



# Ecotoxicological and health implications of microplastic-associated biofilms: a recent review and prospect for turning the hazards into benefits

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## Abstract

Microplastics (MPs), over the years, have been regarded as a severe environmental nuisance with adverse effects on our ecosystem as well as human health globally. In recent times, microplastics have been reported to support biofouling by genetically diverse organisms resulting in the formation of biofilms. Biofilms, however, could result in changes in the physicochemical properties of microplastics, such as their buoyancy and roughness. Many scholars perceived the microplastic-biofilm association as having more severe consequences, providing evidence of its effects on the environment, aquatic life, and nutrient cycles. Furthermore, other researchers have shown that microplastic-associated biofilms have severe consequences on human health as they serve as vectors of heavy metals, toxic chemicals, and antibiotic resistance genes. Despite what is already known about their adverse effects, other interesting avenues are yet to be fully explored or developed to turn the perceived negative microplastic-biofilm association to our advantage. The major inclusion criteria for relevant literature were that it must focus on microplastic association biofilms, while we excluded papers solely on biofilms or microplastics. A total of 242 scientific records were obtained. More than 90% focused on explaining the environmental and health impacts of microplastic-biofilm association, whereas only very few studies have reported the possibilities and opportunities in turning the microplastic biofilms association into benefits. In summary, this paper concisely reviews the current knowledge of microplastic-associated biofilms and their adverse consequences and further proposes some approaches that can be developed to turn the negative association into positive.

**Keywords** MPs · Biofilm · Microbial community · Antibiotic resistance · Plasticsphere · Ecotoxicology

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## Introduction

Plastic and other emerging contaminant litter have emerged as serious pollutants in the aquatic environment due to their slow degradation (Gewert et al. 2015; Okoye et al. 2022b), prevalence, and abundance in our oceans (Eriksen et al. 2014; Horton et al. 2017) and rivers, may pose a severe threat to the entire wildlife and ecosystem (Gall and Thompson 2015; Horton et al. 2017) and maybe a possible atmospheric boundary threat (Jahnke et al. 2017; Galloway et al. 2017). Various assessments aimed at developing an understanding of the distribution and transport of marine plastic debris from coastlines and beaches to remote islands or the big oceans (Dong et al. 2021b; Wang et al. 2021b), including the downward movement from the surface of the sea down the water column to bottom sediments, are currently ongoing (Wang et al. 2022c). The contamination rate caused by plastic debris

in freshwater ecosystems is gaining global attention (Wagner et al. 2014; Okoye et al. 2022a). MPs and nano plastics (NPs) are notorious emerging environmental contaminant that has gained enormous public attention globally due to the threats and potential hazards it poses to the environment (Wang et al. 2020b, a; Chen et al. 2022b). Several studies define MPs as plastic particles < 5 mm (Luo et al. 2022). NPs are plastic particles that fall within the size range of 1 to 100 nm (Gigault et al. 2018). NPs can originate from coatings, biomedical applications, medication delivery, medical diagnostics, electronics, magnetics, and optoelectronics, in addition to being made from plastic fragmentation (Koelmans et al. 2015). The particles may become more reactive as a result of their smaller size and increased surface area, which facilitates the adsorption of various environmental pollutants. The chemical and physical features of nanoparticles alter during their production, affecting their availability and biological influence on aquatic creatures (Rummel et al. 2017; Mattsson et al. 2018). Large plastic debris (i.e., MPs) has apparent negative consequences on wildlife, in addition to the aesthetic problems of littering (Waluda and Staniland 2013). This large plastic debris undergoes fragmentation due to subjection to weathering, giving rise to MPs (Akan et al. 2021; Deme et al. 2022; Okeke et al. 2022). MPs have been shown to exert several adverse effects on both terrestrial and aquatic organisms and many other higher-level consumers in the aquatic ecosystem (Gall and Thompson 2015; Akan et al. 2021; Deme et al. 2022; Okeke et al. 2022). The small size of MPs/NPs makes them very easy to be ingested by many smaller organisms at the trophic level (Cole et al. 2013). The ecotoxicological impacts of NPs have been extensively reviewed (Chae and An 2017; Ferreira et al. 2019). A recent study shows that the growth rate of earthworms was significantly reduced by plastic litter with a consequent reduction in weight and concomitant effect of reproductive toxicity (Huerta Lwanga et al. 2016). Recent data shows that negatively and positively charged plastic fragments could accumulate in *Arabidopsis thaliana*, posing significant threats to agricultural productivity. Furthermore, because they are the largest group, microbial populations harmed by plastic litter should be given special attention (Sun et al. 2020; Chen et al. 2022a).

The natural ecosystems are comparatively rich in microbes accounting for millions of bacterial species per unit volume (Louca et al. 2019; Di Pippo et al. 2020). The abundance of microbes plays a critical role in ecological processes, such as substance metabolism, trophic cycling, and the formation of products (Oberbeckmann et al. 2015). Microbes in complex ecosystems have distinct structures and categories, and the microbial communities are dynamic. Microbial communities can quickly respond and adapt to changing environmental conditions such as climate change and anthropogenic stress-induced environmental conditions (Onrubia et al. 2021). The presence of MPs in the terrestrial ecosystem has been

shown to cause an alteration in microbial community composition based on or influenced by the physical parameters of the soil (Huang et al. 2019; Tu et al. 2020b; Qiongjie et al. 2022). The phyla of Proteobacteria, Gemmatimonadetes, and Bacteroidetes, for instance, were shown to be enriched in polyethylene-amended soil, which could lead to alterations in soil-dissolved organic matter, soil moisture, and bulk density (Wu et al. 2019). Zettler et al. (2013) were the first to coin the term “plastisphere” to describe the complex community, claiming that there were differences in the microorganisms found on the surface of debris and in the surrounding environment. In comparison to the surrounding community, the plastisphere microorganisms had a lower average abundance but a higher homogeneity. Biofilms are functionally and phylogenetically diverse communities of algae, fungi, bacteria, and protozoans collectively known as biofouling community, periphyton, and microbial assemblage (Zhurina et al. 2022). Microorganisms derive some benefits from biofilms, such as nutrient accumulation, formation of stable consortia, protection from toxic chemicals, and horizontal gene transfer (Erni-Cassola et al. 2020). Researchers are recently beginning to pay close attention to the interaction between microbes and MPs in the aquatic environment (Danso et al. 2019; Erni-Cassola et al. 2020). These plastics and plastic debris act as a unique environment for microorganisms that also make use of the surrounding nutrients for reproduction and help in the biodegradation of the plastics. A large amount of nutrients adheres to plastic surfaces in seawater quickly, which attracts microbial colonization to utilize the nutrient substances (Oberbeckmann et al. 2015; Tao et al. 2022). Plastics enhance the colonization of microbial communities, particularly harmful bacteria, in the aquatic environment. The invasion of harmful bacteria can disrupt normal gut bacterial communities and reduce the organisms’ ability to defend themselves (Kurchaba et al. 2020). According to Gong et al. (2019), pathogens made up almost half of the 20 most abundant genera attached to polyethylene, indicating that the plastics functioned as transfer vectors for pathogenic bacteria, posing a risk to human health. MPs in the environment are marked by a wide range of sizes and shapes (Enders et al. 2015; Kanhai et al. 2017; Wang et al. 2022b), which are subject to alterations with age (Jahnke et al. 2017; Potthoff et al. 2017). The natural suspended particles mixed with MPs may interfere with biofilm formation (Ogonowski et al. 2016). Furthermore, other properties of MPs, such as roughness, surface area, surface charges, etc., will definitely be altered upon the formation of biofilm at the surface. When plastic particles or products are released into the aquatic environment, a coating layer of organic and inorganic chemicals forms almost immediately (Oliveira et al. 2020). The subsequent biofilm formation on the plastic surface, which takes minutes to hours, is most probably the initial interaction with ambient biota (Zettler et al. 2013a; Tu et al. 2020a).

In this review, we have extensively summarized the relationship between microbial communities and MPs, nature and conditions favoring microplastic-associated biofilms in the aquatic environment, microbial communities, and structures associated with a biofilm of MPs, environmental and ecotoxicological implications of microplastic-associated biofilms, such as effects on nutrient cycle, impacts on aquatic organisms, trophic transfer of MPs, and hydrophobic organic chemicals and other leached toxic contaminants and their attendant animal and human health implications, trophic transfer of antibiotic-resistant genes as well as an extensive future prospects to reverse their harmful effects of microplastic-associated biofilms. This review will add to the growing body of knowledge on the impacts of microplastic-associated biofilm in the environment and its potential impact on human health.

## Methodology

We followed the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to guide the reviewing procedure.

### Inclusion criteria

Studies were considered if they met the following criteria: (1) microplastic-associated biofilms; (2) full-text articles published in English; and (3) articles from 2015 to 2022 were considered.

