0.007 at Forney's Point (FOR) to 0.092 items per m^2 at Sauces (SAU).

The greatest contribution of debris by mass removed from transects was from the lost fishing gear category, plastics, rubber and other debris (metal, glass, lumber, cloth) also contributed to debris mass. The average mass of macro-debris was 0.003 ± 0.001 kg per m^2 (Mean ± S.E.) and ranged from < 0.001 at Oxnard (OXN) to 0.013 kg per m^2 at Tecolote (TEC).

Initial density of macro-debris

The initial density of macro-debris items on beaches, i.e., the average number of items collected within a transect on the initial collection, ranged from 0.005 - 0.222 items m^{-2}. The mean number of debris items collected during the initial surveys was highest at the Santa Rosa Island sites (SAN, TEC, SOL, SKP) and Ormond Beach (ORM). The mean total number of items per m^2 collected as initial density differed significantly among sites ($F_{8,18}$ = 4.28, p = 0.05). *Post-hoc* Tukey's HSD test revealed that Forney's Point (FOR) had significantly lower mean number of debris items per m^2 than Tecolote (TEC), Skunk Point (SKP) and Ormond (ORM) beaches, which had significantly higher mean number of debris items per m^2 (Figure 2A). The debris items collected during the initial collections were predominantly plastics (PLA) and rubber (RUB), however, a substantial amount of ALDFG (20% of total debris) was collected at the Santa Rosa Island (SRI) sites (Figure 2B).

The average initial macro-debris mass density ranged from 0.001 - 0.041 kg m^{-2} (Figure 3A). The mass of debris collected during the initial survey comprised predominantly ALDFG, particularly on the Santa Rosa Island beaches (SAN, TEC, SOL, SKP) and Forney's Point (FOR) on Santa Cruz Island (Figure 3B). The mean mass of debris per m^2 collected as initial density differed significantly among sites ($F_{8,18}$ = 4.52, p = 0.04). *Post-hoc* Tukey's HSD test revealed that the mass of debris per m^2 at Oxnard (OXN) was significantly different than Sandy Point (SAN), Tecolote (TEC), and Skunk Point (SKP) beaches that had significantly higher mean mass of debris per m^2.

Spatial and temporal variation in macro-debris accumulation rates

Mean accumulation rates of macro-debris at individual beaches were strongly site-dependent and ranged from 0.028-0.337 items m^{-2} yr^{-1} and 0.001-0.051 kg m^{-2} yr^{-1} (Figure 4). Patterns of accumulation rates among sites, seasons and years were complex. A statistically significant two-way interaction between year and site ($F_{22,56.4}$ = 2.8, p = 0.001) and between season and site ($F_{23,\ 64.8}$ = 4.0, p < 0.001) indicated that the effect of site on accumulation rate varied among years and among seasons (Table S3, Figures S2A– C). Rates of marine debris item accumulation varied among sites where some locations, such as TEC and SAU had consistently high rates of marine debris accumulation (items m^{-2} yr^{-1}), and some were consistently low (FOR, OXN). Large quantities of debris items were collected

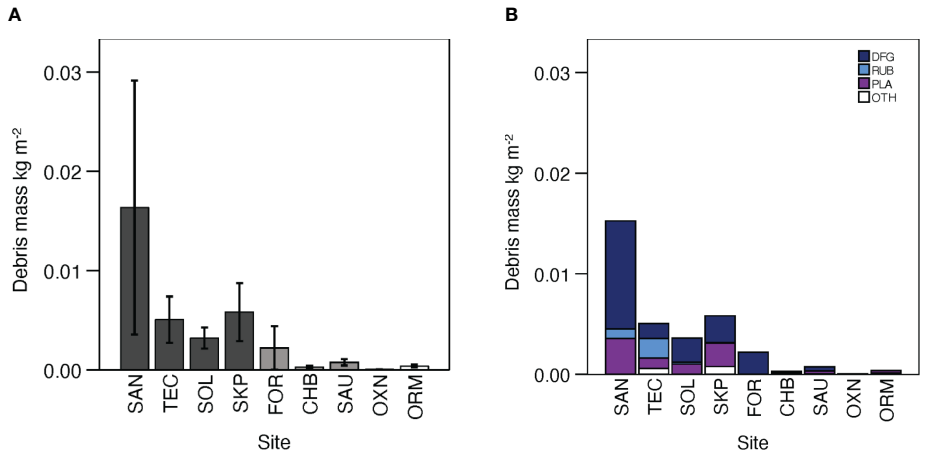

FIGURE 3
The initial density of marine debris **(A)**, represented as the mass of debris in kg per m^2 collected during the initial collection at Santa Rosa Island (SAN, TEC, SOL, SKP - dark gray), Santa Cruz Island (FOR, CHB, SAU - light gray), and Ventura County mainland sites (OXN, ORM – white). Composition **(B)** of initial density of marine debris mass represented as the mean mass of debris (kg $m^{-2)}$ collected during the initial collection at each site. Items were classified as Discarded Fishing Gear (DFG), Rubber (RUB), Other (OTH), and Plastics (PLA).

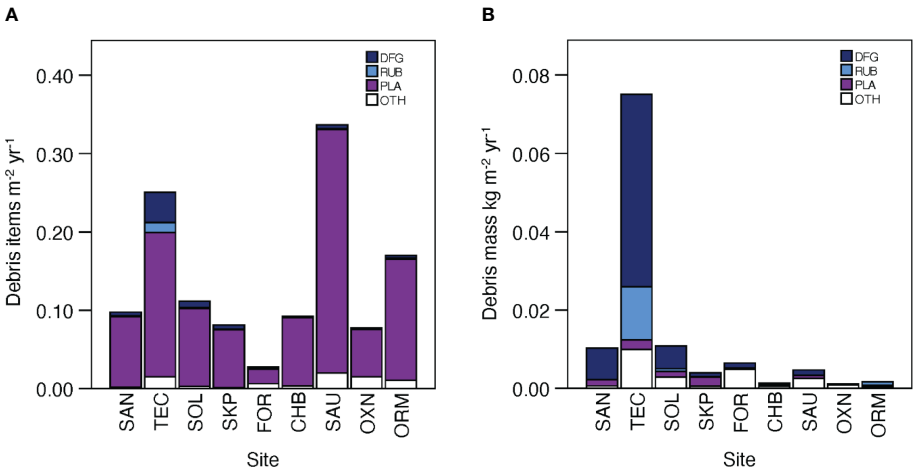

FIGURE 4
Mean number of debris items **(A)** and mass **(B)** collected by site and debris composition. Number of items and mass was standardized by area and by days since the previous survey. Plastics (PLA) was the largest category in terms of number of items removed, and fishing gear (DFG) was the largest category in terms of the mass of debris removed.

