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Microplastic surface biofilms: a review of structural assembly, influencing factors, and ecotoxicity

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As plastic pollution continues to escalate, the widespread presence and potential hazards of microplastics as an emerging global contaminant have drawn increasing attention. In natural environments, microplastic surfaces are prone to colonization by microbial biofilms composed of microorganisms and extracellular polymeric substances (EPS), forming a distinct microecosystem known as the plastisphere. This process not only modulates the physicochemical properties and environmental behavior of microplastics but also significantly changes their ecotoxicity. This paper systematically reviews the biofilm formation process on microplastic surfaces, the succession dynamics of microbial communities, and the key environmental and material factors influencing microbial colonization. On this basis, the regulatory modulates of biofilm formation on the physicochemical properties and environmental behavior of microplastics are analyzed, as well as their effects on bioavailability and ecotoxicological effects. Although there has been an increasing number of studies on the ecotoxicity of microplastics in recent years, most experiments are still limited to the pristine microplastics, that fail to reflect their realistic environmental exposure status. Therefore, this review emphasizes the necessity of incorporating biofilm-coated microplastics into toxicological assessments, to better simulate actual environmental conditions and to elucidate their synergistic roles in compound pollution scenarios.

KEYWORDS

microplastics, biofilm, microbial community, plastisphere, ecotoxicity

1 Introduction

Plastics are widely used across various industries due to their light weight, durability, and low production cost (Adeleke, 2023), which has significantly contributed to the process of industrialization (PlasticsEurope, 2019). In recent years, plastic pollution has become a global environmental issue (Zhang et al., 2021b), among which microplastics with a particle size of

less than 5 mm have become an emerging pollutant in the ecosystem due to their small particle size, diverse sources, and strong persistence (Geyer et al., 2017; O'brien et al., 2023). With the intensification of human activities, microplastics have become widely present in oceans, freshwater, soil, and atmosphere (Chen et al., 2023; O'brien et al., 2023), and have even been detected in remote and pristine regions such as the Arctic Ocean (Kanhai et al., 2020). At the same time, it has been detected in animals (Rebelein et al., 2021; Rakib et al., 2023), plants (Li et al., 2020), and even human tissues (Amato-Lourenco et al., 2021; Nor et al., 2021; Leslie et al., 2022). This widespread presence underscores the high environmental mobility and bioavailability of microplastics (Liu and Zheng, 2025). Moreover, their large specific surface area, surface hydrophobicity, and high adsorption capacity (Raddadi and Fava, 2019), enable them to act as carriers for persistent organic pollutants, heavy metals, and antibiotics (Sorensen et al., 2020; An et al., 2023), which enhance the bioavailability and toxicity of these contaminants (Wang et al., 2019b; Eze et al., 2024), and threatens ecosystem health. Therefore, microplastic pollution has become an urgent global environmental challenge. Although there is an increasing number of studies on the environmental behavior and ecotoxicity of microplastics, most experiments still focus on microplastics in the pristine state, making it difficult to accurately reflect their true exposure forms in the natural environment. In reality, microplastics are highly susceptible to microbial colonization once they enter the environment, and their surfaces rapidly develop biofilms composed of bacteria, fungi, algae, and extracellular polymeric substances (EPS). This attachment process not only modulates the surface physicochemical properties and migration behavior of microplastics, but also establishes a unique microecosystem called the "plastisphere" (Zettler et al., 2013; Amaral-Zettler et al., 2020). This interfacial zone between microplastics and biofilm not only reflects the ecological niche properties of microplastics, but also serves as a critical site for pollutant adsorption, accumulation, transformation, and release, all of which may significantly influence the bioavailability and environmental toxicity of microplastics.

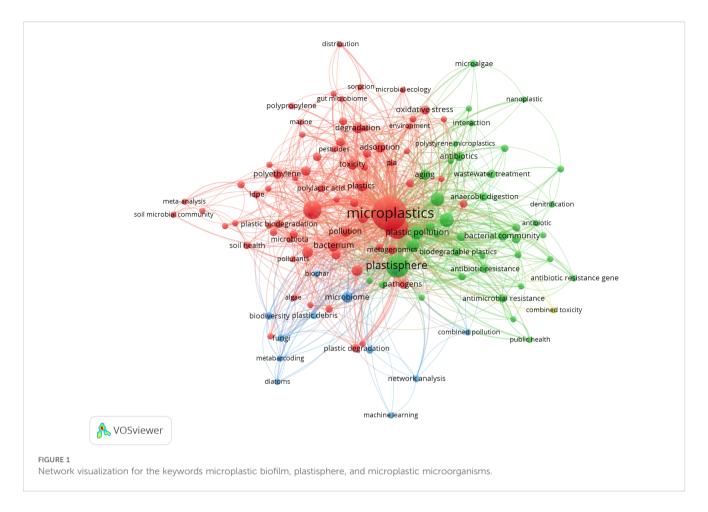
In recent years, the structural characteristics, ecological functions and roles of microbial communities at microplastic interfaces in the process of compound pollution have attracted increasing attention. Studies have shown that the formation of biofilms is driven by environmental factors (e.g., temperature, nutrient level, pH) and microplastic characteristics (e.g., material, surface roughness, polarity, etc.), and plays an important role in regulating the adsorption, release and toxic behavior of pollutants. However, most existing reviews focus solely on ecotoxicity, and there is still a lack of systematic integrated review of "biofilm-coated microplastic". In view of this, this paper takes the biofilm-coated microplastics as the core research unit to systematically sort out the formation mechanism of biofilm, the succession characteristics of microbial communities, and the complex's regulatory function on the environmental behavior of pollutants, and further explore its key role in the bioavailability and toxicity mechanisms. Ultimately, this work seeks to deepen understanding of the environmental risks of microplastics and provide theoretical support for research into compound pollution and ecotoxicity.

