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Review

Fate and transport of biological microcontaminants bound to microplastics in the soil environment

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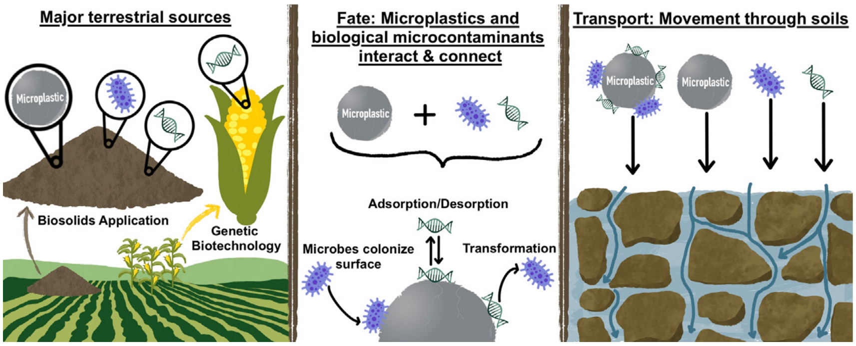
HIGHLIGHTS

- Microplastics serve as reservoirs of biological microcontaminants in soils.
- Sources, pathways, and fate of microplastic complexes in soils are reviewed.
- Novel biological microcontaminant sources are considered (genetic biotechnology).
- Microplastics and biological microcontaminants co-occur in agricultural soils.
- Microplastics increase gene persistence, transport, and transfer to soil microbes.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics, fragmented plastic particles with a maximum dimension <5 mm, are an emerging contaminant of concern that can also serve as a vector of other chemical and biological contaminants. Compared to chemical contaminants, the potential of microplastics to adsorb biological microcontaminants such as antibiotic resistance genes, small interference RNAs, and pathogenic viruses is not well understood. Many current microplastic studies are based in the aquatic environment (freshwater, seawater, and wastewater), even though the terrestrial environment is considered both an important sink and source of microplastics. Microplastics co-occur with biological microcontaminants in many terrestrial environments including agricultural soils, where biosolids containing both contaminants are often applied as a soil amendment. Recent research suggests that microplastics in these environments can increase gene persistence and flow, which could have unintended downstream consequences for environmental microbiome health and resilience. Antibiotic resistance genes and silencing RNAs bound to microplastics, for example, have the potential to increase resistance and alter gene expression in environmental bacteria, respectively. This review evaluates the sources and pathways of microplastics and biological microcontaminants in the terrestrial environment as well as potential sorption mechanisms that can encourage long-range transport and persistence. Novel sources of biological microcontaminants are considered, and the role of microplastics in promoting the persistence and flow of biological microcontaminants evaluated. Finally, future research directions are suggested to increase understanding of the mechanisms that drive the fate and transport of microplastic–biological microcontaminant complexes in the terrestrial environment and better inform risk management.

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Contents

1. Introduction

Plastic pollution is globally pervasive, with plastic wastes detected from the poles to the equator in nearly every environmental niche including air, water, soil, sediment, and even living organisms (Lau et al., 2020; Thompson et al., 2009). Plastics are widely used in consumer products due to their low cost, light weight, flexibility, and durability—the latter a property that also makes them highly resistant to degradation in the environment (Zhang et al., 2021a). As a result, plastics are broken down at very slow rates through solar radiation, mechanical wear, and microbial degradation (Wang et al., 2022). During these processes, they generate increasingly smaller plastic fragments termed “microplastics” (MPs) and “nanoplastics” (NPs). MPs are most frequently defined as plastic particles with a maximum dimension <5 mm, although there is no internationally agreed definition of the term and definitions can be inconsistent across studies. The 5 mm upper limit was likely proposed for biological relevance, as particles of this size are more likely to be ingested by biota. However, there was recently a proposal to align MP terminology with SI nomenclature (i.e., <1000 μm) (Hartmann et al., 2019). NPs, submicron sized particles, are commonly defined as those having an upper limit of 100 nm, although <1000 nm is also sometimes used and is consistent with SI nomenclature (Hartmann et al., 2019). Concern over the ubiquity of MPs and NPs in the environment has increased in recent decades because of their potential to negatively impact environmental and human health (Lamichhane et al., 2023) as these small plastic particles may be easily ingested or inhaled by humans, although the toxicological implications are still not fully understood (Vethaak and Legler, 2021). In addition to being contaminants themselves, MPs also have the capacity to sorb and concentrate many organic and inorganic contaminants from their surrounding environment (Caruso, 2019), contributing to the retention and long-range transport of many chemical and biological contaminants in the environment.

MPs have been detected in a wide variety of aquatic and terrestrial environments, yet research on MP pollution has largely focused on aquatic systems such as freshwater (Arias-Andres et al., 2018), seawater (Bowley et al., 2021; Li et al., 2018), and municipal wastewater (Cheng et al., 2022; Syranidou and Kalogerakis, 2022). The terrestrial environment, however, is considered both an important sink and source of MPs, with an estimated 80 % of MP pollution in the oceans originating from the land (Rillig, 2012; Rochman, 2018). The disparity between aquatic and terrestrial-based research is likely due to the increased complexity of interactions between MPs and contaminants in soils compared to water. In soils, MP–contaminant interactions are often significantly influenced by unique factors of the soil environment (e.g., soil texture, pH, and organic matter) (Chen et al., 2021). Organic matter and other soil particles compete with MPs in offering adsorption sites for contaminants. Due to the greater complexity of the organo-mineral soil matrix relative to water, MP extraction and quantification are also more difficult from soil media (Rillig, 2012). As result, there are currently no standardized techniques for the extraction and quantification of MPs from soils (Zhang et al., 2018), limiting research in this sector of the environment. However, given the ubiquity of plastic pollution in the soil environment, several recent review articles have highlighted the need for more terrestrial-based MP research (Liu et al., 2021a; Song et al., 2022; Wang et al., 2021b).

MPs have been shown to sorb many chemical contaminants, including heavy metals, antibiotics, and hydrophobic contaminants including polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Caruso, 2019). However, only recent studies have begun to establish the sorption potential of MPs for biological microcontaminants (bioMCs). BioMCs include intra- and extracellular genetic material introduced into the environment that has undesired effects, such as altering soil microbiome function or increasing environmental antibiotic resistance (Liu et al., 2021a; Syranidou and Kalogerakis, 2022). The authors of the current review identified only nine articles that evaluated interactions between MPs and bioMCs in the terrestrial environment. Furthermore, the scope of current MP–bioMC complex research is largely limited to sources of DNA—most notably antibiotic resistance genes (ARGs), driven by increasing concern for clinically relevant antibiotic resistance. Far fewer studies have addressed the role of MPs in facilitating the survival and dissemination of human pathogenic viruses, including the severe acute respiratory syndrome virus 2 (SARS-CoV-2) responsible for coronavirus disease-19 (COVID-19) (Moresco et al., 2021; Zhang et al., 2022).

The paucity of MP–bioMC complex research is compounded by the omission of bioMCs that originate from novel sources. The authors of the current review could find no studies to date that have assessed the potential for MPs to interact with foreign genetic material derived from genetic biotechnology. Modern genetically engineered (GE) crops often contain gene silencing RNA interference (RNAi) constructs and ARGs in their transformation plasmids. These genetic elements can be released from living or decomposing plant tissues (Tepfer et al., 2003) and encounter MPs in agricultural soils. Modern biopesticides also include the use of gene silencing double-stranded RNAs (dsRNAs), which limit pest predation, often through foliar applications of dsRNA solutions.

When MPs and bioMCs co-occur, MPs may act as protective microhabitats for bioMCs, shielding them from degradation, transporting them across long distances, and connecting them with competent environmental microbes (Liu et al., 2021a). As environmental microbes are not the intended targets of this genetic material, their uptake of these genes may result in non-target alterations to their function. In the case of RNAi, this could lead to silencing of genes involved in element cycling. The array of potentially concerning bioMCs in the environment—pathogenic bacteria, viruses, and extracellular genetic material, including ARGs and RNA silencing complexes—coupled with the ubiquity of MPs, necessitate future research. This review aims to evaluate the sources, pathways, and fate of bioMCs bound to MPs in the terrestrial environment and the role of MPs in promoting the persistence and flow of bioMCs.