during Fall and Winter collections at some sites including TEC, SOL, and SAU. There appeared to be a decline in debris item accumulation as years progressed from 2017-2020 (Figure S2C) with the lowest accumulation rates at all sites occurring in 2020 (0.028 items m^{-2} yr^{-1}) (note that mainland collections were not conducted at OXN and ORM in 2020).

Accumulation rates of macro-debris mass among sites, seasons and years were also complex. A statistically significant two-way interaction between year and site ($F_{22,56.0} = 2.2$, p = 0.01) indicated that the effect of site on accumulation rate of debris mass varied among years (Table S4, Figures S3A–C). There was a strong effect of site on the rate of debris mass accumulation, with some sites, such as Tecolote (TEC) that had high rates of accumulation (0.051 kg m^{-2} yr^{-1}) across seasons and years (Figure S3). Sauces (SAU), however, although it had a high rate of accumulation of items, these were consistently small, low weight items of debris (frequently Styrofoam fragments) and therefore, this site had consistently low rates of debris mass accumulation (0.013 kg m^{-2} yr^{-1}).

Micro-debris in beach sediments is predicted by accumulation rate of macro-debris mass

We tested if beaches with high densities of marine macro-debris also had high densities of micro-debris in beach sediments. At the nine sample sites, we found a non-significant positive relationship between the mean density of micro-debris (items per 100 mL) in sediment and the mean macro-debris items (items m^{-2} yr^{-1}) ($R^2 = 0.18$, p = 0.26, n = 9). We found, however, a statistically significant positive relationship between the mean density of micro-debris in sediment and the mean of macro-debris mass (kg m^{-2} yr^{-1}) ($R^2 = 0.69$, p = 0.005, n = 9) (Figure S4).

We also assessed if micro-debris in sediment was related specifically to plastic macro-debris items or mass. Similar to the relationship between micro-debris in sediment and total macro-debris items, we found that there was a non-significant positive relationship between micro-debris items in sediment and plastic macro-debris items (Figure 5A) ($R^2 = 0.10$, p = 0.40, n = 9) whereas there was a statistically significant positive relationship between the mean density of micro-debris in sediment and mean plastic macro-debris mass (Figure 5B) ($R^2 = 0.60$, p = 0.01, n = 9). In all cases the positive relationship was strongly driven by the high density of macro- and micro-debris at the Tecolote Canyon beach site.

Rate of accumulation of fishery-related debris reflects trends in lobster fishery effort or changes in fisheries regulation

We compared the rate of fishing debris item accumulation (mean number of items m^{-2} yr^{-1}) during the lobster season (October-March) for the years 2017 to 2020. We found that the accumulation of fishing debris items differed significantly among sites ($F_{8,8.8} = 8.8$, p = 0.002) and among years ($F_{3,21.6} = 4.7$, p = 0.01), with an apparent pattern of decline over time (Figure 6A). Likewise, we found that the accumulation of fishing debris mass

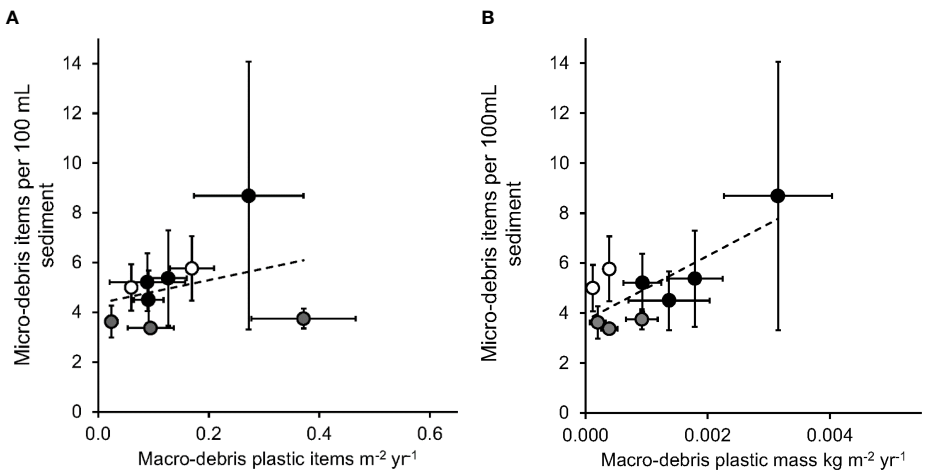

FIGURE 5
Mean plastic macro-debris item count **(A)** (items m^{-2} $year^{-1}$) and mass **(B)** (kg m^{-2} $year^{-1}$) (Mean ± SE) have non-significant positive relationships with the mean number of micro-debris items per 100mL of sediment (Mean ± SE) at sites on Santa Rosa Island (dark grey symbols), Santa Cruz Island (light grey symbols) and the California mainland (white symbols) (n = 9).

differed significantly among sites ($F_{8,5.5}$ = 13.6, p = 0.004) and among years ($F_{3,22.2}$ = 8.3, p < 0.001), where the debris mass rate of accumulation in the 2019-2020 and 2020-2021 seasons appeared much lower than in preceding seasons (Figure 6B).