We performed a bibliometric analysis of relevant literature (2023-2025) using VOSviewer (v1.6.19) based on the Web of Science database. Based on the keywords "microplastic biofilm", "plastisphere" and "microplastic microorganisms", the analysis identified 4,389 unique keywords in total, of which 83 met the minimum occurrence threshold of 20. The red cluster focuses on keywords including "toxicity", "oxidative stress" and "adsorption", emphasizing interactions between microplastics and pollutants alongside and their ecotoxicological effects. The green cluster highlights terms like "antibiotic resistance", "wastewater treatment" and "public health", reflecting growing research interest in biofilm-coated microplastics as vectors for antibiotic resistance genes (ARGs) and human health risks. Meanwhile, the blue cluster covers technical terms such as "network analysis" and "machine learning", suggesting the increasing application of multiomics and computational approaches to resolve complex microplastic related systems. Collectively, the network diagram (Figure 1) demonstrates the multidimensional of microplastics research, spanning pollution behavior mechanisms, microbial ecological responses, and health risk assessments.

2 Formation of biofilm on the surface of microplastics and succession of microbial communities

2.1 Biofilm formation process on the surface of microplastics

In natural environments, microplastics readily serve as substrates for microbial colonization. The development of biofilm-coated microplastics is a dynamic and multi-phased process (Du et al., 2022), which usually includes initial attachment, irreversible attachment, biofilm maturation, and detachment (Datta et al., 2016). Microplastic surfaces exhibit strong hydrophobicity, and rapidly adsorb dissolved organic matter in aquatic environments, forming an organic adsorption layer, that facilitates initial microbial colonization. During early colonization, pioneer bacteria reversibly adhere to the plastic surface, forming loose multicellular aggregates (Harrison et al., 2014; Quero and Luna, 2017), which constitute the first stage of biofilm development. As pioneer bacteria, their metabolic activity and EPS secretion gradually reduced surface hydrophobicity of the plastic (Tu et al., 2020), promoting stable microbial binding. Secondary colonizers enhance irreversible adhesion via surface structures (e.g., fimbriae, adhesion proteins), creating ecological niches for subsequent microbial populations (Dussud et al., 2018; Yang et al., 2020). The continuous introduction, loss and replacement of species in the community led to the dynamic succession of microbial composition, and finally formed a mature and functionally complex microbial community structure. Due to the significant differences in physical and chemical conditions, biological resources and disturbance intensity in environmental media, the colonization process and community structure of microplastic biofilms showed high variability in different



environments. To further understand the differences in ecological behavior, Table 1 summarizes the main characteristics of microplastic biofilms in different environments.

Across environmental media, microplastics rapidly develop surface-attached microbial communities that evolve into biofilms upon environmental exposure. Current research, however, remains predominantly focused on aquatic environments, where substantial data exist regarding microbial colonization dynamics and ecological impacts. Most available studies in these systems are limited to description (Domozych and Domozych, 2008; Liu et al., 2024), lacking systematic characterization of biofilm community structure, functional potential, and ecological roles. Therefore, fundamental aspects including biofilm evolution processes in non-aqueous environments, synergistic pollutant adsorption capacities, and associated ecosystems impacts require in depth investigation.

2.2 Microbial community assembly in microplastic biofilms

The assembly mechanisms governing microbial communities within microplastic biofilms are fundamental to understanding their structural development and functional evolution. These processes not only determine microbial diversity and community stability but also underpin successful biofilm colonization on heterogeneous surfaces. Current theoretical frameworks center on

two paradigms: niche theory, which emphasizes deterministic environmental selection, and neutral theory, which highlights the role of stochastic processes in community assembly.

Niche theory believes that community structure is determined by environmental conditions and species physiological traits, with environmental selection acting as the primary screening mechanism. Distinct microbial taxa showed significant variation in niche preference and spatial distribution patterns in response to pollution-induced environmental stress. For example, Wang et al. employed high-throughput 16S rRNA gene sequencing to compared planktonic archaea and their predominant taxa, Marine Group I (MGI) and Marine Group II (MGII), across varying environmental gradients. Their results showed that the selection processes predominantly governed the assembly of these three archaeal communities, with salinity and nutrient availability identified as key influencing factors. These findings support the central role of niche theory in shaping microbial community structure (Li et al., 2021b). Similarly, Sun et al. collected and analyzed bacterial communities in different ecological niches (including water, sediment, and plant root foliage) in artificial lakes. They found that niche type exerted a significantly stronger influence on community diversity and structure than pollution intensity, Moreover, specific ecological niches, such as sediments and plant-associated interfaces, exhibited more complex and structured community networks, reflecting a clear environmental filtering mechanism. These results further reinforce niche theory, suggesting that environmental

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TABLE 1 An overview of the main microbial communities on the surface of microplastics in different environments.

