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Review

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Impacts of Biofilm Formation on the Fate and Potential Effects of Microplastic in the Aquatic Environment

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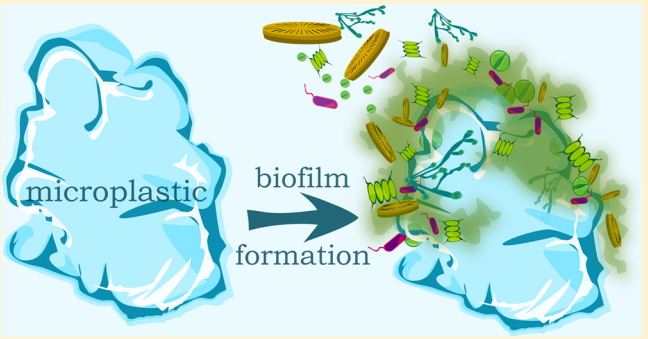
Supporting Information

ABSTRACT: In the aquatic environment, microplastic (MP; <5 mm) is a cause of concern because of its persistence and potential adverse effects on biota. Studies of microlitter impacts are mostly based on virgin and spherical polymer particles as model MP. However, in pelagic and benthic environments, surfaces are always colonized by microorganisms forming so-called biofilms. The influence of such biofilms on the fate and potential effects of MP is not understood well. Here, we review the physical interactions of early microbial colonization on plastic surfaces and their reciprocal influence on the weathering processes and vertical transport as well as sorption and release of contaminants by MP. Possible ecological consequences of biofilm formation on MP, such as trophic transfer of MP particles and potential adverse effects of MP, are virtually unknown. However, evidence is accumulating that the biofilm−plastic interactions have the capacity to influence the fate and impacts of MP by modifying the physical properties of the particles. There is an urgent research need to better understand these interactions and increase the ecological relevance of current laboratory testing by simulating field conditions in which microbial life is a key driver of biogeochemical processes.



INTRODUCTION

In the aquatic environment, plastic litter has emerged as a major pollution issue, because it is only slowly degradable,[1,2] is ubiquitously present in our rivers and seas,[3,4] may represent a hazard to wildlife,[5] and may be a potential planetary boundary threat.[6,7] Current investigations of the fate of marine plastic debris include various surveys that aim to develop an understanding of its distribution from beaches and shorelines to remote islands or the great ocean gyres,[8] as well as downward transport, from the sea surface through the water column[9] to bottom sediments.[10] Also, plastic contamination in freshwaters is currently gaining attention.[11]

Apart from the aesthetical issues of littering, adverse effects on wildlife are obvious for large plastic debris, i.e., macroplastic (>5 mm).[12,13] During its residence in the environment, large plastic debris becomes brittle and undergoes fragmentation due to weathering forces generating so-called microplastic (MP; <5 mm).[14,15] While large plastic debris may have adverse effects on fish, birds, and other top consumers in aquatic environments,[5,13] the size of the MP makes it suitable for ingestion by smaller organisms at lower trophic levels.[16] Although no studies have so far reported any ecologically plausible adverse effects of MP on primary consumers, we know very little about the interactions between these particles and their potential consumers. One of the shortcomings in our current experimental and modeling studies of MP is the missing link of the effects of biofilms on the particle behavior in biological, chemical, and physical interactions. The fate and effects of MP mainly have been investigated in laboratory experiments, using virgin spherical particles with a uniform size distribution. However, environmental MP is characterized by heterogeneous sizes and shapes[17,18] that change with aging.[6,19] Moreover, they are mixed with natural suspended particles that may affect biofilm formation. These parameters should be included in study designs to create more realistic conditions of these mixtures and their exposure.[20] Additionally, particle properties, including those of MP (such as topography or roughness,