Discussion

In this study we assessed the accumulation of macro- and micro-debris on remote beaches of the California Channel Islands and on the adjacent mainland. Upon initial survey and collection of debris on beaches, we found that the initial density of macro-debris was higher on the remote and infrequently visited Santa Rosa Island and Santa Cruz Island beaches, compared to mainland sites. The four sites at Santa Rosa Island, in particular, had large accumulations of debris measured as both items and mass. In the global context, the initial densities of AMD we encountered were relatively modest, and much lower than the global average of 1264.92 items m^{-2} for studies that report density of macro-debris per square meter (Serra-Gonçalves et al., 2019). The majority (62%) of studies from around the world generally reported low densities (0 - 5 items per m^2) (Serra-Gonçalves et al., 2019), similar to the macro-debris densities found in our study (0.005 - 0.222 items $m^{-2)}$ and elsewhere in coastal California (0.03 -17.1 items m^{-2}, Rosevelt et al., 2013). As has been found in other studies (Lavers and Bond, 2017; Ryan et al., 2020; United Nations Environment Programme, 2021), we found that plastics were a dominant component of the accumulated macro-debris items at all sites. Plastics were a relatively high proportion (87%) of beach debris in our study compared to a global average of 70.1% (Serra-Gonçalves et al., 2019).

To enable comparison with previous studies of daily accumulation rate (reviewed by Eriksson et al., 2013), we converted accumulation rates to items per kilometer of *linear* shoreline per day. The overall mean accumulation rate for all sites was 8.69 ± 1.56 items per linear km shoreline per day (items km^{-1} day^{-1}) (Mean ± S.E.). The minimum mean accumulation rate of 1.19 items km^{-1} day^{-1} was found at Forney's Cove (FOR) on Santa Cruz Island and the maximum mean accumulation rate was 16.60 items km^{-1} day^{-1} at Ormond Beach (ORM) on the mainland. The rates in this study were mostly higher than comparable studies of Alaskan Beaches (0.005 – 2.77 items km^{-1} $day^{-1,}$ Johnson, 1990), however, were much lower than rates from Halifax Harbor in Nova Scotia, Canada (175 – 650 items km^{-1} day^{-1} Walker et al., 2006) and Tresilian Bay in Wales, United Kingdom (28.6 – 212 items km^{-1} day^{-1} Williams and Tudor, 2001) (Eriksson et al., 2013). It is worth noting that the relatively low rates of debris accumulation we found may be underestimates. The difficulty in accessing some remote sites, where some beach surveys of marine debris have been conducted, can result in infrequent sampling, on the order of months or years. This can lead to substantial under-estimation of accumulation rates (Eriksson et al., 2013; Smith and Markic, 2013).

We found that the rates of accumulation of macro-debris were highly variable across years and seasons. There were some consistent, site-specific patterns of marine debris density. Mainland sites consistently had the lowest mass densities, some beaches that consistently had high item densities had low mass densities (*e.g.*, Sauces, Santa Cruz Island), and the

beach with consistently high density of marine debris by both number of items and by the weight of items removed was Tecolote Beach, Santa Rosa Island. The sites that had the highest accumulation rates of debris by mass were primarily those with large numbers of heavy lobster traps and trap fragments that accumulate there. There was a consistent pattern of higher rates of fishing debris accumulation on the beaches of northwestern Santa Rosa Island. This correlates with historically high lobster fishing pressure in these nearshore waters (Guenther et al., 2015; California Department of Fish and Wildlife, 2019).

The high spatial and temporal variation in debris abundance (Eriksson et al., 2013) makes it difficult to track the transport and fate of marine debris. A variety of local factors can determine the patterns of debris deposition on beaches (Debrot et al., 2013; Lavers and Bond, 2017; Schmuck et al., 2017; Waluda et al., 2020). Tidal height, wind speed and direction and storm events can strongly influence the accumulation rates of AMD on some beaches (Eriksson et al., 2013). Fishing gear, including traps and pots may be moved, subtidally, particularly during extreme weather events and may be 'captured' by rugose benthic structure such as coral reefs (as in the Florida spiny lobster fishery) (Uhrin et al., 2014; Renchen et al., 2021). On beaches surveyed in this study, the patterns of spatial and temporal variation of debris item and mass accumulation were complex. Patterns of total marine debris accumulation at each site differed among seasons, and among years. It appeared that in fall and winter the rates of debris accumulation were higher at many sites, perhaps as winter storm waves transported debris onto beaches; however, it was precisely these conditions (resulting in challenging sea crossings and washed-out island roads) that prevented access to surveys sites during most winter seasons making it difficult to fully document seasonal patterns of debris deposition. As in other studies, it is likely that winter storms and seasonal fishing patterns influence rates of accumulation during different parts of the year (Rosevelt et al., 2013; Waluda et al., 2020). There is likely also an influence of the prevailing wind direction and the anticlockwise eddy in the western part of the Santa Barbara Channel that are acting in concert with the high fishing effort on the northern coast of Santa Rosa Island to drive high rates of ALDFG accumulation in this area.

Micro-debris, and particularly microplastic, has garnered a great deal of recent attention, and we assessed if the density of micro-debris was predicted by the rates of accumulation of macro-debris. Micro-debris is time-consuming to assess compared to macro-debris, so it is useful to understand the relationship between macro- and micro-debris. Micro-debris was present in beach sediments at all sites and was predicted by the mass of macro- and plastic debris stranded on beaches. Channel Islands National Park had amongst the lowest micro-plastic densities (second lowest of nine U.S. West Coast National Parks) in a study of microplastics in National Park beaches (Whitmire et al., 2017). Micro-debris, including microplastics, are the numerically dominant type of marine debris (Hidalgo-Ruz et al., 2012), and likely have a greater impact on marine ecosystems than macro-debris (Barnes et al., 2009; Bergmann et al., 2015). Anthropogenic micro-debris is more difficult to detect than macro-debris and is virtually impossible to mitigate, therefore, a strong argument can be made to prioritize the removal of macro-debris from the marine environment. Additionally, macro-debris present in the environment is at risk of degrading into micro-debris (Barnes et al., 2009). Even if plastic pollution were halted immediately, we expect to see an increasing density of plastic fragments due to the ongoing physical weathering of plastic items already in the environment, particularly on beaches (Barnes et al., 2009). Despite the challenges associated with removal of bulky and heavy debris from remote locations, regular debris removal constitutes a worthwhile effort in mitigating impacts and improving the ecological value of coastal ecosystems.

