

# Interactions between microplastics and benthic biofilms in fluvial ecosystems: Knowledge gaps and future trends

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**Abstract:** Plastics, especially microplastics (<5 mm in length), are anthropogenic polymer particles that have been detected in almost all environments. Microplastics are extremely persistent pollutants and act as long-lasting reactive surfaces for additives, organic matter, and toxic substances. Biofilms are microbial assemblages that act as a sink for particulate matter, including microplastics. They are ubiquitous in freshwater ecosystems and provide key services that promote biodiversity and help sustain ecosystem function. Here, we provide a conceptual framework to describe the transient storage of microplastics in fluvial biofilm and develop hypotheses to help explain how microplastics and biofilms interact in fluvial ecosystems. We identify lines of future research that need to be addressed to better manage microplastics and biofilms, including how the sorption and desorption of environmental contaminants in microplastics affect biofilms and how microbial exchange between microplastics and the biofilm matrix affects biofilm characteristics like antibiotic resistance, speciation, biodiversity, species composition, and function. We also address the uptake mechanisms of microplastics by consumers and their propagation through the food web.

**Key words:** biofilm, fluvial ecosystem, microplastic, microbial assemblage, transient storage, trophic transfer

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Plastic is one of the most used materials worldwide because of its low cost of production and useful technical characteristics, including elasticity, lightness, resistance to corrosion, and ease of processing (de Souza Machado et al. 2018). However, plastic residues can cause serious environmental problems due to their low degradability (Barnes et al. 2009). These problems will be exacerbated in the near future because experts predict that plastic production will increase (Lau et al. 2020). Plastic litter, especially microplastic particles (<5 mm in length), is a contaminant of global concern because of its potential toxicity (Cormier et al. 2021), their toxic chemical constituents and persistence (Huang et al. 2021, Rai et al. 2021). Microplastics exist in the environment as primary or secondary microplastics, the latter created when larger plastic items fragment, which are the most abundant in the environment (Thompson et al. 2009; Fig. 1A–C).

Growing concern over the presence of microplastics in aquatic ecosystems has prompted research efforts focused on microplastic contamination in water, sediments, and organisms. However, freshwater ecosystems have received

considerably less attention than their marine counterparts. For example, <4% of microplastic studies investigate the role of microplastics in freshwater ecosystems (Lambert and Wagner 2018, Campanale et al. 2020).

Horton and Dixon (2018) proposed the plastic cycle as the 1<sup>st</sup> conceptualization of microplastic pollution within the environment. The model identifies the main sinks for microplastics as agricultural soils, river water, river and lake sediments, and oceans. The main sources of microplastics are direct inputs from land to sea, runoff from urban, industrial, and agricultural areas, and waste disposal sites (i.e., wastewater treatment effluents and sludge spreading). Downstream transport is the most important pathway of microplastic movement from river networks to the ocean. The plastic cycle predicts that the temporal and spatial variability of microplastic transportation within terrestrial and marine landscapes may not be unidirectional. For example, extreme meteorological events may lead to overbank flooding, erosion, deposition of river sediments to land, and coastal deposition of oceanic debris. Finally, these events

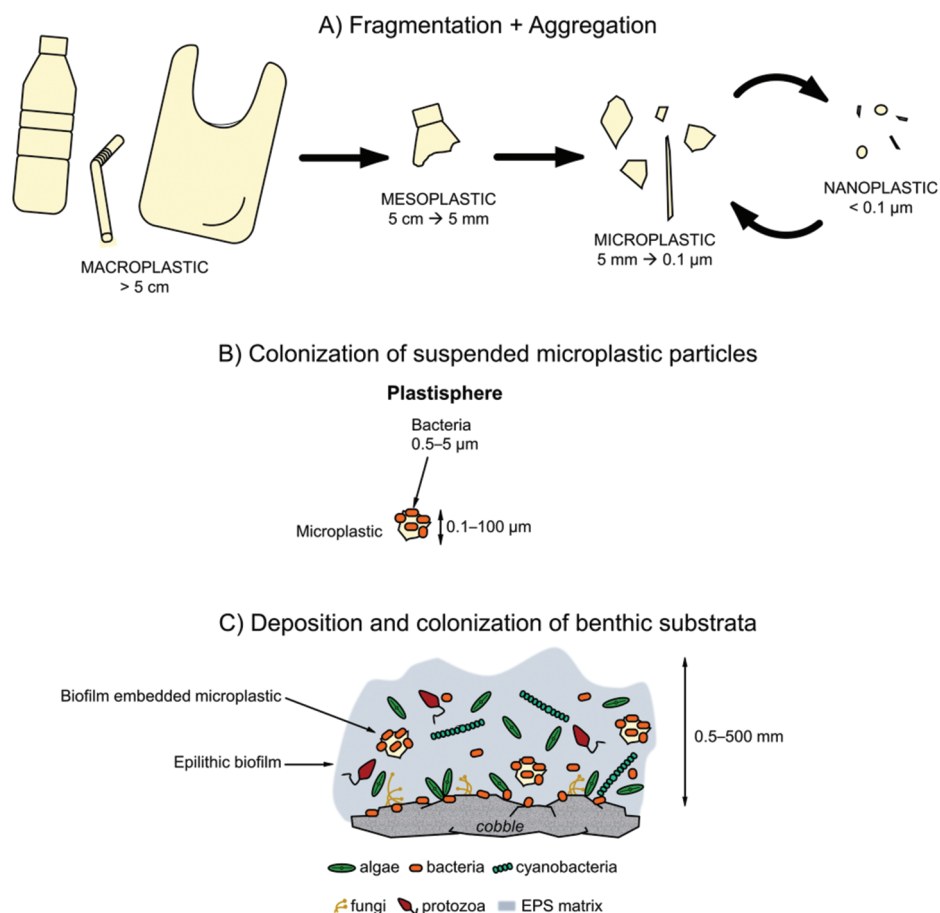


Figure 1. Fragmentation of large plastic items and aggregation of small particles into microplastics with the corresponding length of particles (A). Microbial colonization of microplastics in the water column to form the plastisphere (B). The deposition of colonized microplastics on an epilithic biofilm and embedment inside of the extracellular polymeric substances matrix (C).

may influence the atmospheric deposition of marine microplastics to inland areas (Brahney et al. 2021).