### Search strategy and information sources

The following databases were searched: ScienceDirect, SCOPUS, and PubMed from their launch dates until the end of the searching date of 19 May 2022. The study goal was addressed through a combination of subject headings and keywords in the search, including “microplastic, OR microplastics” and “biofilm OR biofilms.” Other simple subject terms, such as “aquatic,” “ecological,” “trophic transfer,” and other keywords were joined to the major search terms using Boolean operator AND and/or OR to get focus studies on a specific section of this review. For the Embase search to meet our inclusion criterion of full-text articles, conference abstracts were deleted. Our search did not include any other search restrictions, such as a country limitation. The other databases’ searching strategies were originally derived from the PubMed searching approach. Finally, the Google Scholar search engine was adopted for secondary search for a broad scope of relevant scientific papers.

## Selection of studies

Duplication of articles was eliminated after all research results were exported to Mendeley. To organize and finish the screening procedure, the relevant articles were entered into Covidence systematic review software accessible at [www.covidence.org](http://www.covidence.org). First, two authors separately evaluated each abstract and title for eligibility. The eligibility of each full-text publication was then separately evaluated by two authors. Conflicts were settled in both rounds by group talks.

## Quality evaluation

The Mixed Methods Appraisal Tool (MMAT) (Hong et al. 2018), a tool that assesses the methodological quality of studies involving quantitative and qualitative, was used to assess the publications’ quality in its 2018 iteration. Each of the five methodological quality requirements was given could not decide no or yes response rating. Instead of computing an aggregate value from the assessments of each requirement, a thorough report of the assessments of each requirement was utilized to determine the quality of the selected articles (Hong et al. 2018). The caliber of the studies was evaluated independently by two authors. The two authors talked it out and came to a consensus. A third author was engaged when this dialogue failed to resolve the conflict.

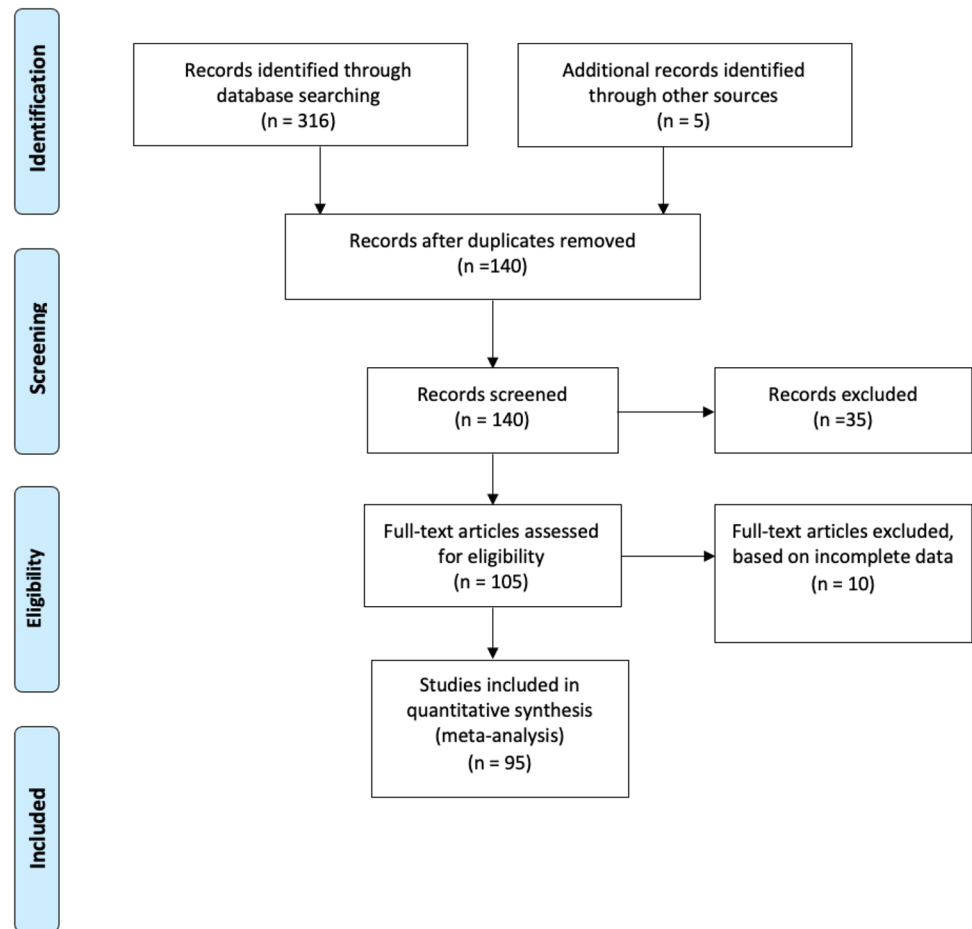
## Results

Initially, 321 publications were found (Fig. 1). Ninety-five studies fulfilled our eligibility requirements after ineligible studies and duplicated studies were eliminated.

## Microplastic-microbe association

There are several reports on the attachment of microorganisms to MPs, such as diatoms (Briand et al. 2012), fungi (De Tender et al. 2015; Zinke et al. 2017; Zhang et al. 2020b), bacteria (Brandt and House 2016; Oberbeckmann et al. 2016), and algae (Masó et al. 2003). Generally, the attachment of microorganisms to surfaces causes a significant alteration in gene expression, which might affect cell behavior. This interaction may impact the expression of genes responsible for surface attachment and motility (Tuson and Weibel 2013; Saygin and Baysal 2022). These microorganisms derive some beneficial effects from this attachment to surfaces. Horizontal surfaces, for example, promote the deposition of suspended particles in the liquid phase, resulting in nutrient accumulation on these surfaces (Tuson and Weibel 2013). As a result, attached microorganisms have access to these nutrients, which promotes growth and

**Fig. 1** Prisma diagram for study selection



development (Free et al. 2014; Shen et al. 2019). Attached microorganisms on surfaces improve access to nutrients and obtain essential metabolites like metals adsorbed on these surfaces (Fig. 1). Metals like these play a crucial role in biological and cellular functions like electron receptors (Maret 2016; Hara et al. 2017).

### Microbial communities associated with a biofilm of MPs

Microbial attachment to surfaces (specifically bacteria) usually occurs through biofilms (Free et al. 2014; Oberbeckmann et al. 2015; Shen et al. 2019). The formation of biofilms on microplastic surfaces and many other surfaces occurs through the secretion of extracellular polymeric substances (Shen et al. 2019). In addition to physical support, biofilm also provides protection from mechanical damages, enhances the bacteria's diffusivity (Fig. 1) (Oberbeckmann et al. 2015; Shen et al. 2019), as well as active protection from evading predators (Tuson and Weibel 2013). Finally, MPs' persistent and buoyant nature may also enhance the survival and spread of pathogens in soil and waters (Keswani et al. 2016; Semcesen and Wells 2021). Numerous

researchers have reported the formation of biofilms in the aquatic environment (De Tender et al. 2017; Ogonowski et al. 2018; Miao et al. 2019c; Wu et al. 2019).

A growing body of evidence has shown that there is a high degree of uniqueness and lesser diversity of microplastic biofilm relative to the microbial diversity of the nearby environment (Bryant et al. 2016; Kettner et al. 2017; Laganà et al. 2019; Miao et al. 2019c; Yang et al. 2021). As a result of the uniqueness of the microbial community attached to MPs, they form a microecological niche, especially in the marine environment, usually referred to as "plastisphere." (Zettler et al. 2013b). Results from preliminary studies have shown that the presence of MPs in the environment improves and enhances the survival rate of the microorganisms and protects them from adverse environmental conditions (Harrison et al. 2014; Bryant et al. 2016). Regardless of the type of aquatic habitat, the most commonly detected bacterial community attached to MPs belongs to the phylum Proteobacteria (Table 1). Cyanobacteria are also prominent in microplastic biofilm in the marine ecosystem (Sgier et al. 2016; Viršek et al. 2017; Dusud et al. 2018). Proteobacteria and Firmicutes have