This study coincided with the implementation of a trap limit and tag system in the 2017-2018 lobster season (14 C.C.R. §122) enabling us to observe trends in ocean-based fishery-related debris during this and three subsequent lobster fishing seasons (California Department of Fish and Wildlife, 2017). Trap loss is common in many of the world's crustacean fisheries and lost or abandoned fishing gear can have ecological impacts on living resources and sensitive coastal habitats (Jeffrey et al., 2016). In the spiny lobster fishery of the Southern California Bight, the fishing season is open from early October to mid-March each year, although 80% of the total landings generally occurs before the end of January (NOAA Fisheries, 2022). In the first half of the season, fishing effort is highest and closest to shore, with most traps usually deployed in less than 31 m of water. The number of active lobster permits has been fairly stable for the last two decades, however the number of traps pulled increased to a peak of 1,179,914 in 2013 (California Department of Fish and Wildlife, 2019; Figure S1). Due to the implementation of the California Spiny Lobster Fishery Management Plan (California Department of Fish and Wildlife, 2016), beginning in the 2017-2018 season fishermen were restricted to 300 traps per permit, with the ability to hold a maximum of two permits. (§122, Title 14, CCR). The number of trap pulls declined annually from the peak in 2013 to 665,436 by the 2019 season (California Department of Fish and Wildlife, 2019). Although our data were limited in the 2016-2017 season, preceding the regulation change, we found that the rates of accumulation of fishing-related debris items and mass was significantly different among years (2017-2018 to 2020-2021 seasons) with an apparent declining trend over time (Figure 6; Tables S5, S6). The sites that had consistently high rates of accumulation, particularly of ALDFG could be identified as sites for future targeted collection efforts, perhaps at the conclusion of lobster fishing season or after strong wave events impacting the northeastern shores of the islands. Reductions in fishing effort can result in local reductions of fishery-related marine litter (Edyvane et al., 2004) and we hope that our continued monitoring of these sites will demonstrate a continued reflection of declining debris deposition.

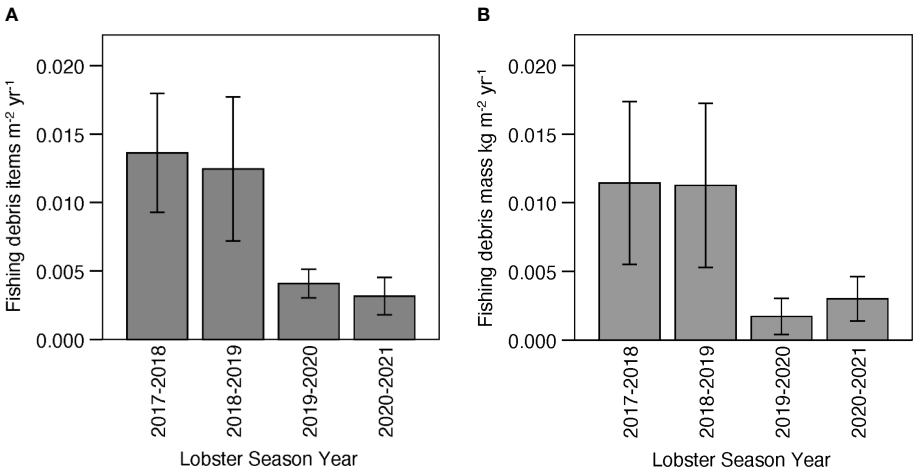

FIGURE 6
Rate of accumulation (mean ± S.E.) of lost and discarded fishing gear items **(A)** and mass **(B)** on beaches of Santa Cruz Island, Santa Rosa Island and the California mainland during the 2017-2018 through 2020-2021 lobster fishing seasons.

Regular monitoring and removal of debris can improve shoreline habitats, reduce risks to wildlife, and can provide useful information on litter composition trends and the impact of measures to reduce marine litter (such as plastic bag bans and fishery-related debris restrictions and incentives) (Lovett et al., 2007; Cho, 2009; Ribic et al., 2010; Blickley et al., 2016; McLaughlin et al., 2022). Information on the density of macro-debris items on shorelines can be helpful to describe spatial or temporal trends but may not be sufficient in a risk-assessment context. The mass and composition of AMD is particularly informative when considering the impacts to ecosystems. High densities of fragmented plastics, as we found at some sites in this study, might have relatively small impacts on recreational use of beaches, fisheries resources, and larger marine wildlife (e.g., pinnipeds), but pose a risk of ingestion by smaller animals. Larger, fishery-related debris might be relatively low in item density but poses a higher risk of entanglement and impacts to larger wildlife and is difficult to remove. Initially, such large debris may cause minimal impacts to small vertebrate and invertebrate inhabitants of beaches, but if not removed, larger debris items may ultimately fragment, increasing the numerical density of debris and becoming bioavailable to a wider range of organisms (Botterell et al., 2019). These are important considerations for management of coastal habitats and unfortunately, the accumulation of debris within National Parks and National Marine Sanctuaries threatens the protections that these protected areas are intended to provide (Renchen et al., 2021). Continued debris removal and monitoring is necessary to provide the reliable information on debris type and patterns of distribution that can support policies aimed at reducing marine litter (Rosevelt et al., 2013).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CS and MM contributed equally to this work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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The first evidence of microplastic presence in pumice stone along the coast of Thailand: A preliminary study

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In February and March 2022, a large amount of pumice stone appeared along the shoreline of Thailand. Pumice is a type of extrusive volcanic rock, and since there are no volcanoes in the Gulf of Thailand, an interesting question was where the pumice stones originated from. Another question was whether the pumice could be a vehicle for microplastics (MPs) which could then journey across the ocean until reaching the coast of Thailand. A preliminary study was begun, which randomly collected samples from seven beaches in five provinces along the coast of Thailand. Grayish-green pumice stones are tiny, porous, and lightweight, ranging from 0.3 to 5.0 cm in size. The examination found 5.7–12.6 MP items per pumice stone. Most of the MP particles observed were less than 1 mm in length. From Fourier transform infrared spectroscopy (FTIR) analysis, the MPs were characterized as polystyrene, polypropylene, poly (ethylene terephthalate) (PET), rayon, and nylon. The MP could have entered the holes in pumice stones while floating on the water surface over long periods. From the seasonal flow patterns, it was revealed that pumice from the South China Sea was more likely to have floated with surface currents into the Gulf of Thailand

