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A comparative, multi-study analysis of plastisphere communities, plasmid dynamics, and antibiotic resistance genes

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Research Article

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Abstract

Microplastics are widespread in aquatic environments and support surface-associated microbial communities. Although antimicrobial resistance in the plastisphere has been reported, the organization of resistance genes across plasmid types and mobility categories on microplastic surfaces remains incompletely characterized. In this study, we analyzed published microplastic biofilm metagenomes to examine microbial community structure, plasmid replicon diversity, predicted mobility, and antimicrobial resistance genes (ARG) distributions across microplastics from various global locations. Phylum-level taxonomic profiles varied by geography, but most plastisphere communities were dominated by *Pseudomonadota*. Core microbiome analysis revealed several genera within the *Pseudomonadota* (*Paracoccus, Pseudomonas, Sphingomonas, Sulfitobacter, Vibrio, Mesorhizobium, Rhizobium, Bradyrhizobium, Roseovarius,* and *Hyphomonas*) as members of the plastisphere core. Plasmid reconstruction revealed differences in predicted mobility profiles, with conjugative plasmids frequently identified in the polyethylene, polypropylene, and polyvinyl chloride samples. A co-occurrence analysis of plasmid mobility, replicons, and ARG showed that conjugative plasmids connected a broader range of replicons to multiple ARG classes than mobilizable or non-mobilizable plasmids. IncFIB and IncFII replicons were prominent with high ARG co-occurrence. Additionally, several less-characterized rep_cluster replicons were detected across microplastic types, indicating diverse and understudied plasmid backbones within plastisphere communities, underscoring the importance of considering plasmid context when evaluating plastisphere resistomes.

Impact Statement

Microplastics are increasingly recognized as microbial habitats that can concentrate antibiotic resistance genes (ARGs) in aquatic environments. While many studies have documented the presence of ARGs within microplastic-associated biofilms, far less is known about the genomic context of these genes. This study advances the field by shifting the focus from simple ARG inventories to the plasmid architectures associated with these ARGs. By integrating metagenomic data from previously published studies spanning freshwater, estuarine and marine systems, we provide the first comparative, cross-system assessment of the plastisphere community, plasmid replicon diversity, mobility potential and ARG co-occurrence across different microplastic polymers. This plasmid-centric perspective reveals that ARGs in microplastic biofilms are often associated with conjugative plasmids, which can facilitate their horizontal transfer within these biofilms. Importantly, this work identifies microplastics as environments where clinically relevant ARGs are linked to plasmid architectures commonly observed in pathogenic bacteria. This work supports more mechanistic risk assessments of plastic pollution and informs One Health-oriented strategies to address antibiotic resistance across environmental, animal and human systems.

Introduction

Antibiotic resistance has emerged as a global health crisis, driven mainly by the extensive use of antibiotics in human medicine and livestock farming (Burow and Käsbohrer, 2017; Chaw et al., 2018; Aslam et al., 2021). Compounding this challenge, no new classes of antibiotics have been discovered in decades, leaving humans and animals vulnerable to infections that were once treatable (Durand et al., 2019; Miethke et al., 2021). It is currently estimated that antibiotic resistance is responsible for approximately 700,000 deaths annually, and this number is projected to rise to 10 million deaths per year by 2050 if no effective interventions are implemented (Tagliabue and Rappuoli, 2018). While much attention has been given to clinical and animal sources of antibiotic resistance, growing evidence indicates that natural environments, such as

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aquatic systems, are significant contributors to the spread and persistence of antibiotic resistance. Aquatic ecosystems have become reservoirs of antibiotic-resistant bacteria (ARB) and their associated antibiotic resistance genes (ARGs), enriched by the influx of untreated or partially treated waste, such as municipal wastewater, pharmaceutical effluents and agricultural runoff (Ferreira da Silva et al., 2006; Ouyang et al., 2015). These contaminants introduce antibiotics and resistant microbes into water bodies, creating hotspots where resistance can evolve and spread (Baquero et al., 2008; Ma et al., 2015).

Alongside these chemical and microbial pollutants, plastic waste accumulation in aquatic environments raises additional concerns. Plastics, produced in large quantities for industrial and consumer use, are highly durable and resistant to degradation (Fayshal, 2024). Over time, larger plastic debris breaks down into smaller fragments known as microplastics, which are defined as plastic particles less than 5 mm in size (Amaral-Zettler et al., 2015; Bajt, 2021). These environmental contaminants have become ubiquitous in marine, freshwater and terrestrial environments. Due to their small size, large surface area, chemical stability and hydrophobicity, microplastics persist and often co-occur with ARBs and ARGs in aquatic environments, acting as substrates for microbial colonization (Reisser et al., 2014; Gong et al., 2019). This colonization leads to the formation of biofilm-associated microbial communities, often referred to as the "plastisphere" (Zettler et al., 2013; Wu et al., 2019). The plastisphere is composed of diverse microbial communities, including opportunistic pathogens such as *Vibrio spp*., *Aeromonas spp., Acinetobacter spp*. and *Pseudomonas spp*., all of which have been identified on microplastics in both marine and freshwater systems (McCormick et al., 2014; Kirstein et al., 2016; Oberbeckmann et al., 2016).