Environment type		Substrate type	Eukaryotes	Microbial community	Reference
Aquatic environment	Wetland	Polyethylene (PE), Polypropylene (PP)	/	Proteobacteria, Pseudomonas fluorescens, Aeromonas caviae	(Liu et al., 2024)
		/	Desmids, Diatoms	Proteobacteria, Bacteroidota, <i>Roseococcus</i>	(Domozych and Domozych, 2008)
		Polyvinyl chloride (PVC)	Nitzschia sicula, Mastogloia Corsicana, M. fimbriate		(Meng et al., 2024)
	Lake	PE, PP	/	Pirellulaceae, Phycisphaerales, Cyclobacteriaceae	(Miao et al., 2019b)
		Poly(3-hydroxybutyrate) (PHB), High- density polyethylene (HDPE), Low- density polyethylene (LDPE)	Cryptomonas, Chlamydomonas (algae); Stentor, Vorticella (ciliates)	Betamyces, Cryptococcus (fungus); Mycoplana, Erythromicrobium (bacterium)	(González-Pleiter et al., 2021b)
		PE, Expanded polystyrene (EPS-foam), PP	Pennales, Bacillariophyceaea, Chlorophyceae	/	(Di Pippo et al., 2022)
		Polystyrene (PS)	/	Proteobacteria	(Guan et al., 2020)
	River	PVC	/	Pseudomonas monteilii, Pseudomonas mendocina, Pseudomonas syringae	(Wu et al., 2019)
		PVC, Polyethylene terephthalate (PET)	Green algae, Diatoms	Cyanobacteria	(Miao et al., 2021)
		PE, Polylactic acid (PLA), PS, Cellulose, Hemicellulose	/	Proteobacteria, Firmicutes	(Zhao et al., 2024c)
	Seawater	PE, EPS-foam, PP, Nylon	Alveolata, Stramenopiles, Radiozoa	Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria	(Amaral-Zettler et al., 2021)
		EPS-foam	Flavobacteriaceae, Rhodobacteraceae, Halomonadaceae, Vibrionaceae		(Zhang et al., 2024a)
		PE, PLA	/	Alcanivorax, Nitratireductor	(Chu et al., 2024)
Soil		PE, Epoxy Resin (EP)	Coccus, Bacilli, Fusarium	PE : Chujaibacter, Rhodanobacter, Mycobacteria EP: Ralstonia	(Tian et al., 2024)
		LDPE, HDPE, PS, PP, PET	1	LDPE: <i>Methylophaga</i> , PS: Saccharimonadales, PET: <i>Sphingomonas</i>	(Chen et al., 2022)
		PP, Tire Wear Particles (TP)	/	PP: Bacillus, Sphingomonas TP: Pseudomonas, Acinetobacter	(Ding et al., 2024)
Atmosphere		PET	1	Proteobacteria, Cyanobacteria, Actinobacteria	(Tu et al., 2022)

In this table, EPS-foam denotes expanded polystyrene. Elsewhere, EPS refers to extracellular polymeric substances; "/" denotes data not reported in the cited source.

conditions and resource availability are critical factors driving the assembly of microbial communities on biofilm-coated microplastics (Sun et al., 2023b). With the deepening of research, the application of this theory was expanded to the study of new artificial ecological niches (e.g., microplastic surfaces) generated by human activities. The surface of microplastics is hydrophobic and nutrient deficient, and the biofilm community structure on it was markedly different from that of the suspended communities in the surrounding water column, showing lower OUT abundance and Shannon diversity index. Additionally, the non-random distribution of biofilm communities and functional taxonomic preferences support the dominance of a species sorting mechanism, indicating that biofilm formation on microplastics is largely influenced by deterministic niche selection processes (Besemer et al., 2012). Some studies have pointed out that the plastisphere can form a stable coexistence pattern by selectively recruiting specific microbial groups, which is clearly different from the microbial composition in the natural environment. In addition, community differences driven by ecosystem background are much greater than physical attributes between plastics and natural substrates, and this trend is also reflected in the global microbial co-occurrence network (Li et al., 2024).

Relatively speaking, the neutral theory emphasizes the equivalence of species in ecological functions, arguing that community structure is mainly determined by random processes such as birth, death, migration, and dispersal (Chen et al., 2019). In certain plastisphere, non-deterministic processes have been found to play an important role in community construction. The role of selective recruitment appears limited. Community assembly is mainly driven by ecological drift and dispersal restrictions. Early dominant taxa, such as Proteobacteria, with broad ecological niches, increase susceptibility to opportunistic colonization. In addition, hydrodynamic processes such as tides further enhance the probability of random attachment of microorganisms on plastic surfaces, reflecting the high randomness of community structure (Yan et al., 2024). In another study of archaeal communities, homogeneous dispersal played a dominant role in the assembly of MGI and MGII communities, with effects 1.63 times and 1.34 times higher than that of the selection process, respectively. This indicates that in these archaeal communities, mass effect leads to the convergence of community structure. The remaining difficult-toexplain differences in the community may be caused by the superposition of multiple weak drivers, manifesting as a nondeterministic random assembly pattern (Wang et al., 2019a).

Accumulating evidence suggests that microbial community assembly is not attributable to a single mechanism, but instead reflects the combined influence of deterministic and stochastic factors. It presents a continuous and unified dynamic balance. Some studies have found that there is no significant correlation between environmental factors and species diversity index in the plastisphere, indicating that the community structure is largely driven by non-deterministic processes such as ecological drift and dispersal restrictions. At the same time, compared with non-plastic substrates, plastic surfaces provide relatively more stable ecological niches, showing certain environmental selectivity, such as the significant enrichment of microorganisms associated with plastic degradation on plastic particles,