The plastic cycle does not take into account the hydrologic and biogeochemical characteristics of potential microplastics sinks, though it does allow them to be largely dynamic over space and time. Some researchers suggest that microplastics and sediments are influenced by hydrodynamic conditions (Kumar et al. 2021) and that microplastic abundance is associated with the distribution of sands and silts along the reach of the fluvial network. In fact, some studies have established a relationship between the abundance of large (>500 µm in length) high-density microplastics particles and sediment grains of similar size (Enders et al. 2019, Pinheiro et al. 2021).

Rivers are reactive, complex ecosystems, not inert pipes that simply transport terrestrial materials to oceans (Peterson et al. 2001, Cole et al. 2007, Mulholland et al. 2008, Bernal et al. 2012). The dynamic and complex nature of rivers has been addressed and added in another conceptual model of the plastic cycle developed at a watershed scale (Hoellein and Rochman 2021). This iteration of the plastic cycle depicts pools and fluxes between the primary source of microplastics, the different types of waste management and freshwater use by humans, and the retention, transformation, and storage of plastic residues in the environment. The model also includes the temporal scale of microplastic dynamics by classifying pools within the catchment as temporary, long-term, or permanent microplastic sinks. This model acknowledges the significance of plastic debris in freshwater ecosystems, which retain a fraction of microplastics instream, where environmental conditions favor the retention and breakdown of plastic particles. However, the watershed-scale plastic cycle does not explicitly account for the intrinsic spatial heterogeneity of rivers and streams and overlooks how the hydromorphology and cover by vegetation (i.e., macrophytes, micro- and macro-algae) of the river channel can influence the dynamics of microplastics at different spatial scales (Frissell et al. 1986, Allan and Castillo 2007, Peipoch et al. 2016).

Most researchers have focused on quantifying microplastic abundance in sediments and the water column (Schwarz et al. 2019, Watkins et al. 2019). The role of fluvial biofilms on the retention of microplastics is still unknown. Three reviews discuss the potential biological and ecotoxicological interactions between microplastics and fluvial biofilms (Yang et al. 2020, Debroy et al. 2021, Kalčíková and Bundschuh 2021) but we lack studies with empirical data (Miao et al. 2019a, b, 2022). The bio-coating growing on plastic surfaces in the environment, on the other hand, has gained substantial attention. Zettler et al. (2013) coined the term *plastisphere* for the microbial assemblages that colonize plastic surfaces immediately after plastic enters freshwater ecosystems. Here, we focus on the interaction between fluvial biofilms and microplastics, and we address

the *plastisphere* because it defines several physicochemical characteristics of microplastics (Fig. 1C).

Fluvial biofilms are benthic assemblages composed of viruses, archaea, bacteria, algae, cyanobacteria, fungi, and meiofauna embedded in a matrix of extracellular polymeric substances (EPS). They develop on streambed substrata, such as sediments, cobbles, wood, leaves, and macrophytes and on artificial substrates of anthropogenic origin like plastic particles of any composition, shape, or size (Mora-Gómez et al. 2016). Fluvial biofilms play an important role in aquatic ecosystems. For example, they influence primary production, C and nutrient cycling, and sediment stabilization (Battin et al. 2016). The structure, composition, substratum type, season, and prevailing environmental conditions affect the structure and composition of fluvial biofilms (Battin et al. 2003). They interact with contaminants and can modulate their fate via sequestration, biotransformation, and biodegradation (Guasch et al. 2012). They are also recognized as a reservoir of bacteria resistant to antibiotics and pathogens (Flores-Vargas et al. 2021).

Microbial assemblages in biofilms are considered main primary producers across freshwater ecosystems, from headwaters to mid-order streams (Vannote et al. 1980). Thus, biofilms are essential in sustaining trophic food webs in these ecosystems. The biofilm EPS matrix, which can get thick and sticky under high light and nutrient availability (Romaní 2010), can trap suspended sediments and microplastics (Flemming and Wingender 2010). Consequently, microplastic trapping within the EPS matrix of the most common fluvial biofilms, like those growing on big substrata (i.e., epilithic biofilms growing on cobbles; Fig. 2A–H), may differ from the deposition of microplastics in fluvial sediments, which is mainly driven by hydrodynamic conditions (Enders et al. 2019). The main aim of this article is to review the current knowledge of the dynamics and impacts of microplastic pollution in river ecosystems, with a focus on fluvial biofilms, to build a conceptual framework that identifies key research findings and avenues for future research. To do so, a workshop was arranged by the Centre d'Estudis Avançats de Blanes of the Consejo Superior de Investigaciones Científicas (in Blanes, Girona, Spain, 9–13 September 2019). The workshop was carried out as part of the *PlasticsInBiofilms* project. *PlasticsInBiofilms* is an international consortium (<http://plasticsinbiofilms.net/>) initiated with funding of the “Consejo Superior de Investigaciones Científicas, Ministerio de Ciencia Innovación y Universidades of Spain” (I-Link project ref: LINKA20169). The main aim of the consortium is to promote the investigation of the complex interlinks between the fate of microplastics and the structure and functioning of fluvial biofilms.

During the workshop, co-authors discussed the topic, drafted the structure of this paper, and distributed the tasks. Here, we discuss concepts of temporal and spatial microplastic transport, storage, and transformation in fluvial ecosystems,