2. The 'plastisphere': a novel niche for biological micropollutants

The hydrophobic surface of MPs provides a unique ecological niche for diverse microbial communities and their associated cell components (Fig. 1) (Yu et al., 2022). The communities that colonize and aggregate on MPs comprise the "plastisphere"—a term coined by Zettler et al. (2013) in reference to microbial colonization of MP surfaces in the marine environment. Microbes capable of colonizing MP surfaces are embedded within a diverse, self-produced extracellular polymeric substances (EPS) matrix consisting of polysaccharides, proteins, lipids, extracellular DNA, minerals, and water, forming a biofilm (Rummel et al., 2017). Microbes (and extracellular genetic material) embedded within this matrix are well-protected from environmental stressors (Santos et al., 2018). As a result, biofilm formation may be largely responsible for the ability of bioMCs bound to MPs to evade degradation. The proximity of plastisphere community members to other microbes and extracellular bioMCs within the biofilm may aid the proliferation and the potential dissemination of bioMCs. Therefore, plastisphere community composition is of particular interest.

The microbial community comprising the plastisphere is distinct from that in the surrounding water environment, with each community comprising distinct taxonomies (Zettler et al., 2013). This is evidenced in a study by Zettler et al. (2013) in which DNA sequence data of microbial communities living on plastic marine debris were compared to those living in the open ocean. Polyethylene (PE) and polypropylene (PP) samples shared a large percentage of their bacterial operational taxonomic unit (OTU) assemblage (up to 40 %). By contrast, seawater samples shared a much smaller percentage of their bacterial OTU with PP (3.5 %) and PE (8.6 %). The greater percentage of bacterial assemblages shared between different plastic substrates indicates that there is a "core" microbiome that defines the plastisphere. Specific bacterial taxa present on both plastic substrates but absent from seawater samples included several groups capable of degrading hydrocarbons, possibly indicating that microbes are utilizing synthetic plastics as a carbon source (Zettler et al., 2013). The plastisphere is also likely to be distinct from microniches formed on naturally occurring surfaces such as rocks and leaves. In one study, the plastisphere of MPs incubated in river water contained two opportunistic human pathogens and one plant pathogen that were absent from biofilms formed on natural substrates (Wu et al., 2019).

Recent studies attempting to bridge the knowledge gap between the aquatic and soil plastispheres have observed similar bacterial microbiomes in the plastisphere that are distinct from those in the surrounding soil environment (Huang et al., 2019; Zhang et al., 2019; Zhu et al., 2022). These studies observed differences in phylum composition in the plastisphere compared to the soil environment, although the relative proportions of groups varied somewhat among studies. In one microcosm study, the relative abundance of Proteobacteria, the dominant phylum in the plastisphere, was significantly lower in surrounding soils (Zhu et al., 2022). A different pattern was observed in a field study, in which the proportion of Proteobacteria was significantly higher in soils than in the plastisphere (although Proteobacteria was still one of the most abundant phyla in the plastisphere) (Zhang et al., 2019). Differences in the relative proportions of microbial taxa between studies indicates a more variable plastisphere community, as was observed by Zettler et al. (2013) in the marine environment. Plastisphere communities may also differ by MP type and size or by specific soil environment. MP size as an important factor affecting bacterial assemblage is supported by differences in bacterial composition between macro- and microplastics observed by Zhang et al. (2019). Another study further observed differences in MP bacterial composition across different MP and soil types (Zhu et al., 2022). Despite these plastisphere communities being somewhat variable, it is likely that there is still a core membership that characterizes the soil plastispheres, similar

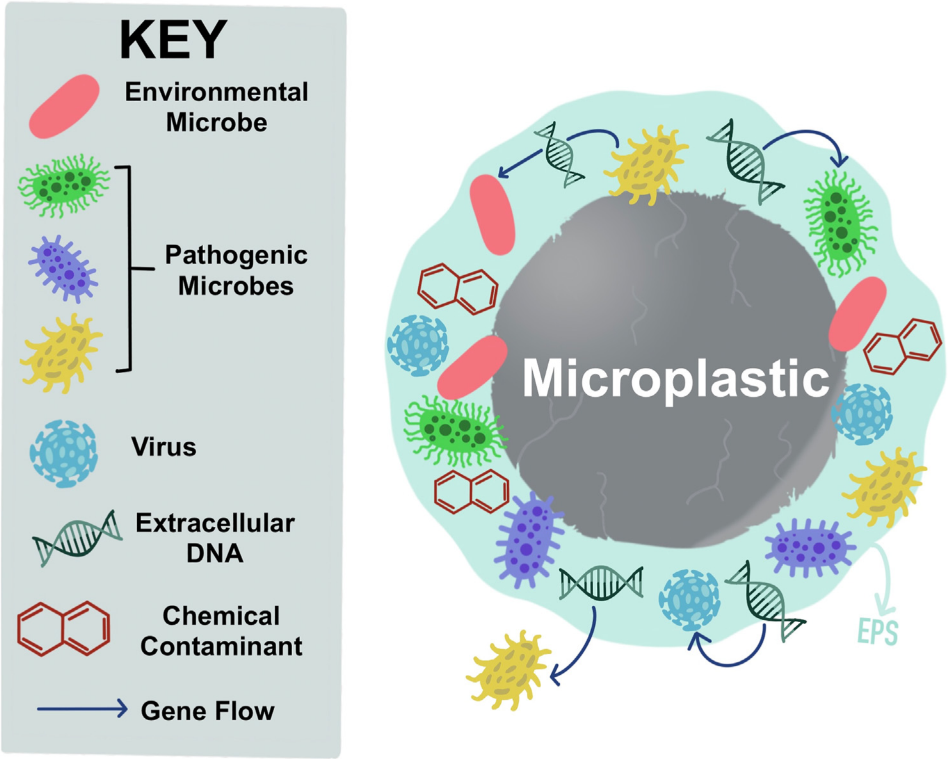

Fig. 1. Soil plastisphere, comprising chemical contaminants (e.g., organic pollutants) and biological microcontaminants (including pathogenic microbes, viruses, and extracellular DNA), embedded within an extracellular polymeric substances (EPS) matrix on the surface of microplastics. Extracellular DNA (or RNA)—potentially containing foreign genetic material—can be transferred to microbes (environmental or pathogenic) within the plastisphere or to microbes that encounter the plastisphere.

to that observed on plastic debris in the marine environment (Zettler et al., 2013).

This core soil plastisphere microbiome likely also consists of microbes capable of degrading plastics or sorbed contaminants (Huang et al., 2019; Zhang et al., 2019). In several studies, Actinobacteria was one of the predominant phyla in the plastisphere (Huang et al., 2019; Zhang et al., 2019; Zhu et al., 2022). This phylum is capable of degrading PE (Abraham et al., 2017)—the main component of plastic mulching films often used in agricultural fields (Zhang et al., 2019). Although the presence of this phylum alone does not conclusively imply plastics degradation, the phylum's prevalence in the plastisphere—in combination with visual evidence of physical degradation following microbial colonization—supports the possibility that MPs attract microbes capable of their own biodegradation (Zettler et al., 2013; Zhang et al., 2019). Several studies additionally identified diverse metabolic pathways that were enriched in the plastisphere community, including those classified under xenobiotics degradation (Huang et al., 2019; Zhu et al., 2022), potentially because microbes associated with the plastisphere are exposed to various contaminants commonly adsorbed to MPs. Because synthetic plastics are physiochemically distinct from naturally occurring substrates, the plastisphere is a unique environment capable of recruiting and supporting microbes with distinct and specialized traits.