**Table 1** Microbial community associated with microplastics in the aquatic environment

Sample matrix	Microplastic/plastic substrate	Size	Microbial community	Reference
Marine	Polystyrene	Macro-sized block (34 × 31 × 5 cm)/symmetric and antisymmetric $\nu(\text{CH}_2)$ stretching modes (10 cm × 10 cm)	Proteobacteria	Laganà et al. (2019)
Marine	Polyethylene Polypropylene Polybutylene Terephthalate		Mucoromycota Ascomycota, Blastocladiomycota, Basidiomycota	Xue et al. (2021)
Freshwater	Polypropylene Ethylene Polyethylene Polystyrene	(0.92 × 0.42 m and 0.36 × 0.41 m)	Proteobacteria, Bacteroidetes, Actinobacteria	McCormick et al. (2014)
Freshwater	Polyethylene	(4 × 4 × 0.2 cm, density 1.00 g/cm <sup>3</sup> ); (4 × 4 × 0.2 cm, density 0.92 g/cm <sup>3</sup> )	Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Gammaproteobacteria, Cyanobacteriaceae, Betaproteobacteria, Ascomycota, Alphaproteobacteria, Basidiomycota, Mucoromycota, Blastocladiomycota	Wang et al. (2021)
Marine	Polypropylene Polyethylene	0.5 × 0.3 m	Cyanobacteria, Proteobacteria, Bacteroidetes	Didier et al. (2017)
Seawater	Polyethylene Polypropylene Polystyrene	0.3~1 1~2 2~5 mm	<i>V. splendidus</i>	Frère et al. (2018)
Seawater	High-density polyethylene	0.22 mm	Proteobacteria Firmicutes	Jin et al. (2020)
Seawater	Polypropylene	2.4 mm, density of 0.9 g/cm <sup>3</sup>	<i>Bacillus</i> sp., <i>Rhodococcus</i> sp.	Auta et al. (2017)
Marine	Polypropylene	250~1000 $\mu\text{m}$	<i>Zalerion maritimum</i>	Paço et al. (2017)
Freshwater	Polyvinyl chloride	(density 1.35–1.45 g cm <sup>-3</sup> , $\phi$ 3 mm)	Bacteroidetes, Chlamydiae, Firmicutes, Chlorobi, Proteobacteria, Gemmatimonadetes, Actinobacteria, Actinobacteria, Fibrobacteres, Planctomycetes, Hydrogenedentes	Wu et al. (2019)
Seawater	Polyethylene Polystyrene	3 mm, density of 0.961 g/cm <sup>3</sup> 3 mm, density of 1.04 g/cm <sup>3</sup>	Gammaproteobacteria Alphaproteobacteria	Kesy et al. (2019)
Marine water	Polyethylene pellets PET	Density of 0.91 g/cm <sup>3</sup> , 3~5 mm Density of 1.68 g/cm <sup>3</sup> , 3~5 mm	Acinetobacter, Sphingobium, Cupriavidus, Brevundimonas, Caulobacter	Li et al. (2020)
Freshwater	Polypropylene Polyethylene Polystyrene Propylene	4 × 5 cm	Veillonellaceae, Moraxellaceae, Campylobacteraceae, Pseudomonadaceae, Flavobacteriaceae, Aeromonadaceae, Comamonadaceae	Hoellein et al. (2014)
seawater	Polypropylene Polyethylene Polystyrene	50.0 mm × 50.0 mm × 1.0 mm	Bacteroides and Pseudoalteromonas	Li et al. (2019)
Freshwater	Polyethylene terephthalate	(38 × 20 cm)	Firmicutes, Proteobacteria, Nitrospira, Chloroflexi, Acidobacteria, Bacteroidetes, Verrucomicrobia	Hoellein et al. (2014)
Marine	Polyethylene Polystyrene Polypropylene	5~2 mm/2~1 mm/1~0.3 mm	Flavobacteria, Proteobacteria	Frère et al. (2018)

**Table 1** (continued)

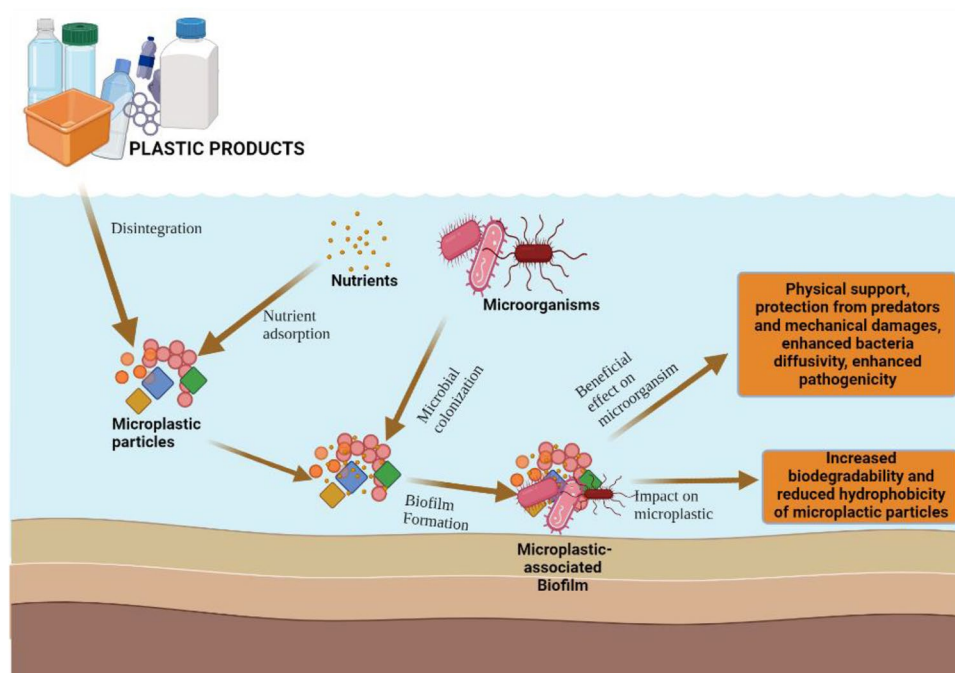
Sample matrix	Microplastic/plastic substrate	Size	Microbial community	Reference
River water	Polyvinyl chloride pellets	Density of 1.35–1.45 g/cm <sup>3</sup> , 3 mm	Proteobacteria, Bacteroidetes, Firmicutes	Wu et al. (2019)
Marine	Polyethylene Polystyrene Polypropylene	> 2 mm	Proteobacteria, Cyanobacteria	Dussud et al. (2018)
Freshwater	Polypropylene Polyethylene	(Diameter 100 nm, density 25 mg/cm <sup>-3</sup> ) (PE; diameter 3–4 mm, density 0.92 g cm <sup>-3</sup> ) and polypropylene particles (PP; diameter 3–4 mm, density 0.91 g cm <sup>-3</sup> )	Proteobacteria, Roseococcus Phycisphaerales, Firmicutes, Cyclobacteriaceae, Pirellulaceae	Miao et al. (2019a, 2019b)

also been detected in marine (Viršek et al. 2017) and freshwater ecosystems (Miao et al. 2019a, 2021b). As a result of the diversity of biofilms attached to microplastic surfaces and the presence of other essential natural substrates in waters, MPs usually have an exclusive selection of the microorganisms that are attached to them (Miao et al. 2019a). In contrast, Oberbeckmann et al. (2016) demonstrated that normal marine biofilm processes were predominantly responsible for the attachment of microorganisms on microplastic surfaces. They came to the conclusion that plastics were just surfaces for microbial attachment, not a selection process based on plastic type or surface. This conclusion was reached based on the findings that there was no significant difference between particle-associated and plastic-associated biofilms despite differences in the surrounding water. The difference between microbial communities attached to particles and free-living microorganisms in water in the same region has been reported (Ortega-Retuerta et al. 2013; Mohit et al. 2014; McCormick et al. 2014). It has also been shown that there is a significant difference between the community structure of biofilms attached to MPs and several other particles present in water (Amaral-Zettler et al. 2015; Luo et al. 2022). The ecological processes of microorganisms attached to MPs are greatly affected, although there is no sufficient data to explain the direct effect of MPs on microorganisms.

### Behavior and fate of microplastic-associated biofilms in the aquatic environment

To understand the behavior and fate of microplastic-associated biofilm, it is necessary to study biofilm formation on microplastic influenced by geographical factors, microbial biofilms, physiochemical weathering, and spatial location (Wright et al. 2020; Zhang et al. 2021). Results from preliminary studies have shown that the presence of MPs in the aquatic environment improves and enhances the survival rate of the microorganisms and protects them from adverse environmental conditions (Fig. 2) (Harrison et al. 2014; Bryant et al. 2016; Qiang et al. 2021). One of the factors involved in surface-programmed biofilm formation is conditioning film (CF), which is formed on the microplastic (substrate) via the deposition of biomolecules (products of aquatic organisms' metabolic activity), such as proteins, glycoproteins, lipids, polysaccharides, nucleic acids, and ions, aromatic amino acids which function by substratum surface modification as well as physicochemical properties, additionally, acting as a chemoattractant to the microorganism, which is required for the complex biofilm formation (Miao et al. 2019a; Bhagwat et al. 2021). Despite CFs, quorum sensing, which serves as

**Fig. 2** Microbial colonization biofilm formation on microplastic surfaces and its effects. Various plastic products used by man disintegrate and find their way into the aquatic environment, where they interact with microorganisms, resulting in the formation of microplastic-associated biofilms. This microplastic biofilm confers some beneficial roles to the microorganisms while also impacting the microplastic itself



the first stage of the contact between the solid surface and microorganisms, is another surface-initiated biofilm development (Lami 2019). Surface thermodynamics and Derjaguin-Landau-Verwey-Overbeek (DLVO)-analyses drive the initial adhesion of bacterium (Carniello et al. 2018). An increase in the number of tethers involved, interfacial water loss, structural changes in bacterial surface protein, and reorientation of bacteria on the surface all lead to irreversible adherence after the initial encounter (McGivney et al. 2020). These adhesion forces cause bacterial cell walls to deform, increasing the contact area of the substratum surface, which activates membrane-located sensor molecules, including exopolysaccharides (EPS) and efflux pumps. Other elements, such as surface conditioning, are also involved in the formation of bacterial biofilms (Carniello et al. 2018).