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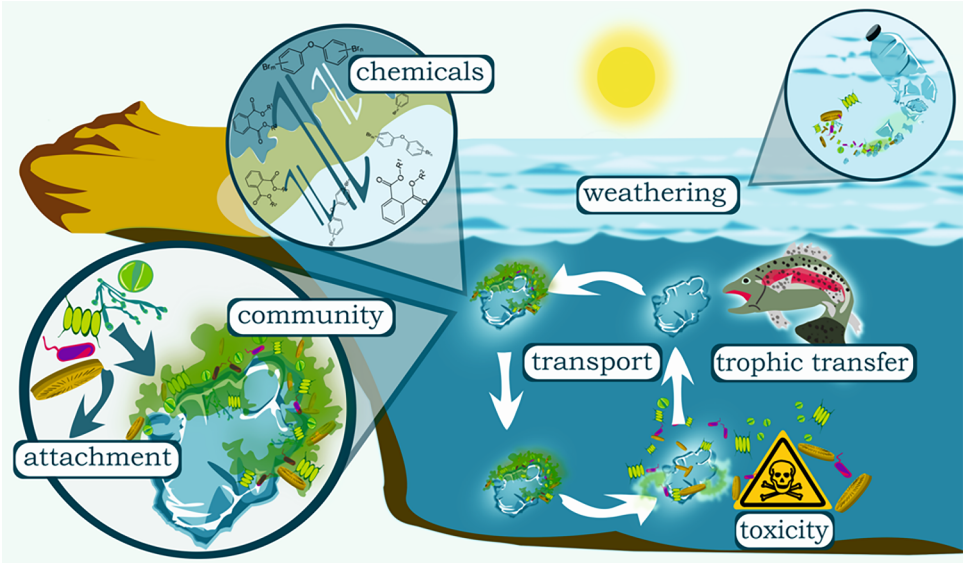

Figure 1. Key processes of the fate and potential effects of MP in the aquatic environment that are modified by biofilm formation. Biofilms on submerged surfaces are the result of selective attachment of microorganisms, facilitation, and interspecific competition in the microbial communities. Weathering processes may favor biofilm growth because of increased surface areas available for settling, which in turn may shield plastic debris from ultraviolet light. However, biofilms have the capacity to biodegrade the polymer. In addition, vertical transport and the uptake and release of plastic-associated chemicals are influenced by biofilm formation on plastic debris. Biological implications of biofilm formation include effects on trophic transfer of MP and associated contaminants, community structure of microbial assemblages, and potential toxicity to grazers.

surface charge, surface area, overall density, and many more), will inevitably change when a biofilm forms on the surface.

Upon release of a plastic item to the aquatic environment, a coating layer of inorganic and organic substances is instantaneously formed.[21] The subsequent formation of a biofilm on its surface is likely the first interaction with ambient biota, taking place within minutes to hours.[22] Biofilms are phylogenetically and functionally diverse communities of bacteria, algae, protozoans, and fungi collectively termed a microbial assemblage, biofouling community, or periphyton. These microorganisms live in spatial proximity of each other on any submerged surface mostly embedded in extracellular polymeric substance (EPS).[23] Life in a biofilm offers a variety of advantages for competition and survival strategies, including possibilities for forming stable consortia, horizontal gene exchange, accumulation of nutrients, and protection against toxic substances and desiccation.[24]

Here, we summarize the different aspects and specific future research needs of the influence of biofilm formation on plastic debris and its potential impact on the fate and effects of MP in the environment. Our specific focus is the physical effect of biofilm formation on the fate of MP and the resulting consequences for biological interactions. We specifically identify current gaps in our knowledge of the early interactions of plastic and biofilm-forming microorganisms and their reciprocal influence on weathering processes, the vertical transport of fouling MP particles, and the potential of biofilms to modify plastic-associated fluxes of chemicals. Biological aspects address trophic transfer, the community structure of the so-called "plastisphere",[25] and potential adverse effects of MP. A detailed description of the systematic literature research by means of the ISI Web of Science performed as a first step in this review is provided as Supporting Information. This query led to insufficient results (see Table S1) because of the virtual lack of studies of the investigated topic. However, several neighboring disciplines like material and medical science, nanotechnology, and food technology provide valuable insights into surface−biofilm interactions. Using the systematic literature research and cross-referencing from these disciplines, we propose some priority areas and important questions for investigating the impacts that microbial colonization may have on plastic debris in the aquatic environment.