MPs are also hubs for pathogenic bacteria (Huang et al., 2019; Zhu et al., 2022). In a microcosm study, MPs incubated in farmland soil comprised a ratio of potential bacterial pathogens to overall bacteria that was 12.4-fold higher than in soils, with the composition of pathogenic species also differing between the two environments (Zhu et al., 2022). The abundance of ARGs and mobile genetic elements was additionally determined in these samples. For three of four tested MP types—PE, polyamide (PA), and polystyrene (PS)—abundances of multidrug resistance genes were significantly greater than in the surrounding soil environment. For all MP types, mobile genetic elements were more abundant in the plastisphere than in soils (Zhu et al., 2022). An abundance of ARGs, mobile genetic elements, and pathogens spatially co-located within the plastisphere is of particular concern, as this increases potential interactions. Interactions between ARGs and pathogenic bacteria may be further facilitated by the ability of MPs to recruit large and complex bacterial networks, which are well-protected from environmental degradation by the large pits and flakes that cover the surface of weathered MPs (Zhang et al., 2019).

3. MP interactions with microcontaminants are influenced by sorbent and sorbate characteristics and environmental factors

Since MPs fall within a similar size range as colloids—nanoscopic to several microns in diameter—colloid science can be used to analyze MPs and predict their behavior in the environment (Al Harraq and Bharti, 2022). MPs interact with other pollutants due to various mechanisms, including hydrophobic interactions, hydrogen bonding, electrostatic interactions, van der Waals forces, and pi-pi interactions (Fig. 2) (Cao et al., 2021; Fu et al., 2021; Tourinho et al., 2019; Wang et al., 2020a). Cheng et al. (2022) used the extended Derjaguin, Landau, Verwey and Overbeek (XDLVO) theory to calculate the total interaction energy between various MPs and both antibiotic resistant bacteria (intracellular ARGs; iARGs) and extracellular ARGs (exARGs). XDVLO theory is the sum of three interactions—attractive van der Waals (VdW) interactions, repulsive electrostatic double layer (EDL) interactions, and Lewis acid-base (AB) interactions—and describes the stability and aggregation behavior of colloids. The attractive VdW interactions are driven by induced electrical interactions between two or more atoms or molecules that are very close to each other; repulsive

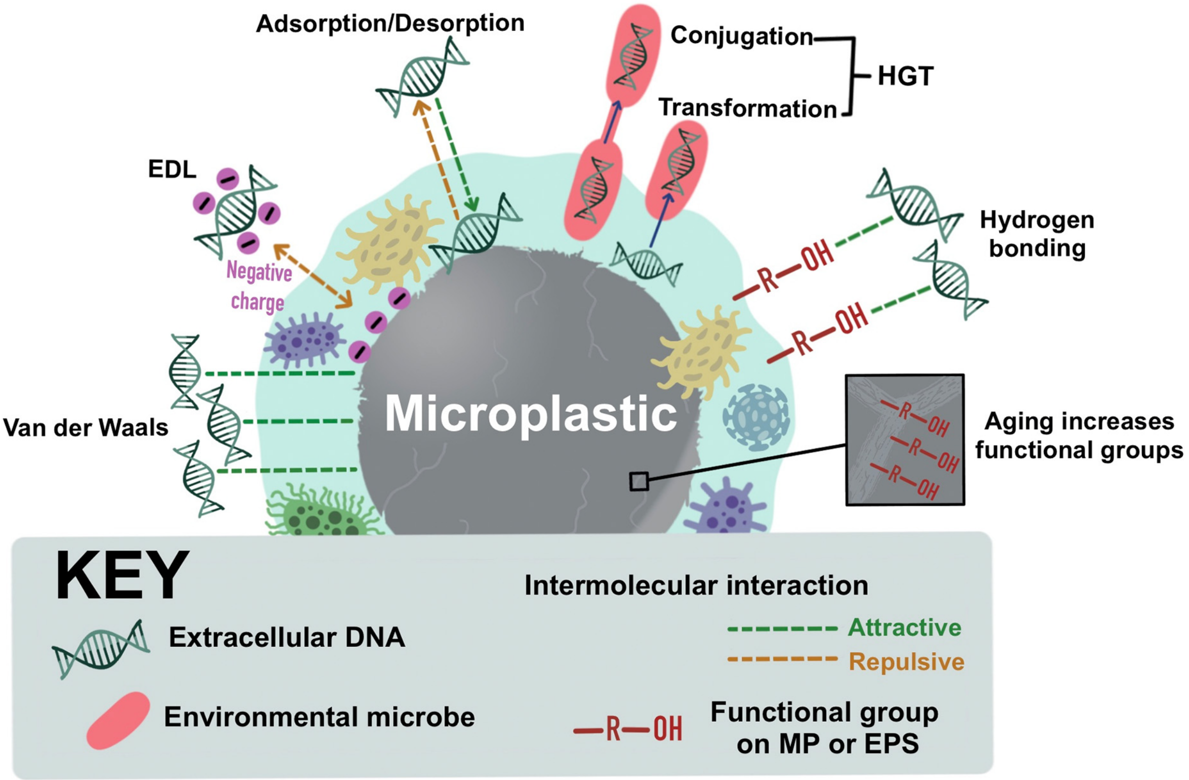

Fig. 2. Potential interaction mechanisms between MPs and bioMCs include attractive van der Waals forces, repulsive electrostatic forces in the electric double layer (EDL), attractive hydrogen bonding, and other adsorption/desorption processes. Through horizontal gene transfer (HGT), extracellular DNA (or RNA) embedded within the extracellular polymeric substances (EPS) matrix may be transferred from the plastisphere to outside microbes (transformation). Intracellular DNA may also be shared between microbes (conjugation). Aging and weathering of MPs can expose reactive functional groups on MP surfaces, increasing hydrogen bonding capabilities. These interactions are dependent on various factors, including MP characteristics (size, shape, composition, and surface properties), extracellular nucleic acid characteristics (size, shape, and structure), and soil environmental conditions (pH, ionic strength, and organic matter). Note that these interaction mechanisms do not have the same bond strength (e.g., hydrogen bonding is much stronger than van der Waals forces), which will impact reversible attachment rates.

EDL interactions arise due to the overlap of electric double layers with the same charge; and Lewis AB interactions describe electron-acceptor/electron-donor interactions. The total interaction energy between MPs and ARGs calculated by Cheng et al. (2022) indicated the presence of an energy barrier (repulsive force) between the two contaminants (with the energy barrier between iARGs and MPs greater than that between exARGs and MPs). This energy barrier—largely due to the strong, repulsive electrostatic force between MPs and ARGs—indicated that ARGs are not easily adsorbed onto MPs. However, the contact probability between MPs and ARGs is very high and can help overcome the energy barrier between the two. Furthermore, once ARGs become attached to MPs due to this high contact probability, the energy barrier is diminished, and the interaction becomes attractive (when the separation distance is ~0 nm) (Cheng et al., 2022).

The following subsections attempt to elucidate the impacts of MP characteristics, bioMC characteristics, and environmental conditions on MP–bioMC interactions. However, given the paucity of studies specific to bioMCs, these impacts are often considered for both chemical and biological microcontaminants. In the absence of MP–bioMC interaction information, interactions between MPs and chemical microcontaminants provide a more general understanding of interaction mechanisms. Moreover, a general understanding of chemical interactions with MPs is important, as chemical and biological microcontaminants often coexist and chemical microcontaminants (e.g., metals and antibiotics) may serve as selective agents in the proliferation of biological microcontaminants (e.g., heavy metal and antibiotic resistance genes) (Imran et al., 2019).