The presence of MPs in the aquatic ecosystem has been reported to provide a new environment for biofilm development resulting in potential antimicrobial resistance gene development. Due to their distinct environmental and ecological impacts on human aquaculture and the ecosystem, the interactions of microplastic-antibiotic resistance genes (ARGs) have raised global concern in the past few years (Dong et al. 2021a; Liu et al. 2022). Plasticsphere, which is predominantly engaged in the accumulation of ARGs and drug-resistant bacteria, has been discovered to carry sulfonamide-resistant genes that are persisting and propagating in the aquatic environment (Debroy et al. 2021; Sathicq et al. 2021). On their long journey from source to sink, microplastics are colonized and enclosed by diverse and complex biofilm-forming

microbial consortia (Xue et al. 2020). Horizontal gene transfer enhances the transfer of ARGs between biofilm-forming microbes and ambient bacteria via various mobile genetic components (Abe et al. 2020), allowing microplastic-associated biofilms to acquire ARGs from far settings and encouraging pathogenicity transmission and antibiotic resistance (AR) in the environment (Laganà et al. 2019). Biofouling has also been found to affect the fate of MPs by altering particle characteristics (e.g., density). Biofilm development raises the density of floating or buoyant MPs, causing sedimentation of these low-density particles (Oberbeckmann et al. 2015). MPs are also most likely included in so-called hetero-aggregates in the environment. Particulate matter (MPs and other suspended solids) and microbes (e.g., protozoans, algae) make up these aggregates, which are bound together by biopolymers. Lagarde et al. (2016) demonstrated polymer-dependent (PP vs. HDPE) aggregations with the algae *Chlamydomonas reinhardtii* in laboratory research. While both HDPE and PP surfaces were colonized quickly, PP was the only one to create growing hetero-aggregates of polymer particles, algal cells, and exopolysaccharides. The ability of small particles to be ingested can be altered by upscaling them via aggregation. Large hetero-aggregates are accessible to macro-feeders, while the abundance of tiny particles and hence the availability to micro-feeders (e.g., protozoans, planktonic crustaceans) declines (e.g., planktivorous fishes). As digestive fluids decompose the biopolymer matrix, the uptake of one aggregate by macro-feeders may result in an internal release and exposure to many particles of various sizes.

## Properties and conditions favoring microplastic-associated biofilm formation in the aquatic environment

Several environmental factors influence biofilm formation in the aquatic environment, such as characteristics of the MPs (substrate-specific), succession/period (time-specific), and environmental conditions. Environmental conditions and microbial community are also collectively called location-specific factors (Oberbeckmann et al. 2015; Amaral-Zettler et al. 2015; Kirstein et al. 2018; Tu et al. 2021). The various microplastic characteristics include the type of polymer (polystyrene, polyethylene polyurethane), plastic additives, and morphology (color, size, virgin or weathered, roughness) (Barlow et al. 2020). The polymer type is the most studied microplastic property since it directly impacts microplastic biofilm formation. A recent study shows that there is a significant difference between the microbial community composition of polypropylene and polyethylene in the Bay of Brest when compared to those on polystyrene at a local scale (Frère et al. 2018; Parrish and Fahrenfeld 2019) and those from the ocean on a global scale (Amaral-Zettler et al. 2015; Sun et al. 2020a; Tarafdar et al. 2021). Available evidence shows that the dominant microbial biofilms on polystyrene, polyethylene, and polyethylene terephthalate MPs are *Gammaproteobacteria* and *Alphaproteobacteria*, unlike the polyethylene MPs dominated by *Burkholderiales* in a garbage patch in the North Atlantic (Didier et al. 2017). The majority of studies, on the other hand, have concentrated on the impact of typical nonbiodegradable plastics on the formation of biofilm in aquatic environments (Koelmans et al. 2019; Akdogan and Guven 2019). Recently, Kirstein et al. (2018) showed that there was a significant difference between the microbial communities present on biodegradable polylactic acid (PLA) and those of 7 other traditional nonbiodegradable plastic polymers (Kirstein et al. 2018). Degradable MPs (such as PLA) can also be found in wastewater treatment plant effluents (Mintenig et al. 2017), but they appear to be highly resistant in the natural, typically nutrient-poor aquatic environment (Napper and Thompson 2019). Research has shown that more MPs are produced by biodegradable and bio-based PLA during degradation relative to the quantity produced by polystyrene (Napper and Thompson 2019; Battulga et al. 2022). It is worthy to note that biodegradable plastics are different and distinct from bio-based plastics, despite the fact that they are frequently confused. Nonpetroleum biological resources are used to make bio-based plastics, while biodegradable plastics can be bio-based or petroleum-based, and their degradation occurs as a result of exposure to naturally occurring

bacteria (Wackett 2019). To better understand the fate, possible toxicity, and other impacts of biodegradable plastic polymers in the aquatic ecosystem, the effects of biodegradable MPs on the formation of microbial should be extensively studied.

The properties of the microplastic surface are greatly influenced by the hydrophobicity and roughness of the MPs, two prominent factors that have an influential effect on the microbial community on the microplastic surface (Mercier et al. 2017; Gong et al. 2019). When compared to virgin samples, aged MPs have a larger surface area, polarity, and roughness, after being exposed to UV light or incubated in water for several weeks (Liu et al. 2019, 2020; Jemec Kokalj et al. 2019). These structural alterations can affect the formation of microbial communities. It has been shown that the roughness of the microplastic surface tends to improve nutrient adsorption and surface area, thereby enhancing microbial attachment (Oberbeckmann et al. 2015). The predominant microplastic type in the environment is usually the aged MPs, which have been reported to have a greater environmental impact on the aquatic environment as a result of their high sorption capacity for most hydrophobic contaminants and their subsequent ingestion by surrounding biota (Fu et al. 2019; Liu et al. 2020; Zhang et al. 2022). Therefore, it is necessary to give a detailed evaluation of the effect of microbial community structure and its role on aged MPs. Currently, available evidence shows that microplastic size has no effect on the composition of the microbial community (Parrish and Fahrenfeld 2019). Consistent with this, there was no significant observable difference in the composition of the microbial community of mesoplastic and microplastic biofilm in the North Pacific Gyre (Bryant et al. 2016). Plastic shape (sheet, monofilament, etc.), just like size, had no significant observable difference in the composition of the bacterial community (De Tender et al. 2015; Feng et al. 2020). Certain constituents added during the production of plastics, such as heat stabilizers, pigments, flame retardants, additives, plasticizers, and antimicrobial agents, are a determinant and cause certain degrees of alterations in some plastics properties of plastics (Smith et al. 2018). These factors are still unexplored and require urgent attention.

In addition to the characteristics of MPs, environmental conditions are another determining factor that greatly affects the formation of microplastic biofilms. Certain environmental conditions like physicochemical parameters (light, temperature, dissolved oxygen, pH, salinity, etc.), availability of nutrients (nitrate, carbon, and phosphorous), and pollutants (antibiotics, toxic metals, etc.) are, to a large extent, essential factors influencing the formation of microbial biofilm and succession on the MP surfaces (Shan et al. 2022). The microbial assemblage on MPs is determined by the nutrient level, temperature, and concentration



of suspended particles in lake water (Chen et al. 2019; Tavşanoğlu et al. 2020). The growth rate of microbial biofilm is also influenced by available nutrients, such as nitrogen, carbon, and phosphorous (Liu et al. 2019). The diversity of bacterial biofilm in the estuary is determined by salinity (Liu et al. 2019). The role played by aquatic animals and plants in the transfer of MPs across the food web cannot be overlooked (Au et al. 2017). Available evidence has shown that the interaction between the rhizosphere of aquatic plants and the microbiome of aquatic animals may affect the formation of microbial biofilm on MPs (Jemec Kokalj et al. 2019). This is further proof that both abiotic and biotic environmental factors can influence the microbiome of the plastisphere (Kettner et al. 2017).