reflecting the directed screening role of functional species (Pang et al., 2024). In addition, there is differentiation in the response of different microbial taxa to the assembly mechanism. Studies have shown that even within the same community, different taxa may be governed by different mechanisms. Dominant taxa are more likely to be shaped by environmental selection, whereas rare taxa are primarily influenced by non-deterministic processes such as ecological drift and dispersal limitations (Xiang et al., 2022). Another study pointed out that eukaryotic communities tend to be controlled by homogeneous selection at specific periods, driven by seasonal changes, while overall random processes and neutral mechanisms remain dominant. In addition, the neutral model had a significantly higher fit for bacterial communities than eukaryotes, indicating that neutral processes have a stronger explanatory power for bacterial community structure formation (Huang et al., 2025). Preference for community assembly mechanisms may also be influenced by organism characteristics. Luan et al. suggested that differences in microbial size affect their ability to spread, with small microorganisms more susceptible to random processes and large microorganisms more affected by environmental screening (Luan et al., 2020). In complex ecosystems, the dynamic balance between assembly mechanisms is significantly reshaped. For example, natural hydrological disturbances can guide communities from decisive screening to random assembly at different time scales, with specific manifestations that vary depending on the ecological context, such as inverse shifts in mechanisms within Mediterranean rivers and northern streams (Sarremejane, 2018). Continuous environmental stress often enhances the dominance of decisive mechanisms, leading to functional stability but reduced structural diversity (Santillan et al., 2020). In addition, plastics can also profoundly affect community assembly mechanisms in the environment. Studies have shown that microplastic introduction increases the relative importance of deterministic processes, particularly in water and sediment samples. This effect is likely due to microplastics providing new ecological niches or altering the local microenvironment, which promotes the selective colonization of specific microorganisms (Wu et al., 2025). Bacterial communities on degradable microplastics are more strongly shaped by deterministic processes, particularly homogeneous selection, compared with non-degradable microplastics. Material degradation alters the microenvironment, which enhances environmental filtering, promotes the colonization of specific microorganisms, and reduces community diversity (Zhang et al., 2024c). It is worth noting that the influence of microplastics on bacterial community assembly and succession is not only affected by their material properties, but also closely related to the shape of microplastics (e.g., fibers, fragments, particles), which have different microstructural characteristics, which in turn change the attachment area and spatial distribution of bacteria, thereby further regulating the colonization process and community structure of microorganisms (Wang et al., 2023). In addition, process such as preferential biological effects and historical contingency are also thought to play an important role in the early colonization stage of communities, further increasing the complexity of assembly mechanisms. The above community assembly process is jointly regulated by a variety of environmental factors and microplastic characteristics, and these key influencing factors are systematically sorted out below.

2.3 Influencing factors of biofilm formation on the surface of microplastics

Microbial biofilm formation on microplastics fundamentally alters their environmental behavior and may amplify ecological risks. The multifaceted process is governed by complex interactions between environmental conditions and microplastic properties. To systematically synthesize current knowledge, we consolidate key influencing factors and their action trends in Table 2. The formation of microplastic biofilm is significantly regulated by multiple environmental factors, mainly including the combined effects of external environmental conditions and the physicochemical properties of microplastics. Existing studies have shown that the initial biomass and diversity on microplastic surfaces are influenced by both the characteristics of microplastics and the surrounding environment, although over time the environmental influence may become more pronounced (Jin et al., 2025). In addition, under specific environmental backgrounds (such as high salinity and low nutrition availability), biofilm communities may show certain substrate specificity. However, this effect is typically governed by environmental factors and its trend is often unstable. Additionally, microbial community structures vary significantly across geographic regions, highlighting the role of spatial heterogeneity in community assembly within the plastisphere (Oberbeckmann et al., 2018). This conclusion is also consistent with the results of previous studies in the North Sea (De Tender et al., 2015).

At the same time, as an important carrier of biofilm colonization, the inherent physicochemical properties of microplastics profoundly

affect the initial attachment and community succession process of microorganisms. Compared to suspended particles in aqueous environments, the rough microscale structure of solid microplastics provides a stable surface for microbial adhesion, facilitating the development of structurally complete biofilms. The microbial community structure on microplastics is significantly different from that of free microorganisms in seawater, and only a few dominant orders coexist in prokaryotes, and the rest are endemic taxa. Eukaryotic microorganisms also showed a clear trend of community differentiation. Although both communities are subject to seasonal variation, microplastic-associated communities remain relatively stable, indicating that microplastics provide a consistent ecological niche for microbes (Davidov et al., 2024). Under the action of external interference, the microbial community on the surface of microplastics showed strong resilience, especially the bacterial community could be quickly rebuilt. The eukaryotic community showed a trend of decreasing diversity after disturbance, which may reflect the differences in ecological strategies between the two communities (Nguyen et al., 2023). In addition, under the same culture conditions, biofilm formation on the surface of microplastics is generally faster than that of natural polymers, and the community succession process is also more rapid, often undergoing multiple dynamic stages in a short period of time (Zhao et al., 2024c). The material type of microplastics further shapes the structure and function of their biofilms. Biofilms on the surface of degradable plastics (such as PLA) exhibit high robustness and low ecological vulnerability. In contrast, petroleum-based plastics such as PE form thicker biofilms, have greater greenhouse gas emission potential, and

TABLE 2 The main factors and mechanisms affecting the formation of biofilm on the surface of microplastics.

	Factors	Mechanisms	Reference
Environmental factors	Temperature	High summer temperatures enhance microbial growth and metabolism, accelerating early biofilm colonization.	(Jin et al., 2020; Zhang et al., 2021a; Song et al., 2024)
	Salinity	Salinity alters microbial community structure and selects tolerant strains, shaping biofilm composition.	(Kesy et al., 2019; Qiang et al., 2021)
	Illumination	Light supports phototrophs (e.g., diatoms, green algae) and stabilizes biofilm communities.	(Tu et al., 2020; Smith et al., 2021; Bairoliya et al., 2024; Deng et al., 2024)
	Rich nutrients accelerate microbial proliferation and biofilm growth. Low nutrients stimulate specific microorganisms, enhancing attachment and stabilizing biofilm formation.		(Stanley and Lazazzera, 2004; Arias-Andres et al., 2018a; Li et al., 2019)
	Time	Over time, biofilm morphology and structure become increasingly complex and diverse.	(De Tender et al., 2017; Guan et al., 2020; Song et al., 2024)
Substrate type	Substrate type	The rough surface is conducive to the early colonization of biofilms; Provide a more stable ecological niche.	(Chen et al., 2022; Tkachuk and Zelena, 2023; Zhao et al., 2024c)
	Plastics type	Different plastic properties affect the selective attachment of microorganisms. The functional tendency to form biofilms is different.	(Chu et al., 2024; Wang et al., 2024; Yan et al., 2024)
	Particle source	Environmentally aged particles generally have biological attachments, which affect the succession process and function of biofilm bacteria.	(Shan et al., 2022; Rozman et al., 2023a; Zhao et al., 2024b)
	Morphological structure	Different morphologies affect the capacity and ecotoxicity of adherent biofilms.	(Lee et al., 2022; Rozman et al., 2023b)
	Particle size	Particle size determines microbial load per unit area, with smaller particles offering greater surface area.	(Klein et al., 2021)