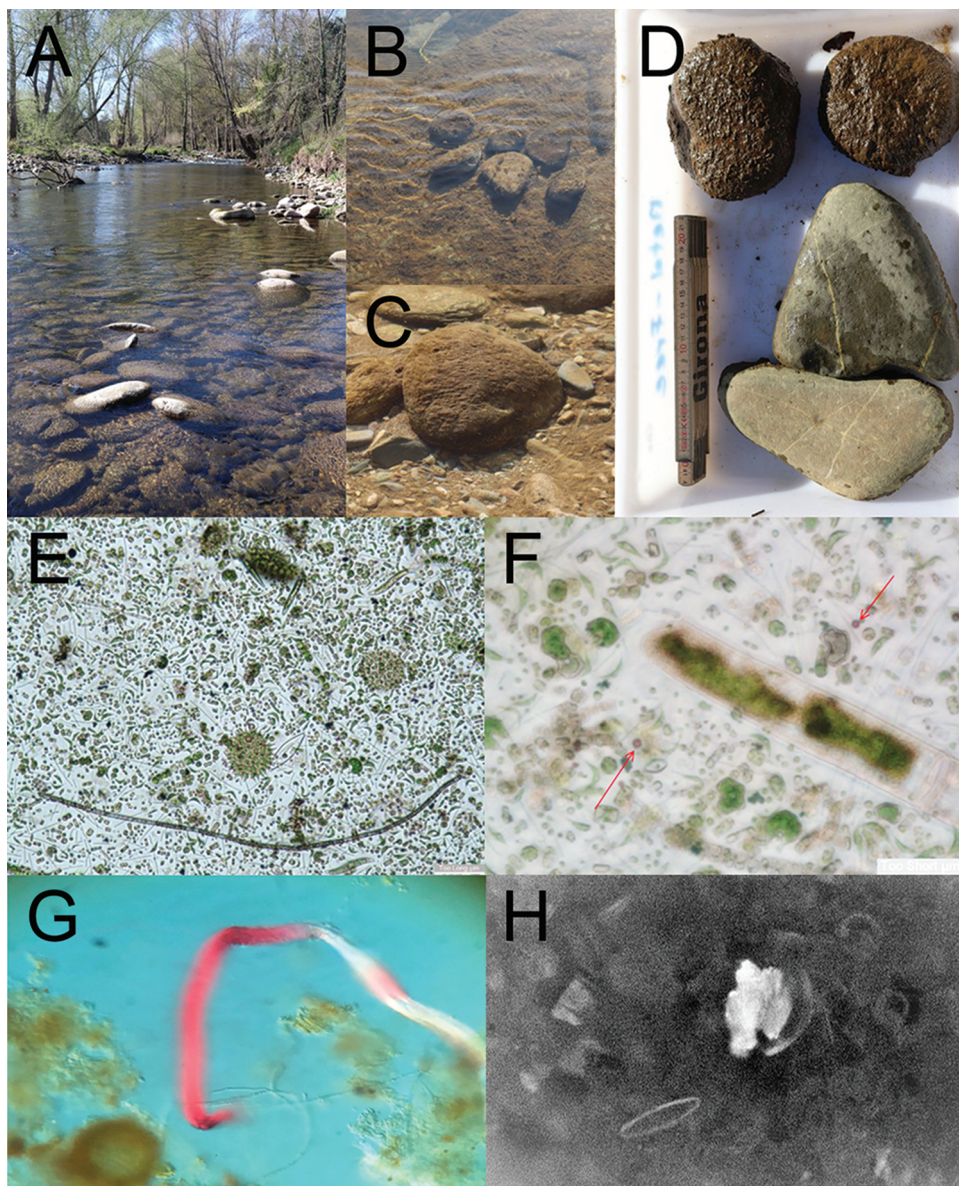


Figure 2. Pictures of a riffle located in the middle part of the Ter River, northeast Spain (A), a detail of epilithic biofilms (B, C), cobbles before and after biofilm sampling (D), a biofilm sample (E), and biofilm samples with microplastics (F, G, H). Photo credit for panels A through D goes to HG, E and F to AT, G to IL, and H to HM.

and review ecological and ecotoxicological interactions between microplastics and fluvial biofilms. Finally, we identify research questions that need to be urgently addressed in the future.

### ROLE OF FLUVIAL ECOSYSTEMS IN THE TRANSPORT AND ACCUMULATION OF MICROPLASTICS

Rivers and streams are hydrologically connected to their catchments through longitudinal, lateral, and vertical dimensions (Ward 1989). These connections determine how sol-

utes, organic particles, sediments, and microplastics are input, transported, and stored within the fluvial network (Hoellein et al. 2019, Bellasi et al. 2020). Longitudinal connectivity promotes the transport of a wide range of natural and anthropogenic compounds, including microplastics, from upstream to downstream. Bidirectional lateral connections are responsible for the exchange of water, solutes, and particles between terrestrial and aquatic ecosystems. Inputs of particles from the catchment to the fluvial network mostly occur during snowmelt and large rainfall events (Barnett et al. 2005), whereas overbank flows induced by floods transfer materials from the stream channel into

riparian and floodplain areas, where they can be retained (Junk et al. 1989, Aksoy and Kavvas 2005).

Vertical exchange between surface water and hyporheic zones determines the balance between the storage and resuspension of particles within riverbed sediments (Boano et al. 2014, Drummond et al. 2016, 2018). Hyporheic zones can act as a transient sink of microplastics, though this storage capacity varies across spatial scales (Castañeda et al. 2014, Hoellein et al. 2017). At the fluvial network scale, erosional forces predominate in headwaters and microplastics can accumulate in lowland depositional zones (Fig. 3A). At the reach scale, riparian vegetation, macrophytes, large woody debris, and coarse organic matter contribute to decreased water velocity and consequently, increased sediment stabilization (Heffernan et al. 2008, Bowden et al. 2017, Riis et al. 2020), which may also favor the deposition of microplastics. Moreover, the 3D structure of macrophytes greatly increases the habitat area for periphytic biofilms (Riis and Biggs 2003, O’Hare et al. 2018), further contributing to the retention of particles (Wijewardene et al. 2022). Therefore, vegetation at the aquatic–terrestrial interface and macrophyte beds might become hotspots of microplastic accumulation (Chen et al. 2021). The structure of epilithic biofilms can also favor the deposition of particles and microplastics in stream beds (Salant 2011). Thick biofilms (e.g., algal mats) increase particle sedimentation by reducing water velocity and modifying turbulence, an effect analogous to the stagnant flow generated by dense macrophyte canopies (Sand-Jensen and Mebus 1996). Algal composition in fluvial biofilms also influences water velocity attenuation (Dodds and Biggs 2002). Finally, the plastisphere influences the retention–resuspension dynamics of microplastics along the stream by changing their buoyancy (Lobelle and Cunliffe 2011), sinking behavior, coagulation to hetero-aggregates, and adhesion to other surfaces like fluvial biofilms (Nguyen et al. 2020).

Stream flow regime determines the temporal variation of microplastic dynamics by influencing patterns of transport, accumulation, and resuspension of sediments and particles (Minshall et al. 2000). During high and extreme flows, microplastics are resuspended and scoured from the streambed, increasing their export towards downstream and coastal areas (Hurley et al. 2018). Intermittent and ephemeral streams illustrate how an extreme and erratic flow regime might regulate microplastic dynamics. In these systems, particle storage predominates over transport during periods of hydrological disconnection (Arce et al. 2019), whereas massive pulses of particles, and likely microplastics, are exported downstream during flush events following dry periods (Shumilova et al. 2019). Moreover, dumping trash in dry stream beds is common because intermittent and ephemeral streams are not designated as official waterbodies by the European Water Framework Directive. Consequently, these streams have less legal protection than their perennial counterparts (Chiu et al. 2017).