The growth of microbial community on MPs biofilms is a perfect representation of temporal succession, which may be split into 3 stages: early colonization, mid-colonization, and late colonization periods (Miao et al. 2022). A perfect example is the case of members of the *Gammaproteobacteria* group, which are the dominant early pioneer community on microplastic biofilm and are subsequently and quickly replaced by members of the *Flavobacteria* and *Alphaproteobacteria* (Pollet et al. 2018; Ramsperger et al. 2020). Generally, members of *Gammaproteobacteria* and *Alphaproteobacteria* are the known early pioneers of the estuarine and marine MP biofilms (Oberbeckmann et al. 2015). Specifically, polystyrene and polyethylene MPs in the marine ecosystem are early colonized by *Vibrio* species (Kesy et al. 2019, 2020). Other microbial families (such as *Planctomycetaceae*, *Rhodobacteraceae*, *Phyllobacteriaceae*, and *Flavobacteriaceae*) are known to be the most abundant in the later phase of microplastic colonization (Pinto et al. 2019). Despite the composition of the microbial biofilm MPs being significantly different from those of the free-living bacteria in the neighboring

environment, they still depend on the surrounding microbial communities for development (Arias-Andres et al. 2018a). As a result of the clear geographical and depth-dependent distribution patterns exhibited by the microbial communities present in aquatic ecosystems like rivers and lakes, the formation of unique microbial biofilms on MPs may be influenced (Liu et al. 2018; Kavazos et al. 2018). It has also been shown that the composition of the microbial community on microplastic biofilms in the natural environments is dependent on the sources of microbial discharge into the aquatic environment (Jiang et al. 2018).

## Environmental and ecotoxicological implications of microplastic-associated biofilms

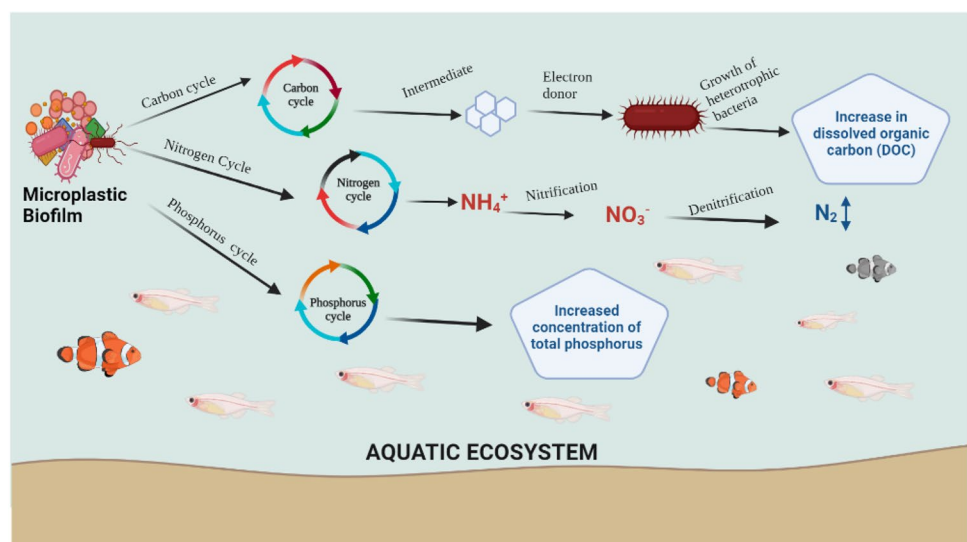
### Effects of MP-microbe interactions on biogeochemical cycles

Microplastic biofilm has been shown to influence the cyclic movement of essential chemical elements, such as carbon, phosphorus, and nitrogen between the living organisms and the surrounding external environment (Fig. 3). This biogeochemical cycle plays a crucial role in synthesizing and degrading organic matter (Rogers et al. 2020).

#### Carbon cycle

The continuous use of plastic products increases their deposition on several ecosystems as a result of inadequate management, thereby enhancing the interaction between MPs and microbes (Roager and Sonnenschein 2019). The

**Fig. 3** Effects of MP-microbe interactions on biogeochemical cycles. Microplastic biofilm has been shown to influence the cyclic movement of essential chemical elements, such as carbon, phosphorus, and nitrogen between the living organisms and the surrounding external environment. The sorption and dissolution of the biofilms lead to changes in soluble and total phosphorus and nitrogen concentrations and an increased level of dissolved organic carbon



intermediate degradative products of plastics could act as substrates, co-substrates, or carbon sources to Mos (Rogers et al. 2020). During the trophic transfer processes, there is a concomitant transfer of MPs, their degradative products and microbes, resulting in the generation of abiotic and biotic chemical reactions in the biogeochemical cycle (Zettler et al. 2013a; Rogers et al. 2020). As a result, oxidation-reduction reactions are essential in determining the fate of MPs in the natural environment. The dissolved organic carbon (DOC) is an important component of the carbon cycle and is known to be one of the largest reduced carbon pools in the world (Peter et al. 2011). Biofilms adhering to plastic particles are rich in heterotrophic bacteria, which promote the degradation of dissolved organic carbon (Peter et al. 2011). About 5.25 trillion pieces of plastic weighing 268,940 tons float at the surface of the sea (Eriksen et al. 2014). The interaction between plastic debris and organic/inorganic matter in the aquatic ecosystem could trigger the production of dissolved organic carbon on the microlayer surface (Galgani and Loiseau 2021). The transformation process is also influenced by the abundance of microbial communities (Huang et al. 2021b). A recent study shows that the leaching of dissolved organic carbon is directly linked to plastic pollution in the ocean (Romera-Castillo et al. 2018). Approximately 23,600 metric tons of dissolved DOC are discharged into ambient seawater each year as a result of microplastic particles (Romera-Castillo et al. 2018).

With more plastic debris entering the oceanic water bodies, dissolved organic carbon leaching from plastics and its possible effects on marine microorganisms and carbon cycling may become increasingly important, particularly in locations where plastic pollution is prevalent. Results from available reports show that many bacteria can potentially transform MPs into dissolved carbon sources, which could be the cause of the high-dissolved organic concentrations detected in environments with high levels of MPs (Huang et al. 2021b). These generated microplastic intermediates resulting from the degradation of MPs significantly impacts the carbon cycling in the ocean. Intermediate products, as potential electron donors, promote the formation of microbe-plastic aggregates, which have an impact on DOC cycling in the ocean (Rogers et al. 2020). Evidence from previous studies has also indicated the effect of released DOC from microplastic particles affecting the growth and carbon cycling of microbes in the ocean (Romera-Castillo et al. 2018; Galgani and Loiseau 2019). These findings showed the ubiquitous nature of MPs in the marine ecosystem could alter the carbon sequestration and turnover with unknown implications for marine biogeochemical cycles and global productivity of the ocean. Therefore, it is evident that the abundance of MPs has a great impact on the activities

of microbes, leading to alteration in their growth and interfering with the ocean's carbon pool, specifically in regions with high microplastic sequestration.

### Phosphorus cycle

There are available reports, which show that the presence of biofilm has an impact on the phosphorous (P) cycle in both the terrestrial and aquatic environment. However, little is known about the effect of microplastic on the P cycle. Recently, a study on the effect of microplastic biofilm on the phosphorous cycle in microcosm shows that the concentration of phosphorus in water increased significantly as a result of the presence of microplastic after 245 days leading to an increase in the activities of alkaline phosphatase activities in the biofilm during the period of cultivation (Chen et al. 2020). The sorption and dissolution of the biofilms were responsible for the changes in soluble and total P concentrations. Another independent research reported a significant increase in total and soluble P concentration due to the addition of MPs (Liu et al. 2017). Pieces of microplastic were found to influence the availability of P in both paddy and red soil (Yan et al. 2021). The available P content increased in red soil but dropped in paddy soil due to the two soils' different microbial communities. In contrast to N cycling, the P cycle circulates chemicals in various forms without causing gaseous loss, resulting in a reasonably stable mechanism (Chen et al. 2020). On the other hand, additional phosphorus cycling processes are required to refine the effects of MPs on terrestrial and aquatic ecosystems.

### Nitrogen cycle

The essential role of nitrogen in the life of an organism in the ecosystem is numerous, ranging from energy metabolism to the formation of materials. Ammonia finds its way into the nitrogen cycle by azofication as an intermediate product during catabolic reactions. According to a prior study, adding PE microbeads significantly raised ammonium concentration and disrupted N cycling, perhaps causing eutrophication (Cluzard et al. 2015). The presence of plastic particles can cause a significant alteration in the two major distinct pathways (denitrification and nitrification) involved in removing excess reactive nitrogen in the ecosystem (Seeley et al. 2020). The effect of 5 types of MPs on activated sludge caused a significant inhibition of the nitrification process (Li et al. 2020). A positive regulation was observed for the denitrification process, and nitrous oxide emission was remarkably increased in the presence of polyethersulfone and PVC (Wang et al. 2022d). The presence of PE MPs significantly accelerated

anammox and denitrification processes leading to an increase in the levels of anammox and denitrification genes in sediments from freshwater (Huang et al. 2021b). MPs peculiarly act as a substrate providing distinct habitat for colonization of microorganisms and biofilm formation (Oberbeckmann et al. 2015). Biofilm has also been reported to play essential roles in biomass production and biological matter cycles microbial respiration. PP microplastic was reported to create an extra anaerobic atmosphere within its inner surface, which could enhance the growth of denitrifying bacteria and consequently improve denitrification (Li et al. 2020).