carry a higher risk of antibiotic resistance. However, some studies have also shown that microbial communities on PLA surfaces are relatively simple and more susceptible to external disturbance, potentially increasing the risk of pathogen enrichment and transmission. This suggests that the impact of plastic material type on biofilm succession may be context-dependent (Yan et al., 2024). In addition, the physical and chemical properties of plastics determine their adsorption preference for microorganisms. PP and PE are more conducive to the development of carbon cycle-related microorganisms and biofilms, while PET tends to enrich microflora with plastic degradation potential (e.g., *Pseudomonas*), further illustrating the material-dependent selectivity of microplastics in shaping microbial ecological processes (Wang et al., 2024).

3 Effects of biofilm formation on microplastics

Beyond biofilm formation mechanisms, the subsequent environmental feedback effects warrant equal attention. Biofilms are prone to form on the surface of microplastics in the environment, which significantly modulates their interface properties and environmental behavior. The adhesion of biofilm endows microplastics with new environmental response characteristics by regulating their surface properties, migration behavior, and degradation processes. The following section explains its key impacts from three aspects. During biofilm formation, microorganisms secrete large amounts of EPS and metabolites containing polar functional groups (e.g., carboxyl, hydroxyl, and amino groups). These substances markedly alter the hydrophilicity, surface charge, and reactivity of microplastics. As microorganisms colonize microplastic surfaces, clear signs of aging rapidly emerge, including reshapes in crystallinity, stiffness, surface roughness, and the colonization of unique microbial taxa. This indicates a close link between microbial community structure and the physicochemical properties of microplastics (Mcgivney et al., 2020). The accumulation of biofilms leads to the gradual formation of irregular structures such as cracks, holes, and grooves on the surface, which not only expands the space available for attachment, but also promotes the expansion and stability of biofilms (Guan et al., 2020). In addition, the hydrophilicity of microplastics is enhanced by microbial secretion of organic matter and degrading enzymes. This effect becomes more pronounced as biofilms mature (Tu et al., 2020). Increased hydrophilicity, in turn, accelerates the adsorption of bioorganic matter, promotes heterogeneous surface deposition, and ultimately strengthens surface roughness (Bhagwat et al., 2021).

The accumulation of biofilms is usually accompanied by an increase in the biomass and density of the surface of microplastics, significantly altering their behavior in water bodies. Microplastics that originally accumulate at the water-air interface due to hydrophobicity have reduced buoyancy and gradually settled to the bottom of the water column or even sediment areas after natural exposure and biofilm attachment (Lobelle and Cunliffe, 2011). With the increase of attached biofilm, the density of microplastics

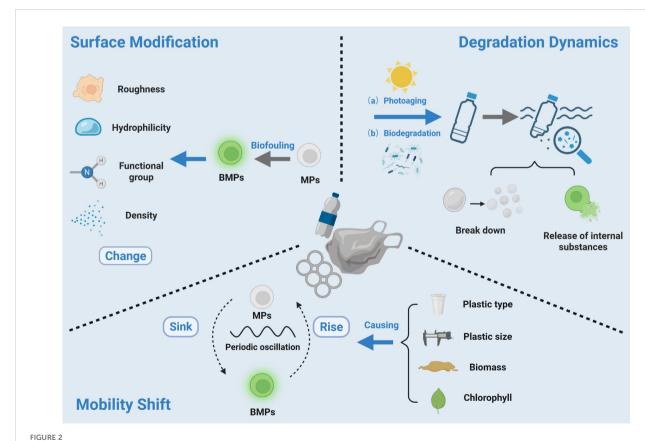
increased, and the sinking rate was affected by the particle size, biofilm thickness and loading. Among them, small-sized microplastics are more likely to lose buoyancy and sink faster than large-sized microplastics (Fazey and Ryan, 2016). Interestingly, this process may show periodic "oscillatory" behavior. As the biofilm load increases, microplastic particles gradually sink. In deeper zones unfavorable for biofilm growth, microbial death and respiration reduce the biofilm, decrease particle density, and increase buoyancy, causing the particles to rise again. Once back in the shallow chlorophyll-rich water body, the biofilm re-accumulates and the particles sink again, showing a periodic cycle of rise and fall (Golubeva and Gradova, 2024). Different types of microplastics exhibit varying responses. The formation of biofilms accelerates the sedimentation rate of high-density plastics such as PET and PVC, while the sinking trend of microplastics with densities close to water, such as PE or PP, is limited by the structural properties and composition of biofilms (Kaiser et al., 2017). However, some studies have shown that biofilm colonization does not significantly alter the sedimentation behavior of particles in some scenarios, suggesting that the density change caused by biofilms may not be sufficient to overcome the buoyancy of the original plastic, or its effect is limited by the physical properties of microbial populations and EPS (Jalón-Rojas et al., 2022). In addition, some scholars have suggested that surfactants produced by microbial metabolism may affect their vertical migration pathways and sedimentation stability by regulating the interfacial tension between microplastics and water bodies (Pete et al., 2023). Compared with its effect on sedimentation, biofilm adhesion also provides an alternative migration pathway. It promotes aggregation with other particles and stable attachment to plants, aquatic animals, or other biological surfaces, thereby influencing migration, spatial distribution, and environmental stability (Kalčíková, 2023).