BIOFILMS ON PLASTIC SURFACES AND THEIR PHYSICOCHEMICAL IMPLICATIONS

Attachment to New Habitable Surfaces. To elucidate the complex interactions between biofilm-forming microorganisms and surfaces available for colonization, we need to understand the attachment processes acting on macro- and microplastic (Figure 1, "attachment"). Within seconds of the first contact between ambient water and a virgin surface, a conditioning layer or film of organic and inorganic substances is formed by adsorption.[21] Microorganisms come into contact with surfaces by repulsive and attractive interactions among the surface, their cell wall, and the medium. The initial conditioning film may have the capacity to govern the colonizing community by modifying the material-specific surface properties.[26−29] The phenomenon of sorbed molecules driving the behavior of particles in fluids was just recently compared to the absorbsome[30] and the so-called eco-corona[31] of nanomaterials in a review by Galloway et al.[7] A key point of these concepts is the rapid establishment of a coating layer consisting of proteins and other biomolecules around nanoparticles in biological fluids such as serum and cytoplasm that affects the physicochemical interaction of the nanomaterials with cells and tissues.[7] Lorite et al.[32] concluded that the chemical nature of the aforementioned conditioning film appears to be more relevant for settlement of organisms than surface roughness or hydrophobicity of the initial substrate surface, which highlights the importance of this very first sorption process. The investigation of the conditioning film on MP and its close link to the concept of the eco-corona seems to be a promising

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field for future research. The universal mechanism of surfaces absorbing molecules may have far-reaching biological consequences because it is the particle's "biologically relevant entity".[33]

From a material perspective, the surface roughness,[34] topography,[35] surface free energy,[36] surface charge, electrostatic interactions,[37,38] and surface hydrophobicity[39] are generally known to be relevant parameters for the attachment process. However, Hook et al.[40] concluded that on the basis of their experiments wettability (or analogously surface hydrophobicity) and polymer topography did not affect the attachment of bacteria to synthetic polymer substrates. On the contrary, Sanni et al.[41] suggested a strong correlation of bacterial settlement and a parameter combining hydrophobicity and molecular flexibility in the specific case of poly(meth)acrylates.

Observing colonization of submerged plastic bags, Nauendorf et al.[42] suggested that surface wettability was probably of minor importance for bacterial attachment compared to surface roughness. Comparative investigations of biofilm succession on polymeric materials and other substrates suggest that the abundance of bacteria on hydrophilic stainless steel, hydrophobic polyvinyl chloride (PVC), and polyethylene (PE) was similar after colonization for 167 days.[43] Although often termed "inert", synthetic polymers exhibit important differences compared to other materials because the amount and composition of additives (chemicals that are intentionally added during manufacturing to improve the material's performance) in the polymer can also affect the species composition of organisms colonizing the surface.[44] In contrast to the work of Pedersen,[43] that of Rogers et al.[45] detected higher bacterial numbers on PE and PVC than on stainless steel during biofilm formation, which they attributed to leaching of additives as a potential nutrient source. Although of high value for our current understanding of biofilms colonizing MP, ongoing research is often observation-based[46−48] rather than mechanistically driven. However, understanding of the underlying mechanisms for eco-corona and biofilm formation and composition is crucial for predicting the behavior and fate of MP in various environmental settings. In summary, plastic materials represent a relatively recent anthropogenic substrate in aquatic ecosystems that can readily be colonized by biofilm-forming organisms. Although many studies have shown that microorganisms attach more rapidly to hydrophobic, nonpolar surfaces (such as plastics) than to hydrophilic surfaces (such as stainless steel),[23] general conclusions about the relative importance of specific mechanisms are difficult to draw, particularly for *in situ* studies. Even in controlled laboratory experiments, physicochemical properties differ between polymer types with varying monomer subunits and copolymers, differing by functional groups and additives. Plastic may also be manufactured as a composite material, further widening the range of (surface) properties. The effects of physicochemical properties driving the early attachment processes have comprehensively been reviewed by Renner and Weibel[38] and Cazzaniga et al.[49]