KEYWORDS

microplastics, marine debris, volcano, monsoon season, South China Sea

Introduction

Plastic waste in aquatic ecosystems has become a global issue because of its toxic effects on marine animals and humans. In recent years, the production of plastic has increased rapidly in conjunction with continued socioeconomic development, urbanization, and industrialization. It has been estimated that 370 million tons of

plastic particles have been produced around the world (Plastics Europe, 2020) and around 4.8–12.7 million tons are released annually into marine environments (Haward, 2018) through river runoff and atmospheric deposition (Chen et al., 2021; Muanyaneza et al., 2022). Plastics are easily broken and fragmented into smaller-sized pieces under the effect of environmental forces, yet they take a long time to degrade. Microplastics (MPs) are usually defined as plastic particles less than 5 mm in size and can be categorized as primary and secondary according to their origin (Bradney et al., 2019; Park and Park, 2021). The greatest concern regarding MPs in the environment is their association with toxic chemicals and the transfer of those chemicals into marine organisms that ingest the debris. It has been found that MP debris can adsorb heavy metals (Brennecke et al., 2016; Pradit et al., 2021; Goh et al., 2022), as well as organic pollutants such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), and DDTs (Jimeenez-Skrzypek et al., 2021), which could be transferred to higher trophic levels and could potentially affect human health (Carbery et al., 2018). Additionally, the impact of MP ingestion on various marine animals has been studied in different parts of the world (Ahrendt et al., 2020; Barboa et al., 2020; Hue et al., 2021; Pradit et al., 2022a).

Pumice is a type of volcanic rock that is created when magma suddenly depressurizes and cools during volcanic eruptions (Whitham and Sparks, 1986). Pumice is characterized as porous and siliceous and has a sponge-like appearance (Sarkar et al., 2017). The composition of pumice is primarily silicon dioxide (SiO2) and aluminum oxide (Al2O3), with trace amounts of other oxides (Manurung et al., 2022). Pumice has been reported to have low bulk density, ranging from 0.35 to 0.65 g cm−3, high porosity (64%–85% by volume), and large pore size (Ersoy et al., 2010; Cekova et al., 2013). The skeleton structure of pumice allows molecules and particles to enter and remain within the pores. Therefore, it is possible that pumice could be a source and sink of MPs and affect the environment where it is found. Pumice has been found in many parts of the world but is most commonly associated with volcanic areas such as Turkey, Italy, Greece, Japan, and Indonesia (Bolen, 2008; Lowensten et al., 2018). Recently, numerous pumice rocks were found on the beaches along the lower Gulf of Thailand in Pattani, Narathiwat, Songkhla, and Chumphon provinces in February 2022 and in Rayong province (eastern part of Thailand) in March 2022 Pumice from volcanic eruption found on southern beaches off Gulf of Thailand, 2022. Many researchers believe they could have originated from a volcanic eruption in Indonesia or Japan (Pumice from volcanic eruption found on southern beaches off Gulf of Thailand, 2022) and were transported across the sea, partly due to the effects of the monsoon and seasonal ocean currents (Yoshida et al., 2022).

If pumice can capture and transport MPs, it could affect ecological systems wherever it is found. Most of the previous research conducted along the coast of Thailand has studied MPs in seawater, sediment, and marine species (Azad et al., 2018; Pradit et al., 2020a; Pradit et al., 2020b; Goh et al., 2021; Jualaong et al., 2021; Pradit et al., 2022b). Therefore, this study was the first to investigate MPs in pumice along the coast of Thailand. It examined the abundance, distribution, and types of MPs, and the findings can be used as baseline data for the presence of MPs in pumice stones throughout this region.

Materials and methods

Pumice stone samples (Figure 1) were collected in February and March 2022 from seven beaches in five provinces in the Gulf of Thailand (Supplementary Figure S1). These beaches were Narathiwat province, Ao Manoa Beach (NTNW, 6.4313°N 101.8498°E), Pattani province, Panare Beach (PNTC, 6.9506°N 101.2864°E), Songkhla province, Bo-it Beach (SKBI, 7.1147°N 100.6654°E), Songkhla province, Maharat Beach (SKMH, 7.4762°N 100.4454°E), Songkhla province, Pak Trae Beach

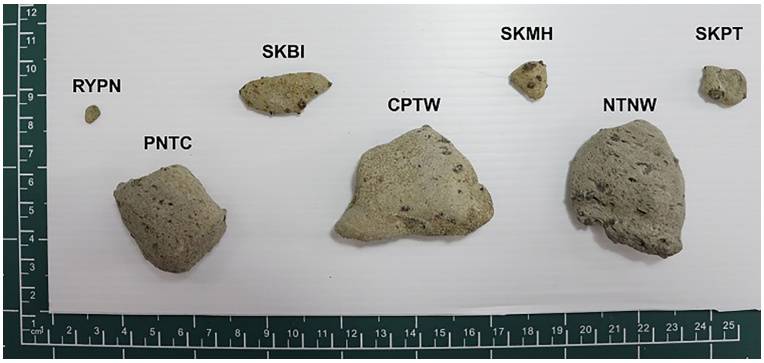

FIGURE 1
Example of pumice stone found on the coast of Thailand.

(SKPT, 7.7784°N 100.3693°E), Chumphon province, Thung Wua Laen Beach (CPTW, 10.5627°N 99.2740°E), and Rayong province, Pak Nam Rayong Beach (RYPN, 12.6551°N 101.2773°E). Samples were manually collected by picking approximately 1 kg of pumice stones from each beach. The samples were put in new, clean, plastic zip lock bags and brought back to the laboratory for analysis.