Microplastics in marine environments primarily undergo degradation through two distinct pathways: abiotic degradation, driven by physical and chemical factors such as ultraviolet radiation, oxidation, and mechanical abrasion; and biotic degradation, which relies on microbial communities that metabolically facilitate the breakdown and transformation of microplastics (Weinstein et al., 2016). Studies have shown that biofilms may play a critical protective role in the photoaging process of microplastics by modulating photoaging pathways and suppressing the generation of reactive oxygen species (ROS) (Zhang et al., 2024b). Emerging evidence demonstrates that plastic-degrading microorganisms preferentially accumulate on plastic surfaces, suggesting that the formation of biofilms on plastic surfaces may actively promote plastic degradation in natural aqueous environments (Pang et al., 2024). To further reveal the role of biofilms in influencing the stability of microplastics, recent studies have explored the underlying mechanistic pathways involved in various degradation processes. For example, on the PS surface, biofilms can induce oxidative reactions that lead to the formation of oxygencontaining functional groups, thereby significantly altering their FT-IR spectral characteristics of the material (Liu et al., 2023b). Moreover, comparisons of microplastics with and without biofilm colonization suggest that key bacteria taxa, including Flavobacterium and

Erythrobacterium may be crucial to the long-term evolution of biofilm communities (Gulizia et al., 2025). However, it is worth noting that Rhusobacter spp. were absent from the microbial consortia associated with polyethylene degradation, indicating that their ecological function may exhibit substrate-specific characteristics (Restrepo-Flórez et al., 2014). Biofilms also showed a significantly influence the release behavior of plastic additives. Experiments have shown that microplastics with smaller particle sizes and higher surface roughness exhibit more pronounced leaching of additives such as FP-127, and this process is positively correlated with the abundance of specific core microbial communities (Pan et al., 2025). In addition, biofilm attachment significantly promotes the release of phthalate additives such as di(2-ethylhexyl) phthalate (DEHP), and their cumulative release increases with biofilm development. Although DEHP can be partially adsorbed and degraded in biofilms, its metabolites (such as MEHP) may exhibit even higher toxicity, indicating that biofilms may not fully eliminate additives and might instead amplify the ecological risks associated with microplastics (Zhao et al., 2024a). In view of the in-depth research on the microplastic-biofilm system in aquatic environments, this paper constructs a schematic diagram (Figure 2) illustrating the environmental changes of microplastics following biofilm colonization. The diagram focuses on key processes such as altered migration behavior, pollutant enrichment and ecological risk. Biofilms not only reshape the environmental behavior of microplastics (e.g., sedimentation and agglomeration), but also greatly enhance the ability of microplastics as pollutant carriers and pathogen transmission platforms, and significantly improve their bioavailability and feeding attractiveness, ultimately profoundly affecting their ecotoxicological effects.

4 Ecotoxicological effects of biofilmcoated microplastics

Biofilm adhesion makes the surface of microplastics appear looser and more porous, which is conducive to the capture and retention of pollutants (Sun et al., 2021). EPS significantly changes the adsorption properties of microplastics by introducing a variety of functional groups, thereby enhancing pollutant enrichment and prolonging their environmental retention time (Liu et al., 2019; Guan et al., 2020). For example, although biofilm biomass on glass beads was initially higher than that on LDPE and PLA, biofilms on the plastic surfaces gradually accumulated over time. This increased biofilm load enhanced metal adsorption, and the final accumulation on LDPE and PLA exceeded that on glass beads, indicating that biofilm adhesion strengthens the pollutant adsorption capacity of microplastics (Richard et al., 2019). Biofilms on microplastics frequently accumulate diverse contaminants, including heavy metals (Forero-López et al., 2022; Xiao et al., 2025), persistent organic pollutants, and antibiotics (Wang et al., 2022). Moreover, microbial communities in biofilms can achieve horizontal transfer of ARGs through close contact, making microplastics an important



The adhesion of biofilms in aquatic environments changes the surface properties of microplastics and affects their environmental behavior

"pollutant-gene" transmission platform, potentially threatening aquatic organisms and public health (Arias-Andres et al., 2018b; Li et al., 2021a). Furthermore, biofilm-coated microplastics act not only as carriers of pollutants and toxins but also as inducers of physiological stress in host organisms (Huang et al., 2020) and cause intestinal microbiota dysbiosis (Jin et al., 2018). To systematically assess exposure pathways and toxicological effects, Table 3 synthesizes findings across pollutant categories, affected species, and associated toxic effects.