3.1. MP characteristics affect MP interactions with chemical and biological microcontaminants

Interactions between MPs and contaminants may be influenced by the size, composition, and surface properties of MPs (Table 1). The large specific surface area (SSA) of MPs is conducive to contaminant interactions, with sorption capacity generally increasing with decreasing particle size and increasing SSA. Sorption mechanisms may also be influenced by the plastic's crystallinity—the arrangement of polymer chains with respect to one another which can affect plastic hardness, density, transparency, and diffusion properties (Mei et al., 2020). Polymer chains that are aligned have a crystalline structure; those that are more disordered have an amorphous structure. The most commonly studied MP types are PS, PE, PP, and polyvinyl chloride (PVC). PS and PVC are classified as amorphous, and PE and PP as semi-crystalline, comprising both crystalline and amorphous regions. Amorphous PS and PVC are denser and more highly cross-linked, reducing the sorption capacity for hydrophobic organic contaminants. Semi-crystalline polymers tend to be rubberier with expanded and flexible structures. As a result, these polymers are typically highly accessible to hydrophobic organic contaminants. In general, higher crystallinity increases hydrophobicity. In batch experiments, PE had a greater capacity than either PS or soil to adsorb the hydrophobic antibiotic triclosan, despite PE having a SSA one order of magnitude lower than that of soil (Chen et al., 2021). In this instance, the strong hydrophobicity and rubbery structure of PE appears to have outweighed the effect of SSA. Crystallinity and hydrophobicity will also likely affect microbial attachment and biofilm formation, as microbes generally adhere more readily to hydrophobic surfaces—via hydrophobic attraction (Donlan, 2002).

Although crystallinity seems to be a determining factor for the sorption behavior of pristine MPs, this may not hold true for aged MPs. In one study, increasing crystallinity lowered the adsorption capacities of pristine MPs for the hydrophilic antibiotic ciprofloxacin (Liu et al., 2019). This aligns with the general principle that higher crystallinity increases hydrophobicity. However, after MPs were artificially aged by exposure to ultraviolet (UV) light for 96 h, the effect was reversed and MPs with higher crystallinities had higher adsorption capacities for ciprofloxacin (Liu et al., 2019). Aging and weathering alter the chemical properties of MPs, affecting MP

Table 1
Known information about the effects of MP and bioMC characteristics on interaction mechanisms and potential risks to human and environmental health.

Major MP types	Characteristics	Potential risks	Complicating factors
Polyethylene (PE) Polypropylene (PP)	Semi-crystalline	Greater hydrophobicity relative to amorphous plastics—greater potential to sorb hydrophobic organic contaminants and hydrophobic antibiotics.	• Age: Increase in oxygen-containing functional groups with aging/weathering. • Size: Smaller MPs have a higher sorption capacity due to increased SSA. Smaller MPs may also be more toxic to microbes, potentially resulting in increased microbial cell permeability and gene transfer (Shi et al., 2020; Sun et al., 2018). • Shape: MP fibers may be transported further in the environment than pellets because of their light weight and small size (Rios Mendoza et al., 2021). • Biofilm formation stage: Biofilms present diverse sorption sites, including cationic and anionic groups in the EPS (Flemming, 1995). • Surrounding environmental characteristics: Ionic strength, pH, and NOM can alter attractions between MPs and bioMCs.
Polystyrene (PS) Polyvinyl choride (PVC)	Amorphous	Greater hydrophilicity relative to crystalline plastics—greater potential to sorb hydrophilic compounds (e.g., metals and hydrophilic antibiotics).	
Major BioMC types	Characteristics	Potential risks	Complicating factors
Single-stranded extracellular DNA	Exposed functional groups (amide and amine) on nitrogenous bases.	May be able to form hydrogen-bonds with functional groups on aged MPs.	• Shape: Super-coiled and open circular plasmid forms may bind to sorbents preferentially over linear forms (Yu et al., 2013). • Length: DNA adsorption increases with decreasing fragment length and decreasing molecular weight. RNA adsorption increases with increasing molecular weight of the bases (Yu et al., 2013). • Homology with recipient: Sequence homology is required for DNA transfer to bacteria (Tepfer et al., 2003). • Surrounding environmental characteristics: DNA molecule becomes positively charged at pH < 5 and negatively charged at pH > 5. When the pH is high, high ionic strength of the surrounding environment enhances adsorption of DNA to soil particles (Yu et al., 2013).
Double-stranded extracellular DNA	Anionic phosphate backbone (electrostatic repulsion for anionic compounds/attraction for cations).	• May be easier for bacteria to integrate into their genomic DNA through homologous recombination. • More stable in the environment than single-stranded DNA. • Potential increased sorption to aged, hydrophilic MPs.	
Plasmid DNA	Genetic transfer requires that recipient has conjugative element.	Preferentially bound to clay minerals over linear form of DNA—greater protection from nucleases (Yu et al., 2013).	
Intracellular DNA	• Conjugation required to transfer genetic material. • Greater energy barrier to binding with MPs relative to extracellular DNA (Cheng et al., 2022).	Able to persist in the environment due to protection of (generally hydrophilic) cell from nucleases.	
RNA	Exposed nucleic acid bases.	Adsorption to MPs via van der Waals forces (Yu et al., 2013).	

interactions with contaminants. Liu et al. (2019) hypothesized that artificial aging (photooxidation) may have exposed reactive functional groups—including oxygen-containing groups—on the surface of MPs, thereby increasing the adsorption capacity for hydrophilic ciprofloxacin. An increase in oxygen-containing functional groups and a decrease in the hydrophobicity of MPs exposed to UV irradiation were similarly observed in other studies (Lu et al., 2022; Yuan et al., 2022). In one of these studies, the higher adsorption affinity of UV-aged MPs relative to pristine MPs for ARG vectors (bacteria, extracellular DNA, and phages) was attributed to an increase in oxygen-containing functional groups (Yuan et al., 2022), which may form more stable hydrogen bonds with bioMCs (Mei et al., 2020). As a result, ARG vectors desorbed at a lower rate from aged MPs than from pristine MPs (Yuan et al., 2022). Aging and weathering also affect the physical properties of MPs. With increased aging, MPs are successively fragmented and roughened, resulting in increased SSA and sorption capacity.

MPs may be further weathered by microbial colonization and biodegradation (Rummel et al., 2017). The hydrophobic surface of MPs is conducive to the formation of biofilms, which act as complex adsorbent systems, trapping and accumulating a wide range of substances (Guan et al., 2020). This may be due in part to the larger SSA of biofilm-covered MPs relative to pristine MPs (Wang et al., 2020b). Biofilms may also affect adsorption by altering the chemistry of MP surfaces. In a study by Guan et al. (2020), biofilm-covered MPs from a freshwater system contained various ionizable functional groups—carboxyl, phosphoryl, amino, and hydroxyl groups—and developed an increase in trace metals adsorption capacity. Another study similarly observed an increase in adsorption capacities of biofilm-covered MPs for copper and hydrophilic tetracycline relative to pristine MPs and soil particles (Wang et al., 2021a). Moreover, after incubation of MPs in microcosms containing agricultural soil, biofilm-covered MPs accumulated higher relative abundances of ARGs, heavy metal resistance genes, and the class 1 integron-integrase gene (*intI1*) (a proxy for the frequency of horizontal gene transfer) compared to pristine MPs (Wang et al., 2021a). Weathered and aged MPs should be the focus of future studies, as these are more reflective of real environments.

3.2. Biological microcontaminant characteristics affect interactions with MPs

Interactions between MPs and bioMCs can also be influenced by the size, shape, and structure of extracellular nucleic acids (Yu et al., 2013). For example, double-stranded DNA is more stable in the environment and more commonly adsorbed to clay than single-stranded DNA (Gardner and Gunsch, 2017). Adsorption of DNA to clay minerals increases with decreasing fragment length and decreasing molecular weight, which could be due to the higher diffusion rate of smaller DNA fragments (Yu et al., 2013). The shape of DNA is also an influencing factor in adsorption. Super-coiled and open circular plasmid DNA are preferentially bound to clay minerals over the linear form, potentially because plasmid DNA may be able to more easily overcome steric hindrances (Yu et al., 2013). For RNA, mechanisms of adsorption to clay are similar to those for DNA. Adsorption of RNA nucleic acid bases increases with increasing molecular weight of the bases, as van der Waals forces become more prominent (Yu et al., 2013). Further research is needed to address how these generalities can be extended from natural adsorbents (e.g., clays) to MPs.