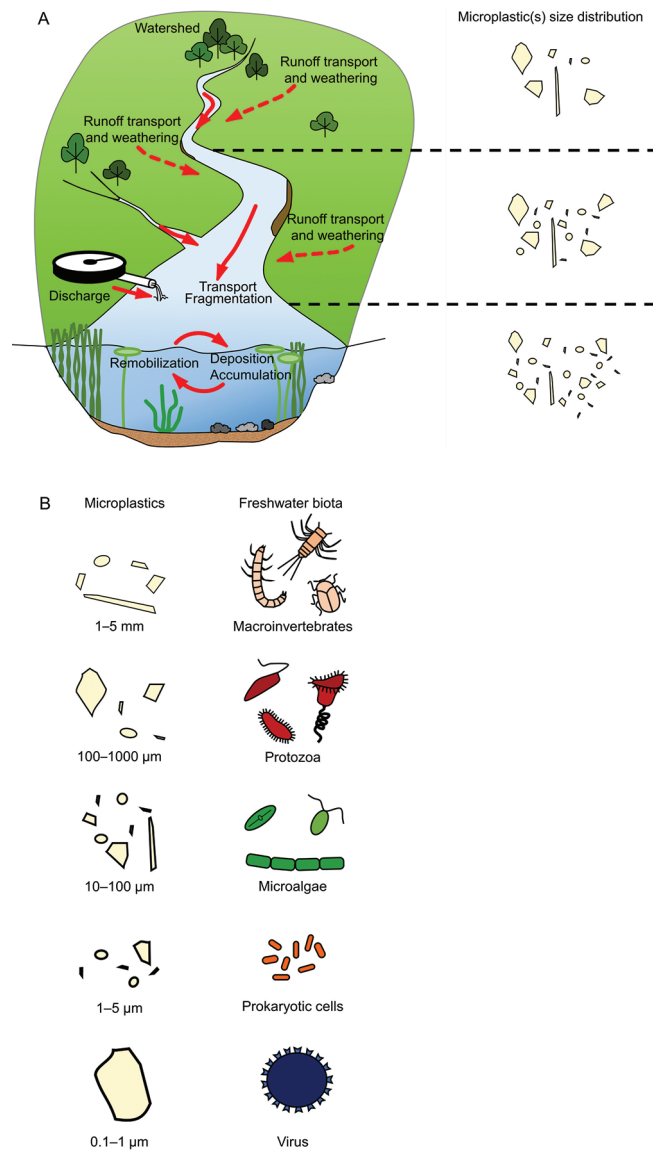


Figure 3. Dynamics of microplastics along the river continuum (A). The largest accumulation of microplastics on the riverbed is expected in depositional zones. At the watershed scale, deposition zones increase downstream. At the reach scale, deposition increases in habitats of low flow. Smaller microplastic size is expected downstream where velocity slows. Arrows indicate microplastic transport (dashed = terrestrial, solid = aquatic). The range of microplastic sizes pictured next to freshwater biota of similar size (B).

Human interventions such as channelization, impoundments, sediment extraction, macrophyte removal, and urbanization strongly modify stream flow regimes, hydrological connectivity, and river geomorphology. Consequently, human intervention has indirect effects on solute and particle dynamics (Elosegi and Sabater 2013, Zhang et al. 2017). For instance, sediments in reservoirs and dams have been identified as a sink for microplastics in river systems

at long timescales (Watkins et al. 2019). In addition, human activities leading to excess bioreactive elements such as N, P, and a cocktail of chemical and organic contaminants (Kaushal et al. 2018, 2020) can alter biofilm composition and structure and affect their capacity to trap microplastics.

### CHARACTERISTICS OF MICROPLASTICS

Unlike natural particles, microplastics are not composed of only one material but rather a diverse array of substances that differ in chemical composition, density, shape, and size. These characteristics influence microplastic partitioning between the water column and the riverbed (i.e., benthic habitats) and affect the specific type and magnitude of environmental impacts that occur.

#### Chemical composition of microplastics

Although microplastics are made of synthetic organic polymers, which are originally produced through the polymerization of monomers derived from oil, gas, or coal, the chemical composition and buoyancy of common microplastics in freshwater ecosystems is rather diverse (Table 1). The most abundant microplastics are low-density ( $<1 \text{ g/cm}^3$ ) materials such as polyethylene and polypropylene, as well as other materials with intermediate density ( $1 \text{ g/cm}^3$ ) such as polystyrene. These polymers are among the most widely used plastics in the world (Bellasi et al. 2020). Other materials that are common but less abundant include high-density polymers ( $>1 \text{ g/cm}^3$ ) such as polyethylene terephthalate, polyester and polyacrylonitrile, and polyamide

(Schwarz et al. 2019). Synthetic rubber particles, like those from car tires and road wear, are also considered microplastics. In some cases, synthetic rubber is the most dominant particle type in systems receiving runoff (Goßmann et al. 2021).

Microplastics constitute highly recalcitrant pollutants (Teuten et al. 2009, Nguyen et al. 2020, Santana-Viera et al. 2021). Because of their high molecular mass, researchers consider microplastic polymers inert and incapable of crossing biological membranes. However, all plastic products contain, to some degree, additives like reactive chemicals. In fact, microplastics may act as long-lasting reactive surfaces and may contain additives, adsorbing and absorbing organic matter, and toxic substances (Rummel et al. 2017). Additionally, toxic substances in microplastics can potentially leach into the environment during manufacture, use, and disposal (Rai et al. 2021).

Plastic products contain thousands of chemicals specific to their polymer type and commercial use. For example, food packaging uses 12,285 listed compounds,  $>600$  of which are potentially hazardous (Groh et al. 2020). For instance, rubber crumb tire and synthetic rubber can be significant sources of microplastics and can contain metals, plasticizers, antioxidants, and antimicrobial agents (Capolupo et al. 2020). Additionally, textile fibers contain bisphenols and benzophenones (Sait et al. 2021). Microplastics may also include impurities from feedstock (e.g., oil) and other unintentionally added compounds that form during production or degradation processes. Microplastics also contain impurities from intentionally added substances that drive polymerization

Table 1. Densities and applications of common polymer types found in freshwater ecosystems. Densities  $>1$  are likely to sink in water and those  $<1$  are likely to float (Horton and Dixon 2018).