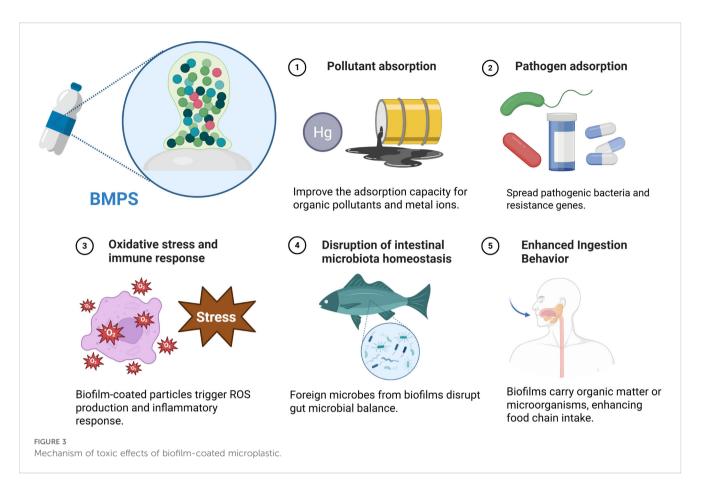
In addition, the complex has a higher bioavailability. Biofilmcoated microplastics not only enhance the adsorption capacity of organic pollutants, antibiotics, and heavy metals, but also improve the bioavailability of these pollutants through their metabolic activity and enzymatic reactions. Studies have shown that biofilm microorganisms can secrete a variety of intracellular and extracellular enzymes, such as oxidoreductase and dehydrogenase, which can convert complex pollutants such as PAHs into small molecule metabolic intermediates, thereby promoting their uptake by organisms (Yadav and Chandra, 2020). In addition, some biofilm formation can also biodegrade the plastic surface by secreting esterases, lipases, etc., and indirectly release secondary pollutants such as plasticizers or additives, thereby changing the exposure form and risk level of pollutants (Behera and Das, 2023). Under specific environmental conditions, microplastics attached to pollutants are more likely to stimulate microbial metabolic reactions and accelerate the transformation of pollutants (Girard et al., 2020). For example, Fajardo et al. found that biofilm-coated polyethylene microplastics triggered microbial oxidative stress reactions after exposure to organic carbon (OCs), suggesting that pollutants may undergo initial metabolic transformations in biofilms (Bonnineau et al., 2021). Secondly, biofilm-coated microplastics are more likely to be ingested by a variety of aquatic organisms, which allows pollutants to be transmitted across trophic levels of the food chain and may lead to bioaccumulation. Biofilms are an important food resource in freshwater ecosystems accumulating metals and organic pollutants, and their high metabolic activity makes them an important entry point for pollutants into the food chain (Bonnineau et al., 2021). Biofilm in rivers can significantly enrich microplastic particles and be ingested by protozoa, indicating that biofilms not only improve particle retention, but also increase their bioavailability at low trophic levels, which may affect their subsequent distribution in the food chain (Hamann et al., 2024). Studies have confirmed that the amount of microplastics ingested by fish through the food chain is significantly higher than that taken directly from water bodies, indicating that the trophic level transfer pathway of microplastics is one of the important exposure pathways faced by high-trophic organisms (Hasegawa and Nakaoka, 2021). Furthermore, biofilm formation produces an "ecological bait" effect on the surface of plastic, enhancing its feeding appeal to benthic and zooplankton. Once microplastics are attached to microorganisms, their feeding rate is significantly increased. For example, benthic filter feeding animals have a much higher ability to ingest microplastics coated with E. coli than pristine microplastics, with average concentrations of 42.360 \pm 23.588 no.g^{-1} and $11.443 \pm 0.4 \text{ no.g}^{-1}$, respectively (Fabra et al., 2021). In addition, the amphipod Orchestia gammarellus significantly

enhances the breaking strength of biofilm-coated plastics, producing up to 8.23 fragments per day, indicating that microplastics are more biologically identified as food after being biofilm-coated. For fish, although active ingestion of microplastics is uncommon, the incidence of accidental ingestion "bycatch" increases significantly when food particles are present nearby, and this probability rises with fish age. These results suggest that biofilmcoated microplastics floating in aquatic environments are more likely to be mistaken for edible prey, thereby increasing their risk of transmission in the food chain (Yagi et al., 2022). This process not only increases the risk of ingestion of microplastics, but may also trigger a "toxicity amplification effect", posing a potential threat to apex predators and even human health (Hodgson et al., 2018). As feeding behavior increases, biofilm-coated microplastics more easily cross the biological barrier and accumulate in the gut, liver, and even brain tissue. The viscous and functional components of the biofilm surface can affect the residence time and distribution of microplastics in the body, inducing microplastics to reside in specific tissues for a long time. Microplastics have been shown to enter a variety of tissues through the digestive system, blood circulation, and even across the blood-brain barrier (Zhu et al., 2023). The presence of biofilms enhances the interaction between particles and biological interfaces, which is one of the important factors for enhancing their transbarrier migration ability. Studies have further simulated the behavior of microplastics that adsorb pollutants in the gastric environment, and found that gastric juice components promote the release of pollutants on its surface, while changing the physical and chemical properties of the particles, thereby enhancing their penetration in the digestive system and the risk of tissue contact (Wu et al., 2023). To more clearly illustrate the toxicological pathways of biofilm-coated microplastics in ecosystems, Figure 3 presents the main mechanistic routes, including pollutant enrichment, pathogen carriage, and enhanced bioavailability.

Although numerous studies have pointed out that biofilm-coated microplastics may exacerbate ecotoxicity in the context of compound pollution, some experiments have found that the formation of biofilms may alleviate the toxic effects of microplastics, showing certain bidirectional regulation characteristics. On the one hand, the attachment of biofilms can effectively alleviate the acute toxicity and physiological interference caused by microplastics. For example, biofilm-coated microplastics exhibit lower lethality and physiological interference effects in a variety of exposure experiments than pristine microplastics. In zooplankton, biofilm-coated microplastics significantly reduce individual mortality (Nik Mut et al., 2024). In fish, biofilm-coated particles also induce less oxidative damage and metabolic disorders than clean particles (Kelly et al., 2024). After exposure in to natural water bodies, the biofilm and humus layer formed by microplastics colonize weaken their ability to enrich hydrophobic pollutants, thereby attenuating their toxic effects on fish (Hanslik et al., 2022). In addition, studies on polystyrene microplastics and nanoplastics of different sizes and surface modifications found that the acute effects of large-sized particles on the freshwater biofilm system were significantly reduced after the formation of biofilms, and the level of oxidative stress induced was significantly lower than that of exposed particles, further indicating that biofilms have a certain

TABLE 3 An overview of the adsorption mechanism and ecotoxicological effects of pollutants on biofilm-coated microplastics.