3.3. Environmental conditions influence MP interactions with chemical and biological microcontaminants

Conditions within the soil environment (e.g., pH, ionic strength, and organic matter) can further complicate interactions between MPs and bioMCs. When the environmental pH is greater than the point of zero charge (pH_{pzc}: the pH at which the net charge of an adsorbent is equal to zero), the MP surface can become negatively charged. A greater negative surface charge generates greater electrostatic attraction for cations, such as metals, and repulsion for contaminants in the anionic form (Tourinho et al., 2019). Environmental pH also influences the charge of DNA molecules. The DNA isoelectric point (the pH at which the molecule carries no net electrical charge) is about 5. When $pH < 5$, DNA bases containing amine groups—adenine, guanine, and cytosine—become protonated and the DNA molecule becomes positively charged. When $pH > 5$, the phosphate groups that form the backbone of DNA contribute to the overall net negative charge of the molecule (Yu et al., 2013). DNA adsorption can also be affected by the ionic strength of the surrounding environment. When $pH > 5$ (i.e., DNA molecule is negatively charged), cationic bridging enhances the adsorption of DNA molecules by soil particles (Romanowski et al., 1991). Soils containing high natural organic matter (NOM) present competitive binding sites for contaminants, potentially reducing the concentration of contaminants on MPs. NOM can also coat the surface of MPs, reducing their hydrophobicity—and thus their affinity for organic pollutants (Wang et al., 2020a). Whether the net effect of interactions between MPs and bioMCs is ultimately attractive or repulsive can be influenced by several factors, including MP and bioMC shape and size and the chemical composition of the medium (Al Harraq and Bharti, 2022). Further research is still needed to fully elucidate these effects.

4. Biosolids application may represent a significant co-transport route to soils for microplastics and antibiotic resistance genes

Agricultural soils are a natural reservoir for two emerging contaminants—MPs and ARGs. Sources of MP pollution in agricultural soils include the land application of biosolids, the use of plastic mulching films, and compost application, as the composting process does not remove all plastic fragments (Wang et al., 2022). Sources of ARGs in agricultural soils include the historical use of ARGs as selection markers in some lines of GE crops, human consumption and misuse of antibiotics, the widespread use of antibiotics in concentrated animal feeding operations (CAFOs), and the land application of biosolids. Since MPs and ARGs have the potential to interact and connect throughout the process of biosolids generation, biosolids application likely represents a significant co-transport route for MPs and ARGs to soils. Other sources of these contaminants already present in agricultural soils may then further promote the retention and proliferation of MP–bioMC complexes (e.g., antibiotics in soils from CAFOs will select for antibiotic resistance in microbes).

Biosolids are produced by wastewater treatment plants (WWTPs) from solids removed during primary and secondary settling processes that are used to generate a nutrient-rich semisolid product. In Europe and North America, approximately 50 % of treated sludge is utilized as fertilizer for agricultural applications (US EPA, 2016). Prior to the physical and chemical treatment needed to produce biosolids from WWTP sludge, this sludge still contains many of the same harmful substances found in influent wastewater—including bioMCs. A survey of >25 WWTPs across the US detected GE crop-derived genes in activated sludge and digestor sludge (Gardner et al., 2018). Theoretically, bioMCs that accumulate in sludge during the wastewater treatment process would be removed during subsequent sludge treatment—US EPA regulations specify that treatment processes for Class A biosolids (those meeting the most stringent requirements) should eliminate pathogens, including viruses.

Despite stringent requirements, sludge treatment processes may not be equipped to fully remove bioMCs. To test the effectiveness of sludge treatment processes in removing these contaminants, Gardner et al. (2019) constructed batch reactors containing anaerobic digestor sludge collected from a wastewater treatment facility and mimicking thermophilic anaerobic digesters (i.e., conditions simulated those necessary to produce Class A Biosolids). After 60 days of operation—30 days beyond the average US digestor residence time—transgenic crop ARG concentrations in the reactors were significantly reduced, but a sizeable fraction continued to persist as extracellular DNA (exDNA) in the sludge (Gardner et al., 2019). In another analysis of bioMCs in biosolids, genes associated with antibiotic resistance, including *sul1* and *bla*, and those associated with horizontal gene transfer, including *intI1*, were less abundant in Class A biosolids (two alkali-stabilized and one heat stabilized) compared to Class B biosolids (dewatered anaerobically digested) (Murray et al., 2019). However, these genes were still detectable in Class A biosolids for two of the three sewage sludge stabilization processes.

Current sludge treatment processes are also unlikely to remove the MP load (Crossman et al., 2020). As a result, approximately 300,000 tons of MPs are applied to North American farmlands each year (Nizzetto et al., 2016). MPs and bioMCs in WWTP sludge can find each other and sorb—potentially increasing the retention and proliferation of bioMCs. In a recent study, MPs in WWTP sludge subjected to aerobic digestion increased the abundance of nine quantified ARGs and the *intI1* gene (Zhang et al., 2021b). This effect may be amplified by the co-occurrence of antibiotics in WWTPs, which exert a selective pressure on microbes in WWTPs to retain or acquire ARGs. Because of the significant volumes of wastewater treated worldwide, even a low concentration of bioMCs in WWTP effluent or sludge could contribute greatly to the total environmental load (Syranidou and Kalogerakis, 2022).

5. Genetic biotechnology is a novel source of biological microcontaminants in the soil environment

Although biosolids application is a well-studied route for bioMC transport to soils (Wolters et al., 2018), other possible, relevant sources of bioMCs to soils are not as well-represented in the literature. If these sources are overlooked, the potential risk of MP–bioMC interactions in the soil environment may be underestimated. One underrepresented, novel source of bioMCs—including ARGs, small interference RNAs (siRNAs), and pesticidal dsRNA—is genetic biotechnology. GE crops are crop plants whose genome has been modified by the insertion of a transgene, a specific segment of foreign genetic material. GE crop technology has been rapidly adopted in modern agriculture as a means of increasing crop yield and quality—using methods that are much less time- and labor-intensive compared to traditional plant breeding methods (Kumar et al., 2020; Saurabh et al., 2014). However, concerns persist regarding the potential environmental risks of GE crops, including the release of foreign genetic material, and its potential for transport and transfer to other organisms (Auer and Frederick, 2009; Tsatsakis et al., 2017).

Plant transformation research to develop GE crops requires the use of a selectable gene marker to confirm the successful transfer of a transgene (Dunfield and Germida, 2004). The first widely used marker was a bacterial gene encoding neomycin phosphotransferase II (*nptII*)—an ARG conferring resistance to several antibiotics. Genetic makers introduced into early plant cells are expressed in all plant tissues—from leaves to roots. Plant roots are surrounded by bacterial populations (rhizosphere microbes) that are nourished by secretions from plant roots (root exudates) and the contents of lysed plant cells. When plant tissues decompose, rhizosphere bacteria can assimilate plant DNA, which is protected against degradation by soil constituents, including clays and soils, and potentially by MPs (Demanèche et al., 2001; Gardner and Gunsch, 2017). Plant roots may also provide a constant DNA source for horizontal transfer through the sloughing off of root cap cells (Tepfer et al., 2003). A study by Tepfer et al. (2003) evaluated the ability of plant donors carrying the *nptII* gene to transfer to the naturally competent *Acinetobacter*, carrying a plasmid containing *nptII* inactivated by a 10 base-pair deletion. The study confirmed the potential for DNA transfer from GE plants roots and leaves in vitro to soil bacterium (Tepfer et al., 2003). Such transgene flow could pose a risk to human health if antibiotic resistance transgenes are transferred to pathogenic bacteria, either as free exDNA or as intracellular DNA (iDNA) through horizontal transfer from other soil bacteria. This is of particular concern because many antibiotic resistance transgenes target antibiotics that are still used to treat a variety of infectious diseases in humans and animals (Khachatourians, 1998).