Polymer	Abbreviation	Density $\text{g/cm}^3$		Main application
		Min	Max	
Expanded polystyrene	EPS	0.02	0.64	Food packaging, construction
Polyurethane	PUR	0.03	0.10	Building and construction
Polypropylene	PP	0.90	0.91	Many applications, mainly packaging
Polyethylene	PE	0.91	0.97	Packaging
Ethylene vinyl acetate	EVA	0.92	0.94	Others
Polystyrene	PS	1.01	1.04	Packaging
Polyamide (nylon)	PA	1.02	1.05	Automotive, textiles
Acrylonitrile butadiene styrene	ABS	1.06	1.08	Electronics
Polyacrylonitrile	PAN	1.09	1.20	Textiles
Polyvinyl chloride	PVC	1.16	1.58	Building and construction
Polymethyl methacrylate	PMMA	1.17	1.20	Electronics (touch screens)
Polyvinyl alcohol	PVOH	1.19	1.31	Textiles
Polyester	PES	1.24	2.30	Textiles
Polyethylene terephthalate	PET	1.37	1.45	Packaging
Alkyd	Al	1.67	2.10	Paints, fibers

reactions or control material qualities, like UV stabilizers, pigments, phthalates, flame retardants, and antimicrobial agents. Furthermore, researchers are increasingly interested in microplastic compounds that are potentially carcinogenic, mutagenic, or endocrine-disrupting (Eerkes-Medrano and Thompson 2018). Alternatively, many companies are showing interest in bioplastics, plastics derived from vegetable fats and oils (e.g., corn starch, wood chips). These bioplastics biodegrade more rapidly than fossil-fuel derived plastics (Chen and Patel 2012), suggesting that their effects on C cycles are more pronounced. However, scientists have not determined the effects of bioplastics on freshwater biota and nutrient cycles.

### Microplastic size and shape

Microplastics encompass a very broad range of sizes, from particles as small as viruses and most prokaryotic cells (0.1–5  $\mu\text{m}$ ) to particles as large as macroscopic aquatic macroinvertebrates such as mayflies (1–5 mm; Fig. 3B). The large variety of microplastics sizes can influence their biological effects (Bellasi et al. 2020). However, we have limited information on the smaller sizes of microplastics (<300  $\mu\text{m}$ ) because many surveys use filtration techniques based on the larger mesh size of traditional plankton nets. Additionally, we are restricted by methodological limitations when determining the polymer makeup of small particle sizes (Shim et al. 2017).

The few studies that assess all microplastic classes indicate that the small microplastics (10–100  $\mu\text{m}$ ) are, in general, very abundant. For instance, Simmerman and Wasik (2020) found a large number (200–2500 parts/L) of microplastics in the water column of an agriculturally dominated stream, the majority of which were very small (~10–50  $\mu\text{m}$ ). In another study, Chanpiwat and Damrongsiri (2021) found that among the 11 size classes included in their study, the most abundant (21–39%) was the smallest fraction (50 to 100- $\mu\text{m}$ ), with the majority (82%) of microplastics <300  $\mu\text{m}$ .

Some researchers have suggested that nanosized plastic particles (solid polymer particles <1000 nm in diameter) should be classified separately from both microplastics and engineered nanomaterials (Gigault et al. 2021, Mitrano et al. 2021) because of their small size and their high heterogeneity. Compared with microplastics of the same material, the small size and high heterogeneity of nanoparticles changes the vertical transport, bioavailability, diffusion, and uptake through biological barriers characteristic of nanoparticles (Gigault et al. 2021).

Microplastic shape is also diverse. Whereas primary microplastics are symmetric, smooth, and mostly spherical, secondary microplastics are irregular in shape. In addition, microplastic fibers, likely derived from synthetic textiles, are the dominant microplastics shape in freshwater samples (Strady et al. 2021). Further, microplastic aging and weathering further fragments the surface, leading to increased sur-

face roughness, further fragmentation, leaching of plastic additives, and changes in the physical structure of the particles (Hartmann et al. 2017).

### BIOFILM-MEDIATED TRANSIENT STORAGE OF MICROPLASTICS IN FLUVIAL SYSTEMS

We can apply fundamental concepts of sediment transport to understand microplastic transport in fluvial systems (Enders et al. 2019, Pinheiro et al. 2021). However, major knowledge gaps still exist in how transport modes, local biota, and the dynamic behavior of microplastics interact to influence microplastic transport. Microplastics enter fluvial systems as suspended particles and can interact with biofilms in 2 ways. First, biofilms can colonize microplastics immediately after entering aquatic systems to form the plastisphere and change the physicochemical characteristics and transformation patterns of microplastics debris (Rummel et al. 2017, Miao et al. 2021). Second, colonized microplastics can accumulate within the EPS matrix of fluvial biofilms, potentially leading to ecotoxicological effects. Here we focus on the ecotoxicological effects of biofilm-embedded microplastics (Fig. 4).

#### Biofilm role in microplastic retention

Biofilms can immobilize microplastics that can potentially be remobilized with detached portions of the biofilm (Kalčíková and Bundschuh 2021), although we lack empirical evidence on the mechanism and nature of the interaction between microplastics and substrate-associated biofilms. For example, river hydrodynamics, microplastic particle size, and microplastic density influence whether microplastics are transported in the water column or retained in the riverbed (Fazey and Ryan 2016; Fig. 4). Research suggests that microplastics in streams behave like suspended sediments (Enders et al. 2019, Pinheiro et al. 2021), the dynamics of which are well understood (Besseling et al. 2017). It is also well known that fine sediment grains may constitute an important fraction of biofilms, and that its proportion is very variable. However, the factors that govern particle trapping and the role this inorganic component of biofilms plays has been poorly addressed (Mori et al. 2018).