To avoid MP contamination, all devices were flushed with distilled water before use. A blank control was created using distilled water poured into a Petri dish and left in the laboratory near the stereomicroscope. At the end of the experiment, no MPs were found in the Petri dish. Furthermore, all sample processing was performed in a clean fume chamber. The pumice samples were rinsed three to five times with clean water to remove sand debris and sediments that could be from the beach environment. In total, 110 pumice stone samples were randomly selected, with a gross weight of approximately 550 g (one stone typically weighs 3–5 g while a small pumice stone typically weighs 0.3–0.5 g). It was expected that the pumice pebbles swarming Thai beaches of several provinces were probably caused by an undersea volcanic eruption and made a long-distance journey across the South China Sea before arrival at Thai beaches. The suspended pumice stones were thoroughly mixed with suspended MPs according to the long-distance journey with long marine residence times and therefore physically homogenized all the stones of pumice containing MPs. This means that the collected pumice of each stone represents one replicate. For our study, approximately 20 stones (replicates) from each province were selected, except for Songkhla province, from which 30 stones (replicates) were required. For samples from provinces where the samples were very small (0.3–0.5 g), the researchers weighed multiple pumice stones with a combined weight of 5 g to equal a single larger stone (5 g). The samples were dried in an oven at 50°C for 3–5 h. Each pumice stone was then ground using a mortar and pestle and put in a 500-ml beaker. MPs were extracted from the pumice stones using a density separation method based on NaCl, which is a widely used technique (Wang et al, 2020; Chinfak et al., 2021; Jiwarungrueangkul et al., 2021). The 250-ml saturated NaCl solution (1.2 g/cm3) was filtered through GF/C filter paper before being added to the ground pumice samples. They were then mixed by stirring the content with a stirring rod for 3–5 min, covered with aluminum foil, and left for 24 h. Subsequently, the samples were poured through a 20-μm mesh filter cloth, and the samples that remained on the filter cloth were transferred to a Petri dish before being oven-dried at 50°C for 3–5 h.

Next, the samples remaining in the Petri dish underwent MP identification by visual examination using a stereomicroscope (Olympus SZ61, Olympus Group, Shinjuku, Tokyo, Japan). The Hidalgo-Ruz et al. (2012) rules for identifying MPs were used to assist in identifying most of the MP particles which were encountered during this analysis. These rules consist of Rule 1: No cellular or organic structures visible; Rule 2: Fibers should be equally thick throughout their entire length; and Rule 3: Particles should exhibit homogenous color throughout the item. All the MPs found were recorded for amount, size [based on three class sizes (>1 mm; 500 μm–1 mm; <500 μm)] (modified from Karbalaei et al., 2019), color, and shape (fiber, fragment, or other) (Li et al., 2016; Jiwarungrueangkul et al., 2021). Polymer type identification was performed using FTIR (Spectrum Two with Spotlight 200i, Perkin Elmer Inc., Waltham, MA, USA). The wavelengths used were 4,000–400 cm−1, using an attenuated total reflection mode. The types of polymer analyzed were compared with the library attached to the FTIR. Data analysis of MP abundance, size, color, and shape was performed using MS Excel 2007 (Office Professional Plus 2019, Microsoft Corp., Redmond, WA, USA). The quantity of MPs within an approximately 5-g pumice stone was calculated to the number of items/stone. Statistical analysis to compare the addressed parameters between sampling sites was performed using the R program.

Results

Pumice stones are porous, light, and can float in water. The pumice stones used in the experiment were approximately around 0.3–5.0 cm in length. Pumice stones are mainly grayish-green, with some stones found with shells within them. From the identification of MPs through visual observation by stereomicroscope, it was found that the MPs found in RYPN = 8.9 items/stone, NTNW = 8.2 items/stone, PNTC = 6.8 items/stone, SKBI = 6.5 items/stone, SKMH = 5.9 items/stone, SKPT = 5.7, and CPTW = 12.6 items/stone. The most common type of MPs found was fiber (Figure 2A) (CPTW>NTNW>PNPT>RYPN>SKPT) except at SKBI and SKMH. The most common color found was blue, followed by white, black, and transparent (Figure 2B), with most being less than 1 mm in size (Figure 2C). FTIR was used to identify the type of polymer. The MP content was characterized as polystyrene, polypropylene, PET, rayon, and nylon, with an example spectrum shown in Supplementary Figure S2.

Discussion

Between February and March 2022, a large quantity of pumice stones was carried by ocean currents and deposited on beaches along the Gulf of Thailand. Pumice is a type of extrusive volcanic rock that is spongy and light and is produced when lava, with a very high content of water and gases, is discharged from volcanoes. When this lava cools and hardens, the result is a very light rock that is filled with tiny bubbles and gas. Based on the results of the analyses, fibers were found to be more predominant in the samples, and this structure type was also very common in previous MP research. Studies suggest that microfibers constitute up to 91% of the entire plastics collected