In addition to ARGs, GE crops also often contain transgenic silencing constructs capable of silencing the production of target crop proteins to produce more favorable crop characteristics (e.g., silencing the PPO-PGAS mechanism in apples to prevent browning (Waltz, 2015)). These silencing constructs rely on an evolutionarily conserved defense mechanism in eukaryotes—RNAi—in which specific enzymes cleave targeted messenger RNA (mRNA) to prevent protein expression of the gene of interest. RNAi has recently been leveraged for targeted gene silencing in agricultural crops to produce favorable phenotypes, including nutritional improvements and enhanced defense against biotic and abiotic stresses (Saurabh et al., 2014). Although there are several gene silencing mechanisms, the siRNA mechanism is one of the most commonly used in synthetic genetic modifications (Un Jan Contreras and Gardner, 2022). This defense mechanism is triggered by the introduction of dsRNA in the cell. This dsRNA—which could be a viral intruder or an introduced transgene—is then processed by a DICER enzyme into siRNAs. The siRNA duplexes are unwound, and the antisense strand bound to a multi-protein structure called the RNA-induced silencing complex (RISC). RISC then catalyzes the cleavage of the homologous mRNA within the cytoplasm to inhibit translation (Zamore et al., 2000).

The RNAi pathway has also been recently leveraged to protect crops against pests (Baum et al., 2007; Burand and Hunter, 2013; Gordon and Waterhouse, 2007; Koch and Kogel, 2014; Price and Gatehouse, 2008). In this application, dsRNA is expressed within the plant's tissue (*in planta* expression) (Koch and Kogel, 2014) or applied exogenously to the crop (e.g., as a foliar spray) (San Miguel and Scott, 2016). When pests, such as insects, nematodes, viruses, and fungi, feed on these plants, they ingest the dsRNA. As a result, targeted mRNA within the pest is degraded, stunting the pest's growth and development, or leading to mortality (Baum et al., 2007; Mao et al., 2007). In one of the first commercial applications of this method, RNAi was triggered in coleopteran insect pests—most notably the western corn rootworm, an especially invasive and adaptable insect pest of maize that causes large yield losses in the United States and Europe (Baum et al., 2007; Gray et al., 2009). A recent study showed that pesticidal dsRNA in soils, like previously studied nucleic acids, can be both degraded and adsorbed to soil particles, although the extent of each of these processes is influenced by soil-specific factors (e.g., organic matter content) and dsRNA-specific factors (e.g., sequence length) (Parker et al., 2019). Like with other nucleic acids, adsorption of dsRNA molecules to soil particles will likely increase their persistence and transport.

One of the risks of RNAi crops is the potential for off-target gene effects—unintended silencing of a gene that is not the intended target—which can occur as a result of sequence homology between GE crop transgenes and genes in non-target organisms (Auer and Frederick, 2009). The likelihood of sequence homology between GE crops and soil microbes is high, given that the genetic code of all living organisms is based on DNA sequences comprised of only four nucleotide bases (Crozier, 1997). Non-target effects to rhizosphere bacterial populations, which play a major role in nutrient transformations and element cycling, could disrupt these critical ecological functions (Dunfield and Germida, 2004).

6. Microplastics can promote gene persistence and transport in the soil environment

MPs have the capacity to adsorb bioMCs at high rates, creating hubs of concentrated genetic material resistant to physiochemical degradation (Cheng et al., 2022; Lu et al., 2022; Yuan et al., 2022). In a study by Cheng et al. (2022), both iARGs and exARGs in samples collected from a WWTP were significantly adsorbed by MPs, with exARGs more highly enriched on MPs compared to iARGs. Viruses can similarly adsorb to MPs, with adsorption increasing their rate of survival and potential environmental transmission (Lu et al., 2022). Adsorption and persistence of bioMCs may become more pronounced as MPs age and their surface chemistry and morphology are altered (Lu et al., 2022; Yuan et al., 2022). In one study, ARGs on MPs from farmland soil samples were more abundant in samples from older field plots and on more highly weathered MPs (Lu et al., 2020).

The persistence of bioMCs bound to MPs in the environment may be largely attributed to biofilm formation. Biofilms are communities of organized microorganisms, embedded within a self-produced EPS matrix, that adhere to abiotic or biotic surfaces—biofilms formed on MPs constitute the 'plastisphere.' EPS account for over 90 % of the biofilm (by dry mass) and serve multiple functions: protection of microbial community members, sequestration of nutrients and other particles from the surrounding environment, adhesion to surfaces, cohesion within the biofilm, and exchange of genetic information (Flemming and Wingender, 2010). Because of the many advantages conferred by EPS, most microbes prefer biofilm community membership over a solitary lifestyle (Santos et al., 2018). Biofilm formation, in some cases, may be a defensive reaction and can be triggered by microbial exposure to antibiotics at subinhibitory concentrations (Hoffman et al., 2005). Biofilm development consists of several steps: adherence of microbes to a surface, microbial proliferation, maturation of the biofilm architecture, and, finally, dispersion (desorption/detachment) (Santos et al., 2018). This last step is important because it can increase the chances of a cell's transport to new regions that may be more favorable for growth and survival, and where it may form a new biofilm (van Loosdrecht et al., 1987).

Microbial activity can also affect the environmental transport of MPs and MP–bioMC complexes. MP–bioMC complexes in the soil environment can be dispersed by runoff or groundwater. It is, therefore, important to understand the transport properties of plastic particles and plastic particle complexes in porous media. Transport in porous media may be affected by solution chemistry (e.g., ionic strength and pH), flow velocity, and media type/size (Kianfar et al., 2022). In a study by He et al. (2018), *Escherichia coli* injected into quartz sand columns were transported further when plastic particles were also present in the influent; but only when the ionic strength of the influent solution was high. At low ionic strengths, the effect of plastic particles on bacterial transport was negligible. Under high ionic strength conditions, NPs (0.02 μm and 0.2 μm) adsorbed to cell surfaces, forming aggregates that were more mobile than bacterial cells alone. No direct interaction was observed between MPs (2 μm) and cell surfaces; increased bacterial transport under high ionic strength conditions was instead attributed to competition for deposition sites between MPs and cells within the sand column (He et al., 2018).

The impact of biofilms on the transport of plastic particles has been largely overlooked in previous laboratory studies. In packed column experiments, He et al. (2020) observed a decrease in (pristine) plastic particle transport in biofilm-coated sand compared to bare sand. Biofilms reduced the porosity and narrowed flow paths in sand columns, increasing the potential of MP–media collisions (He et al., 2020). However, biofilms and/or NOM coating the surfaces of both MPs and porous media could increase steric hindrance, reducing attachment to porous media and increasing the transport distance of MP–bioMC complexes (Zhou et al., 2022). MP–ARG complexes aggregated with soil minerals and NOM have the capacity to travel vertically into deep soil layers—increasing the potential for their dissemination to underlying groundwater (Yan et al., 2020). If physiochemical conditions of the surrounding environment allow for desorption of transgenes from plastics, bioMC transport will be driven largely by advection and dispersion processes (Ceccherini et al., 2007; Poté et al., 2003). Water-saturated column experiments by Poté et al. (2003) indicated that bioMCs can be transported long distances in porous media without significant degradation and while still retaining the ability to transfer genetic material. However, desorption rates for bioMC–plastic complexes are still unknown.

6.1. Exposure of microbes to microplastics increases gene transfer

Bacteria have the potential to undergo three horizontal gene transfer (HGT) mechanisms—transformation, conjugation, and transduction. Of these, conjugation has the greatest number of prerequisites and transformation the fewest. Initiation of conjugation requires that two metabolically active cells establish contact, and that the donor cell contain a conjugative element (plasmid or transposon). By contrast, initiation of transformation only requires that one recipient cell is physiologically active and encounters free exDNA (Lorenz and Wackernagel, 1994).