Total nitrogen concentration is expected to reduce theoretically since denitrification accelerates nitrogen removal in the system. When PP was introduced, the formation of nitrogen-fixing communities contributed to the additional nitrogen input, increasing the total nitrogen content in microcosms (Chen et al. 2020). Conversely, Yan et al. (2021) found that *Microvirga* species (N-fixing root nodule bacteria) in PVC amended soil were reduced. Several parameters, including duration, cultivation system, and microplastic type, may have different effects on azotobacters. Seeley et al. (2020) investigated the association between the microbial population and nitrogen-cycling activities driven by MPs and found that different microplastic types had varying impacts on nitrogen cycling. For example, sediments modified with polylactic acid and polyurethane foam promoted nitrification and denitrification, but PVC had an inhibitory effect on both processes. The addition of various types of microplastic particles (1000 particles/L) leads to an increase in ammonia concentration (Li et al. 2020). The oxidation rate of ammonia was significantly improved by PVC and PP, while polyethersulfone, polystyrene, and polyethylene caused an inhibition of the oxidation rate. Despite the crucial role played by MPs in the nitrogen cycle, research on the subject is still in its early stages. With the support of organisms, nitrogen passes through the biogeochemical cycle, which entails a series of complex processes. The colonized microbial communities are shaped by the microplastic type, surface topography, kind, size, and bioavailability, which have an impact on the circulation of nitrogen. Understanding how different MPs affect these processes under different environmental settings and to what extent these manmade materials influence the natural nitrogen cycle remains a major knowledge gap. Furthermore, the cycling of carbon and nitrogen in ecosystems has been demonstrated to be closely related. These details are essential for a complete understanding of the ecological effects and fate of MPs in the environment. In addition, complete integration of cultivation-independent molecular methods, like proteomics, metabolomics, and metagenomics, will considerably improve

our understanding of the effects of MPs on the nitrogen cycle in different environments.

### Effect on aquatic lives through trophic transfer

There are numerous investigations on the ingestion of MPs by the biota as well as transfer along the food chains and neglected the presence of biofilms under environmental conditions (Phuong et al. 2016; Potthoff et al. 2017). However, available evidence shows that the presence of biofilm enhances the trophic transfer of some nanoparticles within the marine environment (Luo et al. 2022), which most likely applies to microplastic as well. There is high selectivity by primary consumers as they tend to preferentially feed on particles with higher nutritional content, such as microplastic-containing biofilms rich in nutrients (He et al. 2022). This could also be applied to other aquatic feeders like fishes, aquatic invertebrates, and other aquatic predators and birds. This preferential selectivity is more noticeable among selective feeders like shrimps and copepods but to a few extent among passive feeders like cladocerans (Dahms et al. 2007). The probability of MPs adhering to the filtering apparatus in suspension and filter feeders may be increased by biofilm because neutral particles have been demonstrated to be more readily captured than particles carrying negative charges (Fabra et al. 2021). Grazers like copepods and snails may accidentally consume fragments of plastic when feeding on the biofilms on the plastic surface, as evidenced by feeding marks observed on plastic debris sampled on the field (Reisser et al. 2014a). Patches of marine snow could be actively explored by zooplanktons, implying that larger quantities of MPs that are aggregated may be ingested relative to freely scattered particles (Billing et al. 1998). There was an observable enhancement in the uptake of 100-nm polystyrene beads embedded in marine aggregates in suspension-feeding bivalves relative to scattered virgin plastic particles (Ward and Kach 2009). Furthermore, the increased abundance of MPs may lead to a significant alteration in the sedimentation rate of algal bloom, thereby influencing the food supply for benthic and pelagic species (Long et al. 2015). Nanoparticle-mediated flocculation and sedimentation of algal food resulted in a lower rate of feeding in *Daphnia magna* under food-limiting conditions (Campos et al. 2013; Amariei et al. 2022). This process may affect both pelagic feeders in the mixing layer and benthic ecosystems because they may obtain the food of unusual quantity and quality. Conclusively, the formation and potential heteroaggregation of biofilm may alter the uptake and the susceptibility of various organisms to ingestion of MPs through the change in their physical properties, thereby enhancing their availability.

## Trophic transfer of microplastic-associated biofilms (MAB) and implications on human health

Aquatic and marine MPs have been reported in recent times to be associated with microbial colonization, which clogs the MPs forming biofilm and creating a unique biological environment known as plastisphere (Harrison et al. 2018; Dussud et al. 2018; Oberbeckmann and Labrenz 2020; Xue et al. 2021). In addition to several adverse ecological and environmental implications of these microplastic-associated biofilms, they can possibly impact human health adversely, as reported in very few experimental studies (Michels et al. 2018). Despite the sparingly available experimental evidence, this section presents the probable route by which these MABs could negatively affect humans. The association of MPs and biofilms can serve as anthropogenic vectors of several toxic contaminants such as heavy metals (Cao et al. 2021; Wu et al. 2022a), chemicals (Mammo et al. 2020), antibiotics, antibiotic-resistant genes (Li et al. 2021), and pathogenic organisms (Viršek et al. 2017). Humans can come into contact with contaminant MABs directly or indirectly. Direct contact can be through drinking contaminated water and swimming in rivers and other waterbody and skin in contact with such contaminated water (Huang et al. 2021a). Moreover, the transfer of recalcitrant MPs through the trophic levels and across the food chain are indirect means of getting to humans and could serve as a probable prediction of the impact of MABs on human health (Walkinshaw et al. 2020). Studies have proven that the physicochemical properties of MPs change on colonization by microbial biofilms. McGivney et al. (2020) compared the nature of MPs incubated in bacteria-containing water (BCW) and bacteria-free water (BFW) and discovered that MPs in BCW increased in their crystallinity and maximum compression, while a concomitant decrease was observed in stiffness. Contrarily, there were no observed physiochemical changes in plastics incubated in BFW (Reisser et al. 2014b). Another study by Lobelle and Cunliffe (2011) reported that the biofilm formation around buoyant polyethylene MPs causes the MPs to sink. Consequentially, MPs are prone to be wrapped by the feces of sea animals (Zhang et al. 2020a), further colonized by epiblastic organisms, such as diatoms and ciliates, and then ingested by fishes (Reisser et al. 2014b). MP-associated biofilms also accumulate heavy metals, toxic chemicals, antibiotic-resistant genes, pathogenic organisms, hydrophobic organic chemicals (HOCs), leached toxic chemicals, and degraded toxic particulates of MPs (Jin et al. 2020). In this session, we shall review how MAB serves as a portal for trophic transfers of hazards to humans and their potential risks to human health.

## Accumulation and trophic transfer of heavy metals and toxic chemicals

Heavy metals and toxic chemicals can come from diverse sources and accumulate in the aquatic environment as a result of human activities, such as mining activities, leaching of agrochemicals, pesticides and herbicides, and industrial effluent discharges. (Mishra et al. 2019). These heavy metals and toxic chemicals have been implicated in causing severe chronic conditions ranging from cancer, hormonal dysfunctions, and other genetic problems in humans (Rehman et al. 2018). A few studies have reported the accumulation of these chemicals and metals within the plastisphere of MPs. Moreover, the physicochemical properties of the surface of the biofilm favor the adherence, penetration, and accumulation of these chemicals. Despite the significant role of environmental parameters, such as aging, temperature, pH, contact time, ionic strength, and particle size in influencing the native interaction of MPs and heavy metals, the extent of their interaction is heightened in the presence of microbial biofouling and biofilm formation (Leiser et al. 2020).

Microbial biofilms influence the physicochemical properties of microplastic. A recent study by Tu et al. (2020) reported that biofouling of polyethylene microplastic causes a reduction in hydrophobicity as well as a concomitant increase in the abundance of carboxyl and ketone groups on the microplastic surface. The authors reported that these physiochemical changes result in an increase in the affinity for metal ions to get adsorbed onto the surface (Tu et al. 2020a). In general, it was recently shown by a comparative analysis by Wang et al. (2021a) that the sorption of metallic ions onto biofilm-associated MPs is significantly higher than other natural particles, such as sediments, clays, and aquatic particles. Interestingly, some studies reported some mechanisms by which metal gets adsorbed onto the surface of MAB. Electrostatic interaction, ion exchange, and surface complexation are the major and most common mechanisms identified in recent studies (Wang et al. 2020c, 2021a; Guan et al. 2020). Guan et al. (2020) reported that biofilms on the surface of polystyrene showed improved adsorption of trace metal when compared with virgin microplastic. The enhanced adsorption was attributed to the complexation of trace metals with functional groups contained in the biofilms, such as carboxyl, amino, and phenyl-OH, which was revealed by SEM-EDS and FT-IR analysis (Guan et al. 2020; Stabnikova et al. 2022).