The dynamics of microplastics are driven by the erosion, transport, deposition, and consolidation cycle, which describes fine sediment transport (Schmidt et al. 2016). The EPS matrix adhesiveness has an important role in this cycle, allowing deposited particles to stick together, increasing resistance to erosion and its adhesiveness as biofilm develops (Gerbersdorf et al. 2008). Researchers have demonstrated that biofilm stability and EPS content are positively correlated, particularly in the spring (Schmidt et al. 2016). Moreover, fluvial biofilms that produce adhesive EPS also enhance particle deposition (Battin et al. 2003). Therefore, we expect that fluvial biofilms will help retain and remove microplastics from suspension via surface adhesion. The

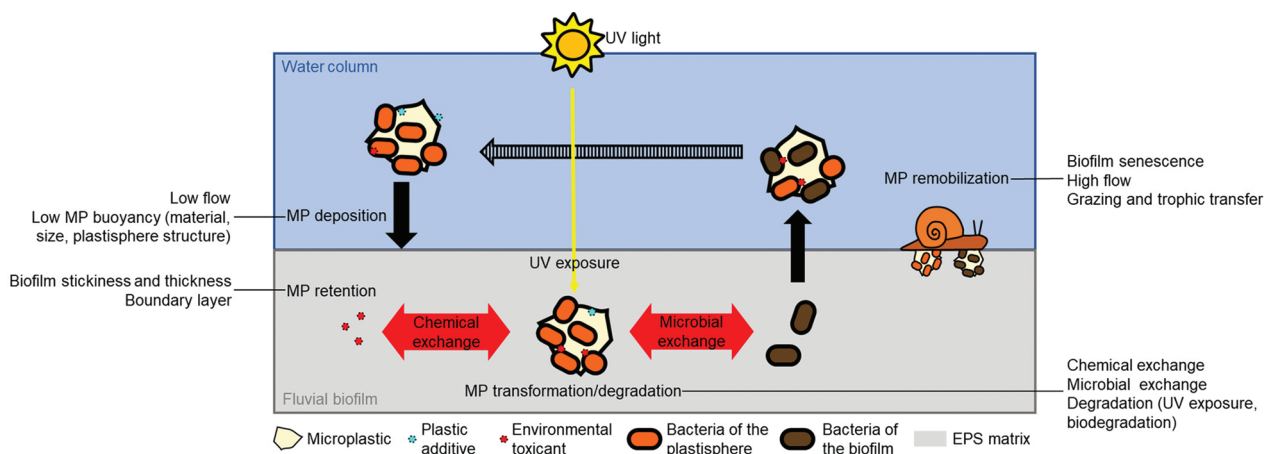


Figure 4. Conceptual model of biofilm-mediated transient storage of microplastics in fluvial systems. Microplastic deposition and the tendency of microplastics to sink and be trapped within the biofilm matrix varies with micro-level hydraulics and the density, size, and shape of the particles. Microplastic retention is affected by biofilm characteristics. Microplastic degradation in the biofilm could occur via chemical, physical, and biological processes. Microplastic remobilization can occur via various detachment processes like scouring, biofilm self-detachment by senescence, and grazing by primary consumers. MP = microplastic, EPS = extracellular polymeric substances.

mucilaginous characteristics of the EPS matrix of biofilms could act as a trap and cause more microplastics to accumulate at transient storage zones within the stream, facilitating microplastic retention under higher-flow conditions (Battin et al. 2003). Moreover, this water–biofilm interface could alter the exchange of nutrients, gases, and particles (Jørgensen et al. 1990) and affect nutrient and C cycling within the biofilm matrix.

#### Ecotoxicology of biofilm-embedded microplastics in fluvial ecosystem

Biofilms accumulate, degrade, and transform organic and inorganic contaminants (Farak et al. 2007, Serra et al. 2009, Guasch et al. 2012, Ancion et al. 2013). In turn, organic and inorganic contaminants can alter species composition and functions of algal (Bonet et al. 2013, Rotter et al. 2015, Morin et al. 2016) and prokaryotic assemblages in biofilms (Tlili et al. 2016, Argudo et al. 2020). Based on these interactions, we expect that biofilms and microplastics influence one another. For example, if microplastics affect the structure and composition of biofilms, it will influence how microplastics accumulate, transform, and degrade.

Few studies have investigated the direct effects of microplastics on the structure and functions of fluvial biofilms despite the fact that fluvial biofilms are widely exposed to microplastics. Miao et al. (2019b) found that polystyrene beads 1 to 9  $\mu\text{m}$  in length had no effects on biofilm chlorophyll concentration and extracellular enzyme production ( $\beta$ -glucosidase, alkaline phosphatase, leucine aminopeptidase), regardless of the concentration tested. On the other hand, Lagarde et al. (2016) showed that biofilms exposed to high-density polyethylene and polypropylene increase their

expression of 2 genes (UGD and UGE) involved in rhamnose and xylose synthesis compared with biofilms without microplastics. The UGD and UGE genes are involved in the exopolysaccharide biosynthesis pathway and are thought to be responsible for the overproduction of sugars during the hetero-aggregation phenomenon (Lagarde et al. 2016).

Researchers studying the effects of microplastics on freshwater microalgae have reported controversial results (Gao et al. 2021). Under certain conditions, microplastics inhibit algal growth (Besseling et al. 2014), negatively affect chlorophyll content (Besseling et al. 2014, Zhang et al. 2017, Prata et al. 2018), reduce photosynthetic activity (Zhang et al. 2017), and possibly decrease the expression of photosynthesis genes (Lagarde et al. 2016). Moreover, microplastics may induce morphological changes in microalgae: unclear pyrenoid, plasma detached from the cell wall, deformed thylakoids, cell wall thickening (Mao et al. 2018). However, they may also enhance algal growth as described by Canniff and Hoang (2018) with large polyethylene beads because they acted as an organic C source. These ecotoxicological investigations provide useful information on the potential impacts of microplastics on microbial organisms. However, these studies used artificially high concentrations of microplastics on primarily planktonic algal cultures. Thus, these studies may not be relevant in studying the toxicity of environmentally relevant concentrations of microplastics on complex natural fluvial biofilms, which differ largely from laboratory cultures.

We used previous knowledge on microplastics and other contaminant particles to identify several physical and chemical mechanisms through which microplastics could impact fluvial biofilms.