Free exDNA is abundant in the environment—actively excreted by live cells or released from lysed cells—and can persist in the environment for long periods (weeks to months) while retaining the ability to transform cells (Lorenz and Wackernagel, 1994; Nagler et al., 2018; Romanowski et al., 1992, 1993). Bacteria likely evolved mechanisms to uptake exDNA as a means of acquiring new genetic information, repairing damaged DNA, and obtaining food (DNA can be used as a source of carbon, nitrogen, and phosphorous). In many environments that serve as reservoirs of bioMCs, exDNA may be more abundant than iDNA (Dong et al., 2019). A study by Gardner et al. (2019) detected ARGs originating from WWTP anaerobic sludge at greater concentrations in exDNA rather than iDNA after thermophilic treatment. These exDNA likely persisted by attaching to particles in wastewater—clays, colloids, and organic matter—or to cellular debris from recently lysed cells (Gardner and Gunsch, 2017; Nielsen et al., 2000). In laboratory studies, MPs also adsorbed higher amounts of exARGs than iARGs (Cheng et al., 2022). Moreover, iARG adsorption to MPs was significantly inhibited in the presence of coexisting contaminants (tetracycline and humic acids), whereas exARG adsorption was not affected (Cheng et al., 2022). Natural and MP surfaces—potential sites of exDNA accumulation—may therefore act as hotspots of transformation, increasing the risk of exDNA propagation.

Bacterial uptake of bound and immobilized exDNA is poorly understood; data comparing gene transformation frequencies of free DNA versus DNA adsorbed to soil are mixed (Lorenz et al., 1988; Lu et al., 2010). Although several studies have demonstrated that host bacteria are able to uptake exDNA directly from mineral surfaces (i.e., desorption of DNA is not essential for successful natural transformation), the direction of this effect is inconsistent (Table 2). In a study by Lorenz et al. (1988), adsorption to sand particles increased the transformation of *Bacillus subtilis* by up to 50-fold. Another study similarly reported an increase in the transformation frequency of exARGs after exposure to sediments (Dong et al., 2019). However, in several other studies, transformation efficiencies for bound DNA were not affected—or even decreased—relative to those for free (unbound) DNA (Chamier et al., 1993; Crecchio et al., 2005; Crecchio and Stotzky, 1998; Gallori et al., 1994; Khanna and Stotzky, 1992; Lorenz and Wackernagel, 1990; Lu et al., 2010). These contradictory finding may reflect differences in bacterial species, solution chemistry, adsorbent surface type, or the size and conformation of adsorbed DNA. However, even in instances when bound DNA was transformed at a lower frequency than free DNA, bound DNA was often more resistant to degradation (Crecchio et al., 2005; Crecchio and Stotzky, 1998; Khanna and Stotzky, 1992). In one study, inhibiting transformation by bound DNA required a DNase concentration 100 times higher than that required to inhibit transformation by free DNA (Crecchio and Stotzky, 1998). So, unlike free DNA, which may rapidly degrade in soil, bound DNA that escapes degradation may have a greater potential to transform cells. However, it has been suggested that there is a tradeoff between protection from attack by nucleases and transformation efficiency in soils—the fraction of bound DNA available for transformation may also be accessible to nucleases (Demanèche et al., 2001). The other fraction of tightly bound DNA can become available—both to bacteria and nucleases—when environmental conditions lead to desorption (Demanèche et al., 2001).

More recent studies support the role of MPs in increasing HGT rates (Arias-Andres et al., 2018; Cheng et al., 2022; Yuan et al., 2022). As a hub of microbes, EPS, and bioMCs, the plastisphere increases contact potential between donor and recipient microbes and provides an ideal exchange site for genes (Cheng et al., 2022). Biofilms that form on the surface of MPs confer many advantages to microbial community members—including adsorptive capabilities, nutritional sources, and protection from environmental stresses—that may be conducive to HGT. In particular, the biofilm matrix holds onto components of lysed cells. This includes exDNA, which in addition to serving as an important structural component of the biofilm and as a source of energy and nutrients, is also available for HGT (Flemming and Wingender, 2010; Santos et al., 2018). In a study by Arias-Andres et al. (2018), gene exchange in MP-associated bacteria was increased relative to free-living bacteria or bacteria on NOM aggregates. Several other studies similarly support enhanced HGT frequencies with MP exposure (Cheng et al., 2022; Yuan et al., 2022). Much less is known about the environmental impacts of extracellular RNAs, although recent

Table 2
Bacterial uptake of different forms of DNA (adsorbate) bound to different soil minerals or MPs (adsorbent).

Transformed species	Adsorbent	Absorbate form	Effect of adsorbent on bacterial uptake relative to free DNA	Reference
Bacillus subtilis	Sand	Chromosomal DNA	Increase	Lorenz et al., 1988
Pseudomonas stutzeri	Sand	Chromosomal DNA	None	Lorenz and Wackernagel, 1990
Bacillus subtilis	Montmorillonite	Chromosomal DNA	None	Khanna and Stotzky, 1992
Acinetobacter calcoaceticus	Sand	Chromosomal DNA	None	Chamier et al., 1993
		Plasmid DNA	Decrease	
Bacillus subtilis	Montmorillonite	Chromosomal DNA	Decrease	Gallori et al., 1994
		Plasmid DNA	Decrease	
Bacillus subtilis	Humic acids	Not specified	Decrease	Crecchio and Stotzky, 1998
Bacillus subtilis	Organo-mineral complex	Not specified	Decrease	Crecchio et al., 2005
Azotobacter vinelandii	Silica & NOM	Chromosomal DNA	None	Lu et al., 2010
Escherichia coli	Polystyrene MPs	Plasmid	Increase	Arias-Andres et al., 2018
Escherichia coli	Sediment	Plasmid	Increase	Dong et al., 2019
Escherichia coli	Polystyrene MPs	Plasmid	Increase	Yuan et al., 2022
Escherichia coli	Polystyrene MPs	Plasmid	Increase	Cheng et al., 2022

studies have attempted to elucidate the fate of dsRNA in agricultural systems (Bachman et al., 2020; Parker et al., 2019; Tsatsakis et al., 2017). Results from Parker et al. (2019) indicate that microbes in soil utilize dsRNA. However, future research is needed to account for potential HGT to soil microbes.

The ability of a cell to take up free exDNA (plasmid or chromosomal element) from the surrounding medium and incorporate it into its own genome using specialized proteins—known as genetic competence—is transient in most transformable bacteria and is developed only under specific conditions (e.g., environmental stresses, including sublethal concentrations of antibiotics) (Johnston et al., 2014). Contaminants that co-occur with MPs in the terrestrial environment can act as sublethal stressors and increase the selection pressure for ARGs, further compelling microbes to acquire or retain these genes (Wang et al., 2021a). Environments with combined pollution, such as farmland soils, may therefore increase selection pressure for these genes. Manure, for example, can contain heavy metals and antibiotics, including copper and tetracycline. In these environments, the co-selection of metals and antibiotic resistance via cross- and co-resistance pathways can drive the proliferation and spread of ARGs (Imran et al., 2019).

Bacterial acquisition of cross- or co-resistance has significant implications for the development and proliferation of multi-drug resistant bacteria. In cross-resistance, a single (or similar) resistance mechanism is responsible for resistance to different compounds. For example, in *Pseudomonas aeruginosa*, an opportunistic pathogen, the heavy metal efflux pump and its regulator genes also confer resistance to a beta-lactam antibiotic (Perron et al., 2004). In co-resistance, two or more genes for different resistance mechanisms exist on the same mobile genetic element in bacteria, and so can be co-transferred from one bacterium to another. For example, *Staphylococcus aureus*, a bacterium that can cause staph infections in humans, co-selects methicillin resistance by acquiring the plasmid carrying both methicillin and metal (cadmium and zinc) resistance encoding genes (Cavaco et al., 2010). Wang et al. (2021a) investigated the effects of the coexistence of tetracycline, copper, and MPs on the fate of nine ARGs and three heavy metal resistance genes in agricultural soils. The presence of MPs increased the relative abundance of ARGs, heavy metal resistance genes, and *intI1* in soils. The total abundance of target ARGs detected on MPs also significantly increased with increasing environmental copper or tetracycline concentrations (Wang et al., 2021a). Therefore, environments that are co-polluted with metals, antibiotics, pathogens, and MPs may pose a significant human health risk.