Adsorption mechanism	Adsorbent type	Affected organisms	Toxic effects	Reference
	Vibrio	Trachinotus ovatus	The abundance of proteobacteria in the intestinal flora was increased, and the abundance of firmicutes was decreased	(Liu et al., 2023a)
	Salmonella, Aeromonas	Zebrafish	Changing the structure of intestinal flora, increasing the diversity and abundance of pathogenic genera and ARGs, and inducing intestinal oxidative reactions	(Yu et al., 2024)
	Vibrio parahaemolyticus	Mytilus galloprovincialis	Causes oxidative stress and apoptosis of blood cells	(Sun et al., 2023a)
Provides an attachment environment	Staphylococcus sp., Elizabethkingia sp.	Amphioxus	Bacteria have the potential to transfer from the plastic intersection to the biological intestine	(Cheng et al., 2023)
	Escherichia coli	Ostrea edulis	Biofilms make microplastics more easily ingested by oysters, creating an immune system response	(Fabra et al., 2021)
	Chloroflexi, Proteobacteria, Basidiomycotina	Zebrafish	Enhanced endocrine system and thyroid toxicity	(Chen et al., 2021)
	Bacillus	Enchytraeus crypticus	Induce intestinal dysbiosis and affect ecological function	(Ding et al., 2024)
	Aeromonas salmonicida achromogenes	Zebrafish	Affects development and interferes with key gene expression	(Missawi et al., 2024)
Provide attachment environment and functional group adsorption	Vibrio, ARGs	Coral reef	Interferes with symbiont metabolism and coral bleaching	(Zhou et al., 2024)
Functional group adsorption	Antibiotic: azithromycin (AZI), clarithromycin (CLA)	Anabaena sp. PCC7120	Inhibited growth and chlorophyll A content	(González-Pleiter et al., 2021a)
EPS complexation	Pb	D. magna.	Enhanced toxicity to Pb(II) and adsorption and combined toxicity, and mortality increased in acute toxicity experiments	(Qi et al., 2021)
EPS complexation and providing an attachment environment	Cu, Pb, Vibrio	Clarias gariepinus	The number of Vibrio in the intestine of catfish increased; Reduces immunity and causes greater toxicity	(Jang et al., 2022)
	Pollutant: triclosan (TCS)	D. magna.	Regulate the adsorption and desorption behavior of microplastics on TCS, and affect the toxicity to organisms	(Verdú et al., 2023)
Hydrophobic interactions	Pollutant: dichlorodiphenyldichloroethylene (DDE), chlorpyrifos, and benzophenone-3	Dicentrarchus labrax	Inflammatory response and increase in potentially pathogenic microorganisms in the intestines	(Montero et al., 2022)



buffering and protective effect (Miao et al., 2019a). On the other hand, biofilms may provide nutritional compensation or act as a physical barrier. In some cases, microorganisms and their metabolites in biofilms can provide secondary sources of nutrients, slightly improving the survival rate of feeding organisms (Motiei et al., 2021). In addition, with the accumulation of biofilms, the roughness and particle volume of microplastic surfaces increase, which may exceed the feeding threshold of some benthic larvae or small zooplankton, reducing their ingestion probability (Nik Mut et al., 2024). Therefore, the formation of biofilm is not simply enhanced or weakened by toxicity, but its ecological effect is obviously context-dependent, which is specifically regulated by multiple factors such as the state of the biofilm (maturity, composition), the nature of pollutants, the physiological and ecological characteristics of exposed organisms (such as species, feeding mechanism) and environmental conditions, which may eventually change the size and mechanism of toxicity. When assessing the ecological risks of microplastics, the serious consequences caused by the above multiple factors should be fully considered.

5 Conclusion and perspectives

This paper provides a systematic review of the formation process, assembly mechanisms, and key influencing factors of biofilms on microplastics in different media and further explores how biofilm encapsulation alters their environmental behavior and ecotoxicity. The results show that biofilm attachment not only significantly changes the physical and chemical properties and environmental orientation of microplastics, but also profoundly affects the ecotoxicity risk by affecting the adsorption and release of pollutants, carrying pathogens and resistance genes, and interfering with host physiology. It is worth noting that the regulation of microplastic toxicity by biofilms is not always linearly enhanced, and its specific effects depend on the composition and structure of biofilms, environmental conditions and exposure scenarios, and may show diverse trends such as amplification, mitigation or neutrality.

At present, the research on biofilm-coated microplastics is still mainly based on the aquatic environment, while investigations into biofilm formation and behavior in other environmental media, such as soil and the atmosphere, remain in their infancy. Moreover, although existing studies have revealed the significant effects of biofilm attachment on the toxic behavior of microplastics, several key challenges remain: Firstly, most experiments are still based on idealized conditions, and the complex process of multi-factor coupling in the real environment has not been fully simulated; Secondly, the research focuses on the adsorption and carriage of pollutants by microplastics, and lacks a systematic assessment of the multi-level toxicological reactions induced by biofilm-coated microplastics in ecosystems. Thirdly, ecotoxicity research is mainly based on acute exposure, and the understanding of long-term exposure, bioaccumulation and genetic risks is still weak.

Future research should further focus on the ecological behavior of biofilm-coated microplastics in the context of compound

pollution and systematically reveal their environmental trends and ecotoxicological pathways from multiple levels such as mechanism of action, dynamic processes to biological effects. At the same time, research should broaden its environmental scope, enabling in-depth comparisons of biofilm formation characteristics and ecological effects in water, soil, and atmospheric systems, thereby establishing a cross-media risk assessment framework for microplastics. In addition, the spatio-temporal scale of ecotoxicology research should be expanded to promote the transformation from short-term experiments to long-term, cross-generational, and multitrophic ecotoxicity assessments.

Author contributions

HC: Methodology, Writing – original draft, Writing – review & editing. XW: Writing – original draft, Writing – review & editing. HY: Writing – review & editing. LS: Supervision, Writing – review & editing. XY: Supervision, Writing – review & editing. WZ: Supervision, Writing – review & editing. LK: Supervision, Writing – review & editing. LL: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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