In response to diverse habitats and ecological requirements, microorganisms have evolved a plethora of attachment mechanisms.[50] Organism−substrate interactions have led to numerous adaptation strategies; for example, surface charge[51] and hydrophobicity of the cell walls and membranes can be adjusted[52] by forming surface structures, such as pili, curli, fimbriae,[53,54] and flagella,[55] and by regulating EPS production,[56] all of which may improve adhesion to a habitable surface. Once the coating and the first colonists of a biofilm are in place, the initial surface properties of the material are modified, which may facilitate colonization for other organisms, as demonstrated by Lobelle and Cunliffe,[57] who observed a decrease in surface hydrophobicity on submerged PE during a 3 week incubation in sea water. Additionally, environmental factors such as ionic strength, temperature, and pH may influence the attachment.[58]

Although the early formation of a biofilm on surfaces has been under scientific investigation for decades,[22,59,60] general conclusions about the underlying physicochemical processes governing early attachment of microorganisms are difficult to draw because a plethora of materials and organisms with different properties exists. As a result of the interactions between substrates and organisms mentioned above, a diverse microbial community colonizes every submerged surface.

Weathering. Among others, the fate of plastic debris in the aquatic environment is governed by weathering processes because these have significant consequences for the condition of the material and its hydrodynamic behavior[61] (Figure 1, "weathering"). Weathering describes the loss of the physical integrity of the material by abiotic and biotic influencing factors and related degradation of the material. For plastic debris, we need to consider several pathways separately, although they usually act in concert. Preceding the biological attack, photooxidation is the most common abiotic degradation pathway, at least for debris exposed to sunlight. Photooxidation may be divided into three main steps: initiation [polymer-chain scission induced by ultraviolet (UV) light and formation of free radicals], propagation (autoxidation), and termination (formation of inert products). The degradation mainly acts on the material surface that is exposed to UV light. As a result, the weathered surfaces may display a modified topography, an increase in surface roughness, and changed chemistry (e.g., becoming more polar because of the formation of carbonyl groups).[62−65] These processes may favour the adhesion of microorganisms,[23] carrying capacity of MP toward biofilm mass and, ultimately, the composition and structure of the microbial communities.[35,49] In addition, successive fragmentation into smaller particles[14,66] with a high surface-to-volume ratio is an important prerequisite for biodegradation. Over time, the surface area of plastic available for colonization by microbes increases,[67] escalating the contribution of biodegradation, changing the particle density, buoyancy, and sinking rate. However, biofilm formation may also influence abiotic aging processes, e.g., by shielding the floating plastic from UV light in the upper water layers[14] or by changing a particle's vertical position in the water column.[68,69] As a result, the exposure to light, shear stress, oxygen, and temperature will be influenced.

In addition to the effect of physical aging caused by abiotic factors, polymers are subject to biological degradation.[25,70,71] While the term biodeterioration refers to a loss of physical integrity, biodegradation encompasses the process of chemical breakdown.[72] Flemming[24] summarized the main biofilm-related processes acting on the aging of synthetic polymers, namely, (i) biofouling, (ii) degradation of plasticizers, (iii) attack on the polymer backbone, (iv) hydration, and (v) penetration of organisms into the polymer structure (e.g., fungal hyphae). Synthetic homopolymers containing C−C bonds in their polymer backbone are least susceptible to biodegradation.[72] During biodegradation, exoenzymes are released by the colonizing organisms and cause the breakdown of the polymer, finally yielding short-chain fragments, such as oligomers,

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dimers, or monomers.[73] These may then pass the cell membrane, become a carbon source, and be mineralized to CO_2, H_2O, and CH_4.[73] Comprehensive reviews of the mechanisms of biological polymer degradation are provided by Shah et al.,[73] Restrepo-Flórez et al.,[44] and Gu.[74]

Essentially unknown are the kinetics of fragmentation and the resulting emission of particles and their size distribution caused by microorganisms in the environment.[67] This is of particular relevance for the fraction of plastic that sinks to the benthos[10,14] and no longer undergoes UV-driven degradation in the euphotic zone. Thereby, high microbial activity in eutrophic waters may increase MP loads in the sediments and may promote its final removal by mineralization both in the water column and in sediments, particularly when bottoms are not hypoxic. There may be a mechanistic trade-off in the fragmentation rate due to biofilms attenuating abiotic weathering on one hand (by shielding from UV light and sinking) and causing biological breakdown on the other (biodegradation). To overcome the current lack of quantitative estimates of the importance of weathering processes for plastic debris,[6] we need to understand the changes in physical and chemical properties due to biofilm formation and thereby driving forces behind the vertical transport of MP.