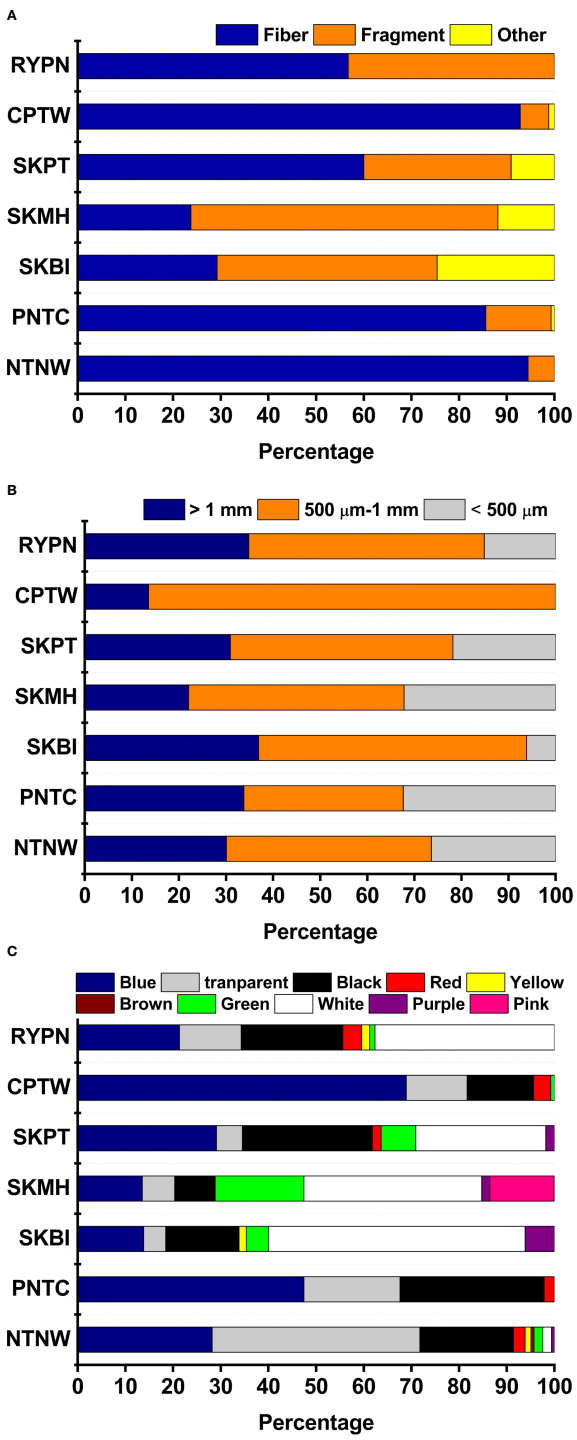

FIGURE 2
The graphs show **(A)** MP type found in pumice stone, **(B)** MP size found in pumice stone, and **(C)** MP color found in pumice stone.

in global seawater samples and are the most ubiquitous type found in ocean surface waters (Barrows et al., 2018). Fibers usually appear to be yarn or threadlike and are either found crumpled or as single threads and are considered degradable plastic waste (Pirc et al., 2016). Commonly, fibers originate from fishing nets, ropes, lines, laundry, and urban waste (Hossain et al., 2020; Severini et al., 2020). Chi-square analysis revealed that the color between the sampling sites was significantly different ($p < 0.01$), which means that blue was mostly found at CPTW and PNTC. White was largely observed at RYPN, SKBI, SKMH, and SKPT, and black was mostly found at SPKT. The most common color found was blue (mostly fiber shape). It would probably be part of the net or fishing gear used in the region. The pumice stones found in RYPN were smaller than the larger and more porous stones found in the other provinces. From one-way ANOVA, size and shape were significantly different ($p < 0.01$), whereas the amount of MPs was not significantly different ($p > 0.05$) among the stations. Most MPs are less dense than seawater and tend to float at the sea surface. Rayon fiber is commonly used to produce artificial silk and other textiles (Pradit et al., 2021). Several broad classes of plastics are used in packaging polyethylene, polypropylene, polystyrene, and PET, while PET and nylons are also used heavily in fishing gear applications (Timmers et al., 2005).

An interesting question is where the pumice stones came from since there are no volcanoes in the Gulf of Thailand. However, February and March mark the end of the southeast monsoon season, which can transport pumice stones from the South China Sea via currents in the Gulf of Thailand. It is therefore highly likely that the pumice stones were carried and driven by ocean currents and wind to the Thai coastline (Figure 3). Ocean currents reach the coastlines of the lower and central Gulf of Thailand (NTNW, PNTC, SKBI, SKMH, SKPT, and CPTW) before changing direction toward the eastern side of the Gulf of Thailand (RYPN). This likely caused pumice stones to be found on the beaches of the southern region earlier in February, and then later (March 2022) in the eastern region of Thailand. By the end of 2021, a massive quantity of pumice stones had been found on the Japanese coasts, which were blown out by the early 2021 eruption of a submarine volcano in the Ogasawara Islands, administratively part of Tokyo and 1,400 km due east of Okinawa. As a consequence, it is possible that the pumice stones found in Thailand originated from the eruption of the undersea volcano in Japan and were then swept by ocean currents in the South China Sea to the Gulf of Thailand.

Ocean currents in Southeast Asia change seasonally due to the influence of the northeast and southwest monsoons during different times of the year. Seasonal variations of the influences of the South China Sea on the water in the central Gulf of Thailand have been reported (Yanagi et al., 2001). Subsurface water intrusion from the South China Sea has been found to develop in the summer during the transition from the northeast to the southwest monsoons, in addition to during the southwest monsoon due to surface heating and Ekman transport (Buranapratheprat et al., 2016). Upwelling along the west coast also occurs during the southwest monsoon season. Strong southwest winds induce the flow of water mass from the Gulf

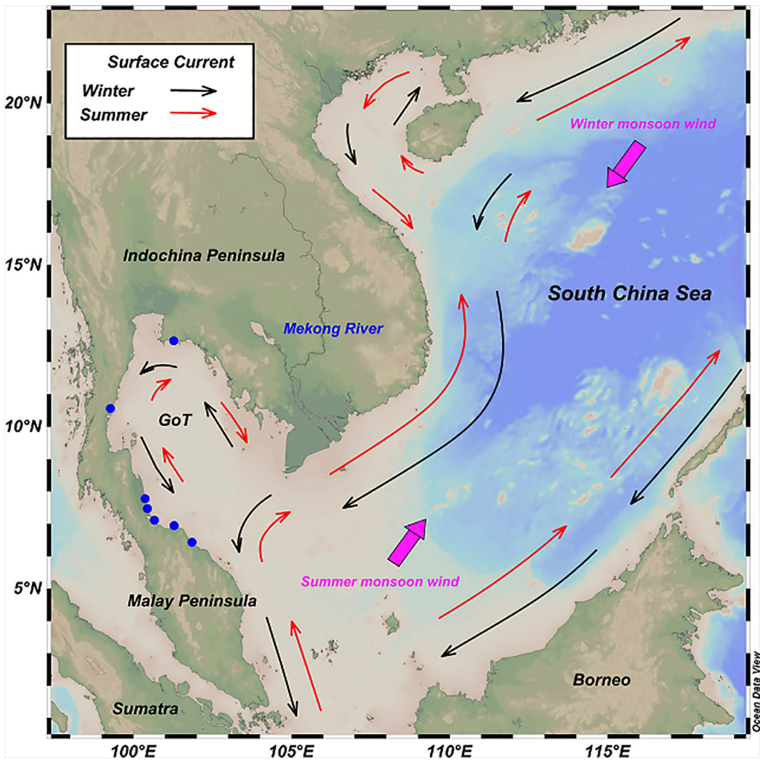

FIGURE 3
Gulf of Thailand (GoT) surface current (modified from Liu et al., 2016). Surface currents in winter (black) and summer (red) surface currents. The blue solid circle represents a sampling station from the bottom–up, namely NTNW, PNTC, SKBI, SKMH, SKPT, CPTW, and RYPN.