**Effects of microplastics settling and siltation on biofilms** Natural particle deposition on biofilms changes light attenuation (Waters 1995, Wood and Armitage 1997), reduces hard substrata available for microbial colonization (Biggs 1995), increases abrasion, and decreases hydraulic connectivity with the hyporheos. Decreased light availability can reduce photosynthetic activity (Van Nieuwenhuysse and La Perriere 1986, Davies-Colley et al. 1992) and affect algal community composition (Newcombe and MacDonald 1991). Izaguirre et al. (2009) demonstrated that the accumulation of fine sediment may temporarily affect biofilm biomass, photosynthetic activity, and community composition. The biofilm adapted to the sediment and fully recovered in terms of chlorophyll *a* (Chl *a*) concentration and photosynthetic activity. However, sediment caused remarkable changes in algal assemblage composition. Diatoms increased 4× and filamentous greens decreased by 50% in high silt treatments, indicating that siltation may ultimately affect stream ecosystem structure and functioning.

We do not expect microplastics to have the same effects on biofilms as sediments because microplastics occur at much lower concentrations than sediment particles. However, microplastics may influence the effect of siltation on biofilms if they co-occur with other particles like fine sediments.

**Microplastics as a nutrient source** Microplastics provide a source of allochthonous organic and inorganic C to aquatic ecosystems (Arias-Andres et al. 2019) and can affect C cycles (Romera-Castillo et al. 2018). The photo-oxidation of plastics leads to the leaching of diverse, organic compounds with low molecular weight. Moreover, microplastics can be photochemically oxidized to CO<sub>2</sub> and dissolved organic C (Hakkarainen and Albertsson 2004, Eyheraguibel et al. 2018), a process that is influenced by water temperature (Ward et al. 2019). Microplastics also contribute nutrients derived from either mineralization or the sorption and desorption of nutrients from their plastisphere. This relatively small C source can still be important, especially in oligotrophic systems with tight nutrient cycles where microplastic pollution may lead to changes in the fate and stoichiometry of nutrients. Moreover, the plastisphere may further contribute additional inorganic nutrients (PO<sub>4</sub><sup>3-</sup>, dissolved inorganic N) to the biofilm that could stimulate algal growth (Mincer et al. 2016), promote denitrification, and influence the sorption of P and microbial-mediated P transformations (Chen et al. 2020).

**Microplastics as vectors for emerging contaminants** Plastic materials and products contain thousands of toxic chemicals. Once in the environment, chemicals will leach out of microplastics and other environmental contaminants will sorb to the plastics until equilibrium is reached (Teuten et al. 2009). The processes regulating the release of these

contaminants are complex, and the degree to which microplastics act as chemical vectors will depend upon the polymer's physicochemical characteristics, degree of crystallinity, size, and surface. Environmental conditions like UV light intensity, temperature, salinity, and the pH of the surrounding medium also affect the release of contaminants from a polymer (Teuten et al. 2009).

The capacity of microplastics to accumulate hydrophobic chemicals is well documented (Hartmann et al. 2017), but the natural tendency for microplastics to release adsorbed chemicals is still under debate. The release of chemicals from microplastics is small compared to the potential of naturally occurring particles to transfer contaminants through the food chain (Koelmans et al. 2016). Plastic additives, such as lead (Pb), can leach from microplastics. In contrast, biogenic coating (i.e., the plastisphere) can contribute to decreasing bioavailability of the metals. Moreover, the plastisphere can enhance the adsorption of several pollutants such as metals (i.e., copper) and antibiotics (i.e., tetracycline) from the water onto polyethylene microplastics (Johansen et al. 2018, Richard et al. 2019, Wang et al. 2020b). This enhanced sorption is more significant in polystyrene microplastics compared to natural particles (Guan et al. 2020). Therefore, we must identify the principal sources and types of microplastics in fluvial systems to understand the potential effects of these compounds and associated contaminants on biofilms.

**Microplastics as vectors for microbial contamination** The physical and chemical properties of a microplastic together with environmental setting can influence colonization and the ultimate microbial assemblage composition (Amaral-Zettler et al. 2020, Eder et al. 2021). However, many studies have not found a difference between the species composition of plastisphere biofilms and those growing on natural surfaces (Oberbeckmann and Labrenz 2020). Alternatively, some research suggests that members of the genus *Vibrio* grow better on plastic than on other surfaces (Zettler et al. 2013, Kirstein et al. 2016, Frère et al. 2018). Pathogens in *Arcobacter*, *Colwellia*, *Escherichia*, and *Pseudomonas* also colonize plastic (Harrison et al. 2014, Keswani et al. 2016, Curren and Leong 2019).

Additionally, plastics and microplastics derived from wastewater treatment plant effluents may already have their own plastisphere (Wang et al. 2020a) and act as vectors for microbial contamination. These microplastics may also act as transport vectors for pathogens such as *Pseudomonas*, *Acinetobacter*, or *Vibrio* and act as hotspots for the spread of antibiotic resistant genes (Sathicq et al. 2021). This inoculation of exogenous microbes could represent a new way of microbial colonization into natural biofilms which could affect their taxonomic composition and the physiological traits and associated ecosystem functions. Yet, the extent to which plastisphere-associated microorganisms can colonize natural

biofilms via microplastics accumulation or plastisphere detachment is still largely unexplored (Fig. 4).

It is worth noting that most research on the microbial contamination of microplastics focuses on bacteria, with fungi and viruses receiving far less attention (Vethaak and Legler 2021).

**Microplastic influence on biofilm 3D structure** Microplastics embedded into the biofilm matrix can alter biofilm 3D structure directly or indirectly by affecting either the biofilm community structure or EPS composition. Microplastics may also directly alter the 3D structure of biofilms by displacement because they have a similar size range as microorganisms (Figs 2A–H, 3B). Experimental data on silver nanoparticles (AgNP) and silver nitrate (AgNO<sub>3</sub>), as a control for Ag ion effects, showed that both AgNO<sub>3</sub> and AgNP had a similar effect on biofilm volume (measured with confocal laser scanning microscopy after staining with a fluorophore coupled to a lecithin) reducing it by ½. The reduction in biofilm biomass (estimated from particulate organic C measurements) was similar for biofilms exposed to AgNP but was not significant for biofilms exposed to AgNO<sub>3</sub> (Kroll et al. 2016). These results suggest that the inclusion of AgNP particles within the biofilm matrix leads to the compaction of their 3D structure. Likewise, surface roughness coefficients were lower in AgNP-treated samples (Kroll et al. 2016). These investigations indicate that microplastics may influence the 3D structure of biofilms. However, to our knowledge, no researcher has studied microplastics and the 3D structure of biofilms at the micrometer scale.