Vertical Transport. From a hydrodynamic perspective, biofilm formation on plastic debris may have substantial implications. First, the fouling organisms may lead to an increase in the density of the particle and a decrease in its buoyancy.[75] The smaller the particle, the faster it can reach its critical sinking density.[76,77] Since the sinking rate is a function of particle size and density, an increase in density above that of ambient water (1.025 g/cm^3 for sea water and 1.000 g/cm^3 for fresh water) implies sedimentation[68] (Figure 1, "transport"). However, the buoyancy of particles that originally had a higher density than water may increase as a result of biofouling, rendering MP susceptible to upward transport. Furthermore, during biofilm formation, MP becomes sticky because of the EPS matrix, which promotes the formation of heteroaggregates, including MP, microbial communities, and detritus.[68] The formation of such heteroaggregates may affect sedimentation rates of algal blooms and associated microorganisms. For example, Long et al.[68] demonstrated that heavy and fast-sinking diatom aggregates displayed substantially decreased sinking rates when low-density microbeads were incorporated, whereas sinking rates of light cryptophyte cells associated with aggregates increased. Furthermore, possible preferential ingestion of MP with well-developed biofilms (see Trophic Transfer) may promote downward transport of MP particles incorporated into fecal pellets of zooplankton.[78,79] However, benthic sediments do not necessarily present an ultimate sink for plastic debris. The extent of biofouling of plastic debris may decrease because of the removal and/or digestion by benthic animals; hence, MP may regain buoyancy, leading to submerging−resurfacing cycles[80] (Figure 1, "transport").

In a recent modeling study of riverine transport of MP, Besseling et al.[81] concluded that biofilm modeled as a 0.4 μm thick monolayer of bacterial cells (1.250 g/cm^3) would introduce no changes into the overall qualitative trends and patterns in particle behavior. In this model, data on attachment efficiencies of biofilm-coated MP particles were derived from the experiments performed by Xiao and Wiesner,[82] who measured an increase in the affinity of engineered nanoparticles for porous media in the presence of biofilms. These experiments demonstrated the affected hydrodynamic behavior of the investigated nanoparticles in the presence of biofilms that may have similar consequences for transport and the ultimate fate of MP in the aquatic environment. The growth of biofilm-forming organisms largely depends on environmental factors, such as light and temperature, as well as on the trophic state of the waters.[83,84] The dynamics of the transport pathways as a function of seasonality, climate effects, and the trophic state of aquatic systems should be addressed if we are to understand and model the distribution of MP in different ecosystem compartments.

Transport of Plastic-Associated Pollutants through Biofilms. The transport of hydrophobic organic contaminants (HOCs) between plastic debris and water may be affected by biofilms because of its sorptive properties on one hand and its ability to metabolize HOCs on the other[85−88] (Figure 1, "chemicals"). In addition to the additives of (recently emitted) plastic debris mentioned above, highly persistent contaminants may be accumulated by plastic from its immediate environment and the plastic may subsequently transport and release them during residence at sea.[89−91] The chemical loads of these contaminants in MP may be enriched up to 10^6-fold compared to those of the surrounding sea water[92] and depend on the polymer/water partition ratios that can be approximated by the octanol/water partition ratios (K_{OW}).[93] Since the capacity of synthetic polymers to sorb HOCs is highly relevant for the environmental risk assessment of MP, we face the question of whether thermodynamic and kinetic processes will be influenced by biofilms, representing a superficial organic phase consisting of water, lipids, and proteins acting as both a potential sorptive phase[94] and a barrier for diffusive uptake and release of chemicals. Furthermore, EPS represents a diverse biological matrix containing polysaccharides, proteins, lipids, and other biopolymers such as humic acids,[95,96] which may contribute to the sorptive capacity of the biofilm-coated MP[97] and heteroaggregates. Humic acids are known to compete for sorptive sites and hence have the potential to attenuate the sorption of PCBs as shown for charcoal.[98] Analogous to the partitioning of HOCs into MP, synthetic polymers, such as PE, are frequently used in the field of environmental chemistry as so-called passive samplers because of their high capacity for sorbing HOCs.[99] The passive samplers are intentionally deployed in the field to sample environmental contaminants and subsequently solvent-extracted and measured in the laboratory. However, biofilm coatings can bias passive sampling rates in the field by increasing the resistance for mass transfer into and out of the polymer[100] as suggested by different sampling rates in fouled and nonfouled sampling devices.[101] In laboratory studies of the kinetics of sorption of HOCs into MP, the influence of biofilms has largely been disregarded,[90,102] despite the observed effects on kinetics in the passive samplers. Diffusion coefficients decreased by ∼4 orders of magnitude upon inclusion of a microbial biofilm during sorption of HOCs to glass beads,[103] which also emphasizes the importance of the biofilm acting as a barrier.