of Thailand to the South China Sea, lowering the mean sea level in the Gulf of Thailand (Higuchi et al., 2020). The northeast monsoon season is the period during which the Gulf of Thailand is influenced by the surface current from the South China Sea according to the wind direction. The mean sea level in the gulf then rises, generating overflowing water levels at the head of the inner Gulf of Thailand. The current flows clockwise during the northeast monsoon and counterclockwise during the southwest monsoon in the Gulf of Thailand (Liu et al., 2016). This water circulation weakens during the transition periods between the monsoons. From the seasonal flow patterns, it has been revealed that pumice stones from the South China Sea are more likely to float along with surface currents into the Gulf of Thailand during the northeast monsoon season, than during the southwest monsoon season.

It is also highly possible that the pumice stones were from the eruption of the undersea volcano in Japan, mixed with MPs floating at the surface of the Western Pacific Ocean, and were carried from the South China Sea, a hotspot of floating MPs (Liu et al., 2021). Basin flow, currents, or regional sea-level processes, such as mesoscale eddies and upwelling, may affect the horizontal and vertical transportation and the distribution of MPs. It is well known that the vesicular texture of pumice rock certainly provides a large number of suitable voids for trapping all suspended tiny particles, making it ideal for use as a filter media in the treatment of municipal and industrial effluents. The low specific gravity and high porosity of pumice make it ideal for these applications and treatment processes. Thus, the floating porous pumice stones immersed in the ocean with MPs under the influence of waves, tides, and currents will definitely provide suitable voids for being bombarded and eventually entered by MPs. Therefore, it is highly likely that they will become a place for small animals that require adhesion, such as shipworms or shellfish, to live (Velasquez et al., 2018). According to the experiment, it can be seen that MPs adhere to pumice stones (Figure 4). Surprisingly, a considerable amount of MP debris could be recovered from destroying the pumice structure by grinding with a mortar and pestle. This means that the MP particles were physically trapped inside the vesicular pumice texture and could not be easily washed away from the pumice surface. Once MP debris is trapped inside the pumice structure, it is not easily released into the environment until the pumice structure is weathered. It will have a very long residence time inside the pumice stone since it takes a few hundred years for the

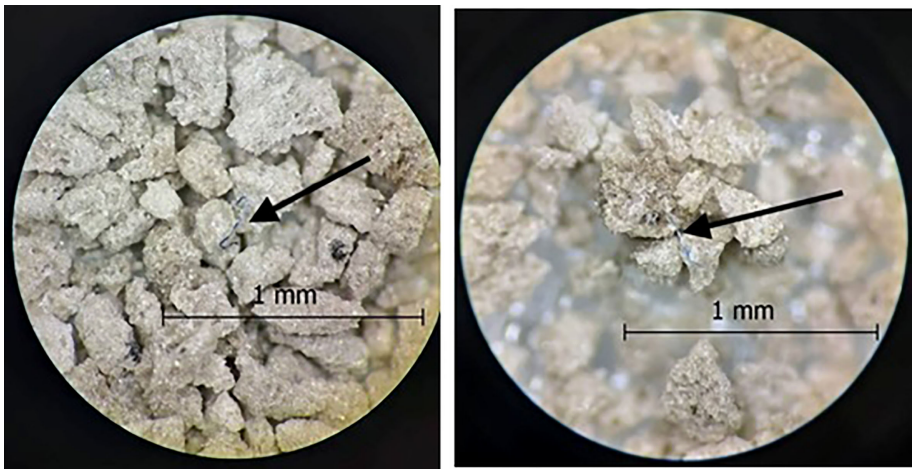

FIGURE 4
Examples of MPs attached to pumice stone (left, right). Note the light blue fiber in the middle of the circle.

pumice structure to break down and release the trapped MPs into the environment.

This is the preliminary report on the presence of MPs in pumice stones found along the coast of Thailand. MPs were found to be attached inside the stones, and it could be said that pumice stones act as a floating home for MPs and, in a way, remove it from the ocean. The polymers found were common types seen in surface water and include polystyrene, polypropylene, PET, rayon, and nylon. It was revealed that volcanic rocks from the South China Sea were more likely to float along surface currents into the Gulf of Thailand. Considering that the MPs found in the pumice stones were tiny (<1 mm), it is very likely that MPs entered the pores of the pumice stones. It is possible that pumice could be a distribution source and sink of MPs. Thus, this amazing feature of the pumice structure probably makes the pumice stone an excellent scavenger of the MP debris suspended in seawater. Although the occurrence of pumice around coastal lines normally has adverse impacts on fishing, transport, ports, and tourism, the appearance of pumice stone at sea will probably lessen the amount of MP contaminants in the ocean since the trapped MP debris in pumice stone will have a lower chance of being released and entering the food chain. This accident investigation finding from the natural field experiment obtained from the collected pumice stone drifting in the South China Sea from our study provides valuable information that terrestrial pumice could probably be employed as a filter medium for removing MP particles from drinking water, and after its service life, it can easily be disposed of by using it as a soil conditioner for growing plants, and the trapped MP particles will weather away before the pumice structure breaks down. Thus, no MP debris will be certainly released into the environment from the disposal of pumice filter waste.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

SP and RP contributed to the conception and design of the study. RP, PN, and SP contributed to sample collection and laboratory work. SP, AB, and PS performed the data analysis. SP wrote the first draft of the manuscript. PS, RP, AB, PN, and SP wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.961729/full#supplementary-material

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