**Biofilm grazing can introduce microplastics to the aquatic food web** Trophic transfer of biofilm-embedded microplastics may explain the presence of microplastics in a large range of environments and in some marine taxa (Wright et al. 2013, Taylor et al. 2016, Hermsen et al. 2017). Scientists have reported the presence and ingestion of microplastics for a wide range of marine and freshwater taxa (Gregory 2009, Farrell and Nelson 2013, Wright et al. 2013). Given their small size, microplastics are easy to ingest by even the smallest taxa. Additionally, the plastisphere mimics natural food sources of small organisms at low trophic levels. For example, researchers have observed feeding marks in the plastisphere of benthic plastic debris (Reisser et al. 2014). These observations could indicate that small grazers, such as snails or copepods, ingest plastic fragments while feeding (Reisser et al. 2014). Xue et al. (2021) observed freshwater rotifers ingesting microplastics found in microalgae contaminated with polyethylene.

Information on the effects of microplastics on the food web for low trophic levels is scarce, despite documented cases of microplastic ingestion by primary consumers (Krause et al. 2021). Microplastics reduce nutritional food quality,

block intestines, and translocate accumulated particles from one part of an organism to another (Browne et al. 2008, Kohler 2010, Tourinho et al. 2010, Avio et al. 2015). For example, research studying the effects of microplastics on marine top consumers like fish and crabs showed that microplastic ingestion reduces growth, survival, feeding, and reproduction. Additionally, because benthic biofilms act as a sink for particles, amounts of accumulated microplastic are expected to be higher than in the water column. Consequently, effects on food webs through dietary exposure will be more important than exposure through water. Therefore, we must examine and understand the mechanisms underlying microplastic uptake, pathways, and effects on the food web.

Studies of the effect of microplastics on organisms usually ignore the fact that many particles are naturally present in the environment (e.g., sand, clay, vegetal fibers). Therefore, we must distinguish the effects of microplastics on consumers from those of natural particles to better manage and mitigate the effects of microplastics on primary consumers.

## OUTSTANDING KNOWLEDGE GAPS AND FUTURE RESEARCH DIRECTIONS

Our review reveals crucial but unexplored physical, chemical, and biological interactions between microplastic and benthic biofilms. Here, we highlight knowledge gaps that must be addressed to improve our understanding of the processes that govern the fate of microplastics in rivers and the potential interactions with benthic biofilms and their consumers (Table 2).

Several unanswered questions remain concerning the transient storage of microplastics in biofilms. In particular, we must address how the spatiotemporal characteristics of the biofilms (e.g., spatial organization, species succession) and the inherent physicochemical characteristics of the microplastics themselves (e.g., size, shape, polymer) influence microplastic transformations within the biofilm matrix. For example, would a thick biofilm composed of long, filamentous green algae be more prone to retain plastic particles than a thinner matrix dominated by diatoms? Likewise, can we expect thick biofilms with pH and dissolved oxygen gradients to be less efficient in degrading microplastics compared with thinner biofilms where UV light easily penetrates?

Under our framework, tight biotic and abiotic interactions between microplastics and biofilms are expected to occur, although these interactions are largely unexplored. Moreover, even though leaching of chemicals from microplastics as well as the sorption and desorption of environmental contaminants to the particles have been investigated for microplastics in suspension, it remains unclear how such processes could affect biofilms. It is also important to investigate the exchange of microbial assemblages between microplastics and the biofilm matrix and study the impact

Table 2. Open research questions about the interactions between microplastics and benthic biofilms in fluvial ecosystems. EPS = exopolymeric substances.

Interactions	Research questions
Biofilms and the transient storage of microplastics in fluvial ecosystems	<p>How does the transient storage of microplastics in biofilms change with different seasons, hydrological conditions, and along the river continuum?</p> <p>Are all materials trapped in the same way?</p> <p>What is the influence of biofilm characteristics such as EPS composition, thickness, species composition, consumers and palatability on microplastic retention, degradation, and remobilization?</p> <p>How does biofilm exposure to UV light affect microplastic degradation?</p>
Microbial and chemical interactions between microplastics and biofilms	<p>In the biofilm matrix, how does the leaching of chemicals out of microplastics and the sorption and desorption of environmental contaminants to microplastics affect the structure and functioning of biofilms?</p> <p>How does the exchange of microbial assemblages between microplastics and the biofilm matrix affect antibiotic resistance, speciation, biodiversity, species composition, and function of biofilms?</p>
Interactions between microplastics and biofilm consumers	<p>Do microplastics have different effects on biofilm consumers than naturally occurring particles?</p> <p>Are biofilm consumers able to avoid the ingestion of microplastics from their food?</p> <p>Are spherical microplastics more easily consumed and excreted by biofilm consumers than fibers or non-spherical fragments?</p> <p>Is the use of more biodegradable plastics having a stronger impact on fluvial biofilms and food webs than traditional plastics?</p>

of these interactions on key biofilm characteristics such as antibiotic resistance, speciation, biodiversity, and species composition.

We also expect that microplastics will affect biofilm consumers. Future research should differentiate between the effects of microplastics from those caused by naturally occurring particles. Improving our ability to predict the effects of microplastics will allow us to accurately assess the risks microplastics pose to consumers feeding on contaminated biofilms. It is also not clear whether biofilm consumers can select and avoid microplastics in their food. Certain invertebrates have the ability to select food particles based on size, shape, and nutritional value. However, most studies on the preferential selection of microplastic types by invertebrates have been conducted in the pelagic zone of marine ecosystems, and we have very little information on benthic invertebrate feeding behavior, especially in fluvial environments.

We expect microplastics to affect consumers in various ways. For example, microplastics with varying physicochemical characteristics likely change food palatability and alter feeding habits. Additionally, we hypothesize that particles spherical in shape pass through the gut system of consumers more easily than long filamentous microplastic. Indeed, filamentous microplastic ingestion by small consumers may result in higher risks of obstruction. However, the effects of microplastics on consumers have not been largely studied, particularly for microplastics that are in-

corporated into fluvial biofilms. Similarly, most studies have focused on spherical microbeads even though microplastic fragments may cause more serious internal injuries. Overall, it is clear that the uptake mechanisms of microplastics and the effects of microplastic propagation through the food web should be addressed to define efficient management strategies for microplastic pollution in fluvial ecosystems.

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