The release of additives may even promote microbial growth by serving as a nutrient source.[72,104] A wide range of bacteria, fungi, and algae are capable of degrading HOCs,[105] which is why they can be used, e.g., for bioremediation of surface waters *in situ* or as engineered bioreactors.[106,107] This demonstrates the high relevance of biofilms for the accumulation and/or removal via metabolization of plastic-associated chemicals,[87] which may affect their bioavailability for consumers ingesting MP. Another concern is the addition of antimicrobial agents to

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polymer materials by manufacturers to hamper microbial settlement;[108] these substances may leach and promote the spread of resistance adaptations in microbial communities.[109]

In summary, sorptive processes may lead to faster uptake and release of chemicals in MP compared to macroplastic litter due to higher surface-to-volume ratios. At the same time, however, colonization by microorganisms is facilitated because of the enlarged and weathered surfaces available for colonization that can influence the kinetics and persistence of HOCs. These two-way interactions can influence the kinetics of uptake and release of contaminants into and from the polymeric bulk phase through the active microbial interface need to be considered further to predict a more realistic scenario for risk assessment of MP being a transport and emission source of HOCs in the aquatic environment.

BIOLOGICAL EFFECTS

Community Structure. Since environmental factors and material and organismal surface properties govern the attachment of organisms, any community inhabiting a submerged surface is a result of selection processes. Microbiologists are currently investigating communities present on MP surfaces and the underlying factors that determine the community structure and succession patterns (Figure 1, "community"). Zettler et al.[25] introduced the term "plastisphere", implying that plastic-associated communities are distinct from the surrounding surface water. This assumption supports the view that plastic is a novel ecological habitat.[25,46,110,111] Studies using high-throughput sequencing showed that bacterial assemblages colonizing MP are taxonomically distinct and often less diverse than those in the water column, suspended organic matter or sediment.[110,112,113] Current studies, however, often lack a proper comparison to co-occurring natural substrates, both polymeric (e.g., cellulose, chitin, or lignin) and mineral (e.g., clay). Adequate particle controls are essential in field and experimental studies[20,114] that aim to address the specific effects of anthropogenic particles.

Although the composition of microbial communities on plastic surfaces may largely be influenced by geographical, spatial, and seasonal factors, an additional selection of a distinct community by the polymer substratum may occur.[115,116] Dang et al.[27] showed that the early microbial colonization is similar on plastic and glass surfaces during the first few days of succession. However, the lowest diatom diversity was observed on plastic, concrete, and rubber compared to that on the hydrophilic surface of iron plates and the seagrass *Posidonia oceanica*.[117] This observation indicates that plastic as a habitat may be less favorable for some species, such as diatoms, than other substrates. A recent study investigated the succession of microbial assemblages on PE in coastal sediments, suggesting a selection for specific bacterial taxa.[111] By contrast, Oberbeckmann et al.[118] concluded that the community structure on plastic surfaces is driven by conventional marine biofilm processes rather than selection of plastic-specific microbial colonizers. Interestingly, a different pattern of gene expression in microalgae grown on polypropylene and PE was demonstrated by Lagarde et al.,[75] indicating substrate-specific adaptations. The polymer-specific gene expression of sugar-synthesizing pathways may have important implications for the EPS production and subsequent formation of aggregates, which may result in a differential transport and fate of plastic particles.[75] Knowledge of the community structure and the underlying forces driving these assemblages at each succession stage will help us to elucidate the impact of plastic pollution on aquatic microbial load and diversity.[119] We need to integrate community structure and functions of the microbial communities on plastic debris because microbial activity is a crucial link between pollution as an anthropogenic pressure and the resilience of ecosystems.