Similarly, other studies have shown that with the increase in the maturation of biofilm, there is a concomitant increase in biofilm formation. A recent study by Richard et al. (2019) investigated the effects of biofouling of

MPs to sorb environmental pollutants and heavy metals. They reported that the concentration of heavy metals, such as Ba, Cs, Fe, Ga, Ni, and Rb progressively accumulated as the biofilms developed around the polylactic acid and low-density polyethylene MPs. Moreover, mature biofilm around MPs was reported to harbor other metals, such as Cu, Pb, Al, K, U, Co, Mg, and Mn at varying concentrations (Richard et al. 2019; Wang et al. 2022a). Similarly, Wang et al. (2020a) reported that biofilm formation onto polyethylene enhances the adsorption of copper ( $\text{Cu}^{2+}$ ) and tetracycline in MPs. The Freundlich adsorption and desorption isotherm model was adopted to show the enhancement of heavy metal adsorption by biofilm association with the MPs (Wang et al. 2018, 2020c).

Several pieces of evidence on the enhancement of heavy metal adsorption onto MABs and the ease of the trophic transfer of microplastic up to humans as consumers present a picture of the potential toxicity to humans that the association can cause (Aghoghovwia et al. 2016; Zong et al. 2021; Cao et al. 2021). Recently, Qi et al. (2021) reported that MPs act as an anthropogenic vector for the heavy metal Pb (II), and its adsorption onto MPs are enhanced by the formation and maturation of biofilms (Guan et al. 2020; Li et al. 2022b). Moreover, from their studies, they discovered that biofilm heightens the combined toxicity of both the heavy metals and MPs in *Daphnia magna* (Qi et al. 2021; Cao et al. 2021). Metal sorption onto MABs results in the loss of buoyancy and sinking of the consortium (Miao et al. 2021a). Another study reported that plastic-associated biofilms strongly adsorbed radioactive metals— $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  cations, which cause microplastic-biofilm consortiums to potentially emit radioactivity (Johansen et al. 2018, 2019). Several pieces of evidence have shown that unscrupulous exposure to radioactive emissions could have chronic effects resulting in genetic mutation, cancers, hormonal dysfunction, brain damage, and many more (Carbery et al. 2018; Wang et al. 2018; Mammo et al. 2020; Qi et al. 2021).

In the same vein, toxic chemicals and pollutants, such as antibiotics, hydrophobic organic contaminants (HOCs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) can also adhere more suitably to MABs due to the improvement of the MP hydrophobicity, density, roughness, and functional groups by the biofilm association (José and Jordao 2020). Wang et al. (2021a) reported that biofilms developed on MPs are potent vectors for aquatic pollutants to foster their trophic transfer through the food web to higher organisms. In their report, they highlighted that biofilms, which enhance the adherence of these pollutants, emit olfactory signals for sea organisms to ingest consortiums. Secondly, biofilms enhance the bioavailability of the pollutants and evade their degradation by the host defense system. Finally, the bioavailable pollutants are transmitted up the food web until they get to humans (Naik et al.

2019; Wang et al. 2021a). There are sparsely available studies on the toxic effects of pollutants associated with MABs on humans and on higher animals. More so, the fate of these chemicals needs to be—to know if they remain intact or metabolized by the microbial consortiums. There are possibilities that the microbial members of the biofilms could metabolize the toxic and recalcitrant chemicals into less toxic or more toxic derivatives. Alternatively, the association may serve as a vector of the intact pollutants. Therefore, future studies should look into elucidating the vast possibilities in the association of microplastic and biofilms.

### Accumulation and trophic transfer of antibiotic resistance genes and pathogenic organisms

Microbial biofilms are known for their recalcitrant nature and invasiveness in colonizing susceptible surfaces. Biofilms are most often resistant to antibiotics and other bactericidal agents due to the coating of the biofilm microbial community by EPS as well as the acquisition of antibiotics genes (Ghosh et al. 2019). Individual organisms in the consortium of the MAB may acquire these resistant genes from their environment and from other organisms through either vertical or horizontal gene transfer (Marathe and Bank 2022). Parthasarathy et al. (2019) opined that plastic pollution in both the aquatic and terrestrial environments is the vector for transmission of pathogens as well as antibiotic resistance. Pathogenic bacteria and fungi, such as *Pseudomonas*, *Escherichia*, *Acinetobacter*, *Candida*, *Cryptococcus*, and *Rhodotorula* were reported to be present on the surfaces of domestic plastic appliances. At the same time, other genera, such as *Pseudomonas*, *Aeromonas*, *Arcobacter*, *Zymophilus*, *Aquabacterium*, and *Campylobacter* spp. have been associated with MPs in aquatic environments (Parthasarathy et al. 2019; Boni et al. 2021). Pathogenic organisms and antibiotic genes can emanate from different sources. Hospital wastewater is one interesting hub for antibiotic resistance. Antibiotic-resistant genes, such as *ermB* (macrolides resistant), *tetW* (tetracyclines resistant), *bla<sub>TEM</sub>* ( $\beta$ -lactams resistant), *qnrS* (fluoroquinolones), *ermB*, and *sulI* (sulfonamides) have been reported to populate hospital wastewaters (Rodriguez-Mozaz et al. 2015). Similarly, effluents from antibiotic-producing industries are another portal for antibiotic resistance to gain entry to water bodies at their disposals (Felis et al. 2020).

A growing body of evidence has shown that the association of MPs in a consortium of biofilms enhances the transfer of antibiotic genes from one organism to another within the biofilm consortium. Polyamide MPs have been reported to be more prominent as a vector of antibiotic genes in freshwater than in seawaters (Arias-Andres et al. 2018b; Wang et al. 2021a). Furthermore, from their study, plasmids, especially those bearing antibiotic resistance, were more easily

transferred among MP-associated bacteria than those free-living bacteria. Finally, the gene transferred occurred across diverse phylogenetic bacteria, which potentially suggests the ease to which human pathogens could gain antibiotic resistance in a MP-associated biofilm association. These pathogens potentially gain entry into humans via ingestion of raw or poorly cooked aquatic foods and drinking unpurified waters. (Arias-Andres et al. 2018b; Li et al. 2022a). Xiang et al. (2019) reported that the ingestion of polystyrene (2–2.9 µm) by *Foliosomia candida* significantly altered its gut microbiome and antibiotic resistance gene profile. Similarly, Y. Zhang et al. (2020), in their studies, opined that the antibiotics resistance bacteria on MPs were 100 to 5000 times higher than those in fresh water and that about 25% of the microplastic biome showed multidrug resistance of TET-SFX-ERY-PEN (Zhang et al. 2020c; Deng et al. 2021). Similarly, another study from King George Island, South Shetlands, Antarctica, showed that 7 strains from 27 bacteria that were part of the biofilm of a macroplastic surface exhibited multi-antibiotic resistance against cefuroxime, cefazolin, cinoxacin, ampicillin, carbenicillin, and mezlocillin (Laganà et al. 2019). Conversely, Wu et al. (2019) compared MP surfaces to other natural surfaces, such as rock particles and leaves, for their ability to form biofilms and ease of disseminating antibiotic resistance. With the aid of high-throughput sequencing experiments and metagenomic analysis, they reported that microplastic uniquely distinctive microbial communities with the highest node connectivity (Oberbeckmann et al. 2021). More so, the metagenomics analysis revealed that microplastic biofilms possess broad-spectrum bacteria, distinctive resistome, and two opportunistic human pathogens, *Pseudomonas monteilii* and *Pseudomonas mendocina*. In summary, their results suggest that MP surfaces are the most preferred aquatic surface for biofilm formation and gene transfer of antibiotic resistance genes (Wu et al. 2019; Cholewińska et al. 2022).

In addition to the upregulation of antibiotic-resistant genes in MP-associated biofilm consortiums, several studies have reported a concomitant increase in the expression of integrons gene (*int11*) as well as metal accumulations. Integrons are static genetic elements that facilitate the integration of gene cassettes and horizontal transfers and dissemination of antibiotic-resistant genes (Zhang et al. 2020c; Wang et al. 2021c). Class 1 integrons in many studies have been associated with antibiotic resistance gene transfers in the fish pathogen *Aeromonas salmonicida* (Eckert et al. 2018; Li et al. 2021). A study conducted by Pham et al. (2021) reported that polyethylene and polystyrene MPs promoted selective colonization of antibiotic-resistant and pathogenic taxa, such as *Raoultella ornithinolytica* and *Stenotrophomonas maltophilia*. The consortium around MPs was discovered to express enriched sulfonamide resistance genes (*sul1* and *sul2*) as well as class 1 integrons (*int11*) (Pham et al. 2021). In conclusion, antibiotic-resistant

genes, when transferred to human pathogens, could cause serious health challenges, especially for high-risk individuals comprising the aged population, children, and immunocompromised patients.

## Future prospects: reversing the harmful effects of microplastic-associated biofilms

The ubiquitous nature of microplastic and its attendant adverse effects are a significant concern of many environmental scientists. Moreover, the advent of biofouling and the formation of biofilms quickly around microplastic raises more problematic issues, as discussed in the previous section. Little attention was given to the advent of harnessing the underlining biological and physicochemical principles of biofilm formation around MPs for beneficial purposes. This section briefly highlights future research perspectives on reversing the negative effects of MP-associated biofilms.