6.2. Bacterial cell membrane damage is a potential mechanism of improved gene transfer in microbes exposed to microplastics

The exact mechanisms that increase HGT in microbes exposed to MPs are still unclear. Do these mechanisms differ from those of bioMCs bound to natural adsorbents (e.g., clay)? What properties of MPs might make these artificial adsorbents more conducive to HGT than natural adsorbents? Biofilms that form on the surface of MPs provides an ideal site for gene transfer—sheltered protection from environmental stresses, close contact with other microbes, and a reservoir of exDNA. Relative to the surrounding soil environment, the plastisphere may contain greater abundances of multidrug resistance genes—and the genetic elements needed to transfer them (Zhu et al., 2022). MPs may also act as a weak toxicant, inducing a level of sublethal stress in microbes sufficient to induce genetic competence.

The results of several recent studies propose another mechanism—bacterial cell membrane damage as a result of increased intracellular reactive oxygen species (ROS) production in microbes exposed to MPs (Cheng et al., 2022; Hu et al., 2022; Yu et al., 2023; Yuan et al., 2022). In many organisms, exposure to foreign substances, such as MPs, may trigger innate immune defense mechanisms and, in the process, generate large quantities of ROS (Hu and Palić, 2020). ROS may also be generated through the interaction of electron donor sites on the surface of MPs with molecular dioxygen (O_2). This process may be exacerbated in aged (e.g., UV-exposed) MPs—which have a greater number of surface reactive functional groups—and in smaller-sized MPs—which have a greater surface area for displaying reactive groups and (at the nanoscale size) can penetrate lipid membranes (Hu and Palić, 2020; Wei et al., 2019). Intracellular ROS accumulation (leading to oxidative stress) can damage cell membranes, increasing cell permeability and potentially allowing for increased transfer of DNA from donors to recipients (Beaber et al., 2003; Cabiscol Català et al., 2000; Cheng et al., 2022). Qiu et al. (2012) hypothesized that this mechanism of gene transfer—damage of bacterial membranes by oxidative stress—could explain their observation of increased HGT between bacteria exposed to nanomaterials (nanoscale sized engineered materials). In this study, aluminum oxide nanoparticles (nanoalumina) promoted conjugative transfer of a plasmid by up to 200-fold between bacteria of the same genus, and up to 50-fold between bacteria of a different genus. Nanoalumina exposure also damaged the integrity of bacterial cell membranes and induced an oxidative stress response in parent bacteria—as evidenced by an increased production of hydroxyl radicals, as well as antioxidant defense system indicators (Qiu et al., 2012).

Compounds released by certain MPs can also induce oxidative stress. As MPs age, they often release plastic additives and organic compounds (e.g., depolymerization byproducts) (Yuan et al., 2022). In one study, the release of these compounds by UV-aged PS-MPs induced intracellular ROS generation, increased cell permeability, and upregulated HGT-associated genes in recipient bacteria (Yuan et al., 2022). In another study, MPs containing phthalates—a plasticizer—increased the abundance of ARGs in farmland soils relative to a no plastic control; MPs without phthalates decreased ARG abundance (Lu and Chen, 2022).

Bacterial cell membrane alterations may result not only from the indirect effect of oxidative stress with (nano)microplastic (N/MP) exposure (Cheng et al., 2022; Yu et al., 2023; Yuan et al., 2022), but also from direct interactions between N/MPs and bacterial membranes (Hu et al., 2022).

These direct interactions may be more relevant for plastics in the nanoscale size range (NPs), as plastics at this size range are able to cross biological membranes (Liu et al., 2021b). Results from Hu et al. (2022) suggest that plastic particles interact directly with bacterial cell membranes, with NPs able to bind to the lipid membrane through van der Waals forces and electrostatic interactions. A portion of NPs that interact with the cell membrane may become internalized by the cell. Hu et al. (2022) also observed size-dependent effects of N/MP exposure on bacterial HGT, oxidative stress, and the formation of nanopores within cell membranes. These effects were induced in bacteria exposed to NPs (75, 90, and 100 nm), but were absent with exposure to MPs (1 and 10 μm) (Hu et al., 2022). This contrasts findings from several other studies in which HGT and oxidative stress were increased in the presence of MPs around 200 μm (Cheng et al., 2022; Yu et al., 2023; Yuan et al., 2022). However, several studies support the impact of plastic particle size on interactions between plastics and bacteria, with smaller-sized particles more relevant to toxicity (Shi et al., 2020; Sun et al., 2018). Changes to bacterial community, ARG and mobile genetic element abundance, and ROS production were observed in landfill leachate samples in the presence of MPs (9–9.9 μm) and NPs (50–100 nm and 200–500 nm) (Shi et al., 2020). Although the production of intracellular ROS was increased in all samples exposed to N/MPs, the increase was only statistically significant with NP exposure. However, MP exposure resulted in the greatest increase in 16S rRNA gene copy numbers, indicating that MPs with larger surface areas might be more conducive to bacterial enrichment (and plastisphere formation) (Shi et al., 2020). Therefore, plastics of different sizes might be acting through different mechanisms to facilitate gene transfer. Particle concentration is also an important factor in plastic–bacteria interactions. Although bacterial transformation was enhanced or unchanged when NP concentrations were low, at higher concentrations, NPs interfered with and reduced the transfer of plasmids with low replication capacities (Hu et al., 2022). Yu et al. (2023) similarly observed an increase in conjugative transfer in bacteria exposed to MPs, which then decreased to levels similar to those for control treatments at higher MP concentrations.

7. Conclusions

MP–bioMC complexes pose a major threat to environmental and human health, with the potential to protect and transport genetic material (including silencing RNAs and ARGs). ARG persistence and transport in the environment, facilitated by MPs, threatens to increase the resistance of environmental (and potentially pathogenic) bacteria; and silencing RNAs have the potential to perturb essential native environmental microbiomes. Several recent lab-based studies have proposed that improved gene transfer in MP-exposed microbes may be the result of cell membrane damage—resulting from oxidative stress induced by MP exposure. The plastisphere also serves as a reservoir of genetic material, placing microbes in close contact with one another and with extracellular nucleic acids. As MPs and bioMCs co-occur in several environments (most notably in agricultural soils), there is a high potential for these two contaminants to interact.

Despite the efforts of recent studies to better characterize the soil plastisphere, there are still several knowledge gaps. More quantitative studies are needed to better elucidate the mechanisms of bioMC adsorption to MPs, and the subsequent fate and transport of MP–bioMC complexes in the terrestrial environment. The few current mechanistic studies are either lab-based or focused on aquatic ecosystems. In terrestrial systems, MP recruitment of bioMCs may be influenced by soil properties (e.g., soil pH, nutrients, temperature, and moisture) and MP properties (e.g., polymer type, size, shape, surface properties, and additives) (Wang et al., 2022)—factors which are not well-studied. MP–bioMC complex fate and transport is also influenced by MP weathering and aging, which can expose new functional groups and lead to biofilm formation. These effects are overlooked in shorter-term studies or those working with pristine MPs.

N/MPs are a diverse class of contaminants, spanning seven orders of magnitude in size. Most current studies focus on MPs, despite the fact that MPs and NPs likely have distinct behaviors and transport properties in the environment (Gigault et al., 2021; Sharma et al., 2022). NPs—smaller than microorganisms—may be less suitable for microbial colonization (Sharma et al., 2022), but may instead interact directly with microbial cell membranes to induce toxicity (Hu et al., 2022). A lack of reliable analytical methodologies for analyzing NPs in the environment has likely biased studies towards larger particles (Cai et al., 2021).

Current studies are also limited in bioMC absorbate type and source. ARGs are the focus of the current (limited) research related to MP–bioMC complexes—and for good reason, as these bioMCs present a major human health concern. However, other bioMCs, such as RNA silencing constructs, have the potential to disrupt critical ecological functions and should be considered in future research. The effects of MPs on bioMC persistence, transport, and transfer warrant further and more thorough investigation in soils. A better understanding of the mechanisms that drive the fate and transport of MP–bioMC complexes in the terrestrial environment will enable future solutions for bioMC risk mitigation.

CRediT authorship contribution statement

Lane Maguire was responsible for the following contributions: Conceptualization; Data curation; Formal analysis; Visualization; Draft writing, review & editing.

Courtney Gardner was responsible for the following contributions: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Visualization; Draft writing, review & editing.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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