Trophic Transfer. Most studies that have investigated the ingestion of MP by biota or transfer along artificial food chains used spherical, virgin MP particles and ignored the presence of biofilm under field conditions.[19,120] However, biofilm was found to facilitate trophic transfer of nanoparticles in marine systems,[121] which most likely also holds true for MP. Primary consumers may preferentially ingest particles of higher nutritional quality, such as MP carrying nutrient-rich biofilms.[122] This discrimination would be particularly pronounced in the selective feeders, such as copepods and shrimps, but also, at least to some extent, in passive feeders, such as cladocerans.[123−125] Biofilm may also increase the probability of MP adhering to the filtering apparatus in filter and suspension feeders, because neutral particles have been shown to be captured more readily than particles with a net negative charge.[126] Grazers, such as snails or copepods, may also ingest plastic fragments accidentally while feeding on the surface biofilm, as indicated by feeding marks observed on field-sampled plastic debris.[127] Zooplankton can actively explore patches of marine snow,[128] suggesting that potentially larger quantities of MP (and a broader size spectrum) incorporated into aggregates may be consumed compared to freely dispersed particles. Indeed, in suspension-feeding bivalves, enhanced uptake of 100 nm polystyrene beads embedded in marine aggregates was observed compared to that of the dispersed virgin particles.[121] Moreover, increased MP abundance may alter sedimentation rates of algal blooms, thus affecting the food supply for pelagic and benthic animals.[68] Campos et al.[129] reported nanoparticle-mediated flocculation and sedimentation of algal food resulting in a reduced rate of feeding in *Daphnia magna* under food-limiting conditions. This mechanism may potentially affect both pelagic feeders in the mixing layer and benthic communities because they may receive food of unusual quality and quantity. To conclude, biofilm formation and potential heteroaggregation may affect the uptake and susceptibility of organisms to ingesting MP by changing the physical properties and/or increasing the availability of MP particles. Biofilm coating has so far been disregarded in study designs but should be included in future studies to derive reliable uptake and ingestion rates in a more environmentally realistic scenario.

Toxicity and Adverse Effects. Because of their structural role as an interface between the overlying water and the sediments, biofilms are often used in ecotoxicology to evaluate the effect of chemicals in aquatic ecosystems.[130] In a recent study, flow cytometry was successfully applied for the detection of MP in ecological biofilms but no structural or toxicological effect was reported.[131] However, limited attention is paid to the direct adverse effects of plastic debris and the associated chemicals on the biofilms (Figure 1, "toxicity"). Potential effects may result either directly from physical and/or mechanical stress by the presence of solid particles (e.g., via adsorption of particles to the cell wall) or indirectly from plastic-associated chemicals leaching out of the polymer. Zhang et al.[132] revealed a negative effect of micrometer-sized PVC particles on the microalgae *Skeletonema costatum* only for the highest and environmentally unrealistic exposure concentration (50 mg/L).

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As they excluded shading effects by their experimental design, both physical adsorption and aggregation might have caused toxicity.[132] The available reports on suspended algae suggest effects of nanoplastic exposure on planktonic microalgae, such as inhibition of photosynthesis, promotion of reactive oxygen species,[133,134] growth inhibition, and reduced chlorophyll *a* content.[135] Until now, the exposure scenarios applied in such experiments were beyond being environmentally relevant and do not distinguish between the direct and indirect effect mechanisms. Indirect effects of plastic debris on biofilm-forming organisms may result from leaching of HOCs to the biofilm. The toxicity of plastic additives such as flame retardants[136] and plasticizers[137,138] as well as HOCs[139−141] toward microalgae was demonstrated in laboratory studies. In addition, the ingestion of plastic covered with biofilms may increase the dose of HOCs to consumers because of increased capacity to carry HOCs (with biofilms acting as an additional sorptive phase mentioned in Transport of Plastic-Associated Pollutants through Biofilms).