### Use of biofilms to coagulate plastic microparticles into macroparticles for easy mop-up

Plastic waste and MPs have caused severe environmental and health worries for several decades. Several approaches have been developed to help manage and control plastic litter and MPs from water bodies. A very recent review highlighted coagulation/flocculation, degradation, separation, and filtration as the significant methods for mopping up plastic wastes from water bodies, having varying limitations and successes (Krystynik et al. 2021). However, mopping up MPs is usually difficult because of their minute sizes, variations in their buoyancy, and ease of dispersal. Moreover, the coagulation and flocculation approach has been reported as one of the most efficient methods for MP mop-up. The coagulation/flocculation chemical-based methods adopt the principle of charged neutralization to cause MPs to aggregate into insoluble floc that can be easily filtered or mopped up. The limitation of this method is the adverse effect of the residual chemical on water life and ecosystem and cost ineffectiveness when trying to treat or mop up MPs from large water bodies.

The ease by which microplastic facilitates the formation of biofilms, which have been perceived as serious environmental and health challenges, can be turned into an advantage for a more effective microplastic mop-up. Several studies have reported the changes in the physicochemical properties, such as buoyancy, roughness, and tensile strength of microplastic in biofouling by microbial communities (Djaoudi et al. 2022). Free-floating MPs could easily become sediments when covered with biofilm, enhancing the ease of coagulating or forming aggregates. There are very sparse studies on developing novel biogenic particles that

could improve the rapid aggregation of MP-associated biofilms. Michels et al. (2018) reported that biogenic particles collected from the southwestern Baltic Seas stimulated the rapid aggregation of MP-associated biofilms. Future studies could focus on engineering nonpathogenic microbial strains with exciting characteristics and abilities to colonize MPs, displace or inhibit the growth of pathogenic counterparts, and efficiently aggregate to form clusters when inducers are introduced. A recent and novel study by Prof Yang Liu and colleagues from Hong Kong Polytechnic University reported the exciting abilities of engineered *Pseudomonas aeruginosa* biofilms to trap and aggregate MPs. Furthermore, the engineered biofilms also possess control release abilities, promising reuse and sustainability for microplastic mop-up (Liu et al. 2021; Ayush et al. 2022; Wu et al. 2022b).

### Engineering biofilms for microplastic degradation

Another exciting aspect of turning our problems into solutions is the engineering of biofilms to degrade MPs into harmless, beneficial, or less recalcitrant products (Lu et al. 2022). The advent of modern genetic engineering techniques has paved the way for achieving virtually any form of microbial genetic manipulation to bestow any desired traits on organisms. Microbes, which are part of the consortium of biofilms around microplastic, can be engineered to degrade hazardous MP particles into products for their metabolism. More so, there are possibilities that microbes within the biofilm could be engineers to form a syntrophic association—where another group of microbes uses the product at different stages of microplastic degradation (Sturm et al. 2022). Lastly, biofilm consortiums can be manipulated to accommodate and serve as vectors for in situ lab-engineered organisms targeted toward plastic degradation. When in the biofilm consortium, these engineered organisms have more improved specific targets for MPs (Chen et al. 2021).

Despite these research possibilities, there have not been many studies that have investigated this interesting approach. Morohoshi et al. (2018) reported that the biofilms form around poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) when incubated in freshwater samples, causing the degradation of the plastics. Using next-generation sequencing, the researcher reported 28 plastic-degrading strains, of which the majority were from the genus *Acidovorax* and order Burkholderiales (Morohoshi et al. 2018). Furthermore, Ghosh Saheli recently reported a possible modeled pathway through which biofilms could degrade plastics and MPs (Ghosh et al. 2019).

To wrap up this session, if studies can achieve the possibility of degrading MPs by engineering its biofilms, several benefits apart from environmental remediation may abound. Some interesting applications, such as the

application of degraded products from MABs could serve as supplementary nutrients for aquatic plants and animals as well as agriculture in swampy soils. When degraded, MPs could be an attractive source of metabolizable carbon and hydrogen, which are useful for maintaining the trophic level of food chains and the environmental geochemical circle.

### Microbial enzymes from microplastic-associated biofilms associations—naturally occurring and engineered

Microbial biofilms around microplastics could become an interesting source of extracellular enzymes for wide industrial and remediation purposes. Moreover, some of these enzymes contribute to the clearance of microplastics revenging freshwater and marine habitat (Menzel et al. 2021). The microbial population within the biofilm consortium could produce two different types of enzymes—the surface modifying enzymes (SME) and plastic polymer degrading enzymes (PPDE). While the SMEs contribute to making the surface of microplastics more hydrophilic and coarse for biofilm attachment, PPDE acts on plastic polymers to degrade them to monomeric units and forms that microbes can metabolize as energy sources (Wright et al. 2020). Examples of SMEs are majorly hydrolases, such as lipases, carboxylesterases, cutinases, and proteases. On the other hand, PPDE comprises oxidases, amidases, laccases, and peroxidases (Othman et al. 2021). Due to the possibility of production of SMEs and PPDEs within the microbial biofilms, the consortiums efficiently foster the enzymatic degradation of microplastic compared to organisms existing in isolation (Gao and Sun 2021).

Some of the very interesting and well-studied enzyme groups for remediating several microplastic pollutions are the polyethylene group-degrading enzymes, polyethylene terephthalate-degrading enzymes, polystyrene-degrading enzymes, and polypropylene-degrading enzymes (Othman et al. 2021; Menzel et al. 2021; Nur et al. 2022). These enzymes have been extensively studied in recent years, and a wide range of organisms comprising bacteria, fungi, actinomycetes, and algae have been implicated in extracellularly releasing these enzymes (Chattopadhyay 2022). Biofilms formation around microplastic promotes the synergy among this wide range of organisms, especially for microplastic degradation. Moreover, microbial members of the plastisphere could be improved for microplastic mop-up through genetic and metabolic engineering. Recent advances in enzymatic degradation have gained applicability in the remediation of thermos-microplastics, such as polyethylene terephthalate (PET) by polyethylene terephthalate-degrading enzymes (Maity et al. 2021).

## Other potential applications

There are other potential applications of MP-associated biofilms. Kalčíková and Bundschuh (2021) reported that biofilms around microplastic could act as a sink or source of microplastics depending on the environmental conditions. As a microplastic sink, it accumulates microplastics from a polluted environment, and as a source is the release of the accumulated microplastics from the biofilms under certain conditions (Kalčíková and Bundschuh 2021). One very recent and interesting study developed a biofilm association having an inert “trap and release” mechanism. The engineered biofilm microbial strains release exopolymeric substances (EPS) to trap and accumulate microplastics in the aquatic environments. Moreover, the biofilm association easily disintegrates on induction to release the accumulated microplastics. This bioengineered system can be of valuable applicability in microplastic recovery and removal from polluted aquatic environments (Liu et al. 2021).

Another interesting application is the engineering and fostering of the formation of periphytic biofilms around microplastic for microplastic degradation. Periphytic biofilms comprise a consortium of algae as the first colonizer, followed by bacteria, fungi, and micro- and meso-organisms (Wu et al. 2018; Shabbir et al. 2020). Periphytic biofilms are self-sufficient and more efficient for the degradation of biofilms because of the greater diversity of microbial species involved (Shabbir et al. 2022). A previous study by Shabbir et al. (2020) reported the development and application of engineered and immobilized periphytic biofilms for enhanced microplastic degradation. Other studies have shown several artificial substrates favorably support the formation, development, and immobilization of periphytic fungi, of which plastic such as PVC are interesting alternatives (Miao et al. 2019b, 2020; Wright et al. 2020). More efforts should be directed toward turning the hazards of MABs into benefits.

## Conclusion and other future prospects

MPs are ubiquitous; many recent studies have evidently proved their impact on the environment and human health. Contemporary studies have shown that the favorable biofouling of the surface of MPs has resulted in worsening negative consequences. This paper has extensively reviewed the impact of MPs on the environment, geochemical cycles, and aquatic lives. It also expounds on the heightened consequences and long-term effects on human health that may result from the favorable trophic transfer of MPs associated with biofilms. The reviews have shown that a lot of research interest is being focused on the detrimental effect of the microplastic biofilms

association, with very little attention on ways the association could be harnessed for the benefit of mankind. The review paper finally highlighted some prospects researchers could focus on turning the hazards of the microplastic biofilms association into benefits. In conclusion, rather than just directing research efforts toward only investigating and understanding the detrimental impacts of biofilms associated with microplastics, future studies should focus more on proactive measures in developing sustainable solutions to the environmental challenge or harnessing their benefits. As detailed in the manuscripts, scientists could consider advancing or developing biofilm-microplastic association to foster coagulation of plastic micro/nanoparticles into macroparticles for easy mop-up. Moreover, unexplored possibilities are available through the genetic engineering of the biofilms to promote microplastic degradation and produce degradative enzymes for environmental pollutants. Finally, the microbial constitute of the biofilms could be engineered to become avirulent and outcompete pathogenic organisms in aquaculture, aquatic, and agroecosystem.

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## Declarations

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