Another aspect of biofilm growth on MP may be its infectious capacity caused by its transport of pathogens.[142] It is known that even free EPS fragments, called "transparent exopolymer particles" (TEP), facilitate the uptake of pathogens by biota.[143] MP may present an additional vector for the dispersal of rafting communities. Plastic-associated biofilms may cause such concerns as potentially pathogenic *Vibrio* spp. were detected on floating MP.[25,144−146] However, it is unclear whether the potential for pathogen dispersal is different between MP and natural particles and whether this route can increase the rate of infection of consumers. In conclusion, knowledge of the toxicity and potential adverse effects of MP and their associated chemicals on biofilm-forming organisms and primary consumers is currently lacking.

RESEARCH PRIORITIES

As biofouling of submerged surfaces is a long-standing cause for concern in pharmacology, medical and material sciences, and food technology,[147−150] knowledge of the colonization processes from these fields can contribute to our understanding of the behavior of plastic in the environment and facilitate technical approaches to studying this behavior. The formation and succession of a biofilm on MP particles involve multilateral processes determining the respective fate of MP in the environment and the responses of biological systems to MP pollution. On the basis of the literature discussed above, we identified the following research priorities.

(1) As every submerged surface is subject to microbial colonization, we need to better understand the basic processes that are involved in the formation of a biofilm, with a particular focus on biofilm−MP interactions. Following the eco-corona concept from nanotechnology,[7,33] experiments should be designed to identify key factors that influence the physicochemical behavior of MP (e.g., particle properties and surface characteristics and/or absorbing molecules). It should be evaluated whether these factors differ for different MP materials and whether they are comparable to those of natural particles of similar size. Further, experiments should consider changes in physicochemical properties after weathering. These investigations should be performed under different weathering conditions like UV, temperature, or mechanical abrasion.

(2) Our understanding of the biofilm−plastic interactions for hydrodynamic processes, such as vertical transport, needs to be improved to parametrize predictive models of the transport and exposure of MP particles and their associated pollutants in aquatic systems. Thus, sinking and flocculation studies with environmentally representative biofilm−MP complexes are needed, on micro- and mesocosm scales.

(3) The sorption of HOCs to MP has attracted an increasing amount of attention. However, a realistic concept accounting for the effect of biofilm formation and its consequences for the kinetics of chemical partitioning is still lacking, which hinders experimental evaluations. Modeling studies in a three-phase system (water−plastic−biofilm) should be complemented by experimental studies.

(4) Virtually all experiments published to date about the effects of MP on biota lack the proper preparation of the test particles that would simulate natural biofilm coating. MP coated by biofilms (e.g., derived from preculture incubations) should be included, and the influential characteristics of different biological materials like bacteria, fungi, and different algal strains should be tested. Furthermore, particle controls need to represent natural particles similar in size, density, and biofilm colonization.

(5) The relevance of biofilms for the mode and rate of MP uptake by consumers should not be ignored when estimating feeding uptake and exposure effects under realistic conditions. Differential uptake of MP due to biofilm formation should complement the current (ecotoxicological) research on MP ingestion in artificial food chains.

(6) We need to understand the intricate interactions between microbial assemblages in water and their capacity to sustain biofilm formation on various polymer materials ("plastisphere") if we are to assess the resilience of aquatic systems to MP pollution. Therefore, the investigation and analysis of biofilms on plastic debris are encouraged so we can gain functional insight into its productivity and diversity as well as its vector role in carrying and dispersing microorganisms for reliable hazard assessment.

In conclusion, the challenge for the MP research is to account for the interactions between diverse plastic materials undergoing weathering and colonization by microorganisms in various environmental settings to provide a science-based risk assessment for the effects of plastic debris in aquatic environments.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.7b00164.

Description of the literature research by means of the ISI Web of Science and the summarized results (Table S1) (PDF)

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