These colonized microplastics not only provide a habitat for microbial proliferation but also facilitate horizontal gene transfer (HGT), a process by which bacteria exchange ARGs, often mediated by plasmids, integrons and transposons (Luo et al., 2023). Thus, the plastisphere can act as a hotspot for ARG exchange. Moreover, microplastics can travel long distances across water bodies, contributing to the dissemination of ARB and ARGs across ecosystems (Horton and Dixon, 2018). These particles can then be ingested and bioaccumulated by aquatic organisms across various trophic levels (Miller et al., 2020). Through such pathways, they may ultimately reach humans *via* contaminated seafood or drinking water, a concern supported by evidence of microplastics detected in human feces (Yan et al., 2022). Thus, highlighting their emergence as a growing public health concern.

Metagenomic studies investigating plastisphere-associated microbial communities have revealed the complexity of resistome profiles. However, comparative, multi-study analyses remain limited, especially those that integrate plasmid dynamics, ARG profiles and taxonomic diversity across different aquatic environments. To address this gap, we collected published metagenomic datasets generated from across different geographical locations and aquatic systems. These datasets were analyzed using a standardized bioinformatics pipeline to identify key microbial taxa within the plastisphere, assess ARG diversity and characterize associated plasmid types.

Methods

Data curation

In August 2024, we performed a literature search in Google Scholar using the key terms "microplastics," "biofilm," "metagenome," and "aquatic" to identify metagenomic datasets generated from microplastic biofilms (Supplementary Table S1). Specifically, we focused on metagenomes from microplastics collected from natural bodies of water (Bryant et al., 2016; Delacuvellerie et al., 2022; Di Cesare et al., 2024). Thus, we excluded engineered or built environment systems (*e.g*., wastewater treatment plants). However, to expand the number of included studies, we retained studies that set up bioreactor incubation experiments using microplastics with water from a natural system (Wu et al., 2022) or *in situ* incubations of microplastics in natural bodies of water (Bhagwat et al., 2021; Oberbeckmann et al., 2021). To ensure data relevance, each dataset was manually reviewed to confirm that the sequencing reads represented untargeted metagenomes and matched the metadata descriptions. Studies were excluded if the study metadata did not match the metadata in the sequence read archive (SRA), if the metagenomes were not generated from microplastic biofilms, or if the sequenced reads were not publicly available. Additionally, we excluded amplicon sequencing-based datasets. Only datasets meeting these criteria were retained for subsequent analyses.

Bioinformatic analyses

The summarized bioinformatics workflow used in this study is shown in Figure 1.

Read quality control and taxonomy: To ensure comparability across studies, all raw metagenomic reads were imported and analyzed using a standardized pipeline on the Department of Energy (DOE) KnowledgeBase (KBase) platform (Arkin et al., 2018) with default parameters, unless otherwise specified, similar to approaches used in other integrative metagenomic studies relying on data mining from previous published studies (Yi et al., 2022; Subirats et al., 2023; Kang et al., 2024; Zhu et al., 2025). All publicly available raw sequence data from the selected studies were imported from the SRA using the Import SRA File as Reads from Web tool with the SRA identifiers compiled in our literature search. Initial quality assessments of the raw sequence reads were performed using FastQC (v0.12.1) (Andrew S. Andrew, 2010) to detect low-quality bases and identify adapter sequences. Adapter sequences were first removed using Cutadapt (v4.4) (Martin, 2011), after which reads were further trimmed, if needed, using Trimmomatic (v0.36) (Bolger et al., 2014) before the quality of the trimmed outputs was reassessed using Fast QC (v0.12.1).

To identify the core plastisphere microbiome, genus-level taxonomic classifications were obtained using Kaiju (v1.9.0) (Menzel et al., 2016) against the RefSeq Genomes (no Euks) database. Reads classified as "unclassified," "viral," or "unassignable" to a bacterial genus were excluded from downstream analysis. To ensure comparability across samples, relative abundances were calculated as the proportion of reads assigned to each genus relative to the total number of classified bacterial reads per sample. A genus was considered present in a sample if its relative abundance exceeded 0.1%, a threshold applied to minimize the contribution of sequencing noise and low-abundance classifications. Prevalence was calculated for each genus as the proportion of samples in which it was detected above this threshold. Genera were classified into a strict core (prevalence ≥90%), a dynamic core (prevalence 50–90%) or a non-core (<50%), following a previously described framework (Risely, 2020).

Metagenome assembly, binning and annotation: We used metaSPAdes (v3.15.3) (Nurk et al., 2017) to assemble the trimmed reads to generate contig sets. These contigs were binned using MaxBin2 (v2.2.4) (Wu et al., 2014) to produce binned contig objects. In a mock community, metaSPAdes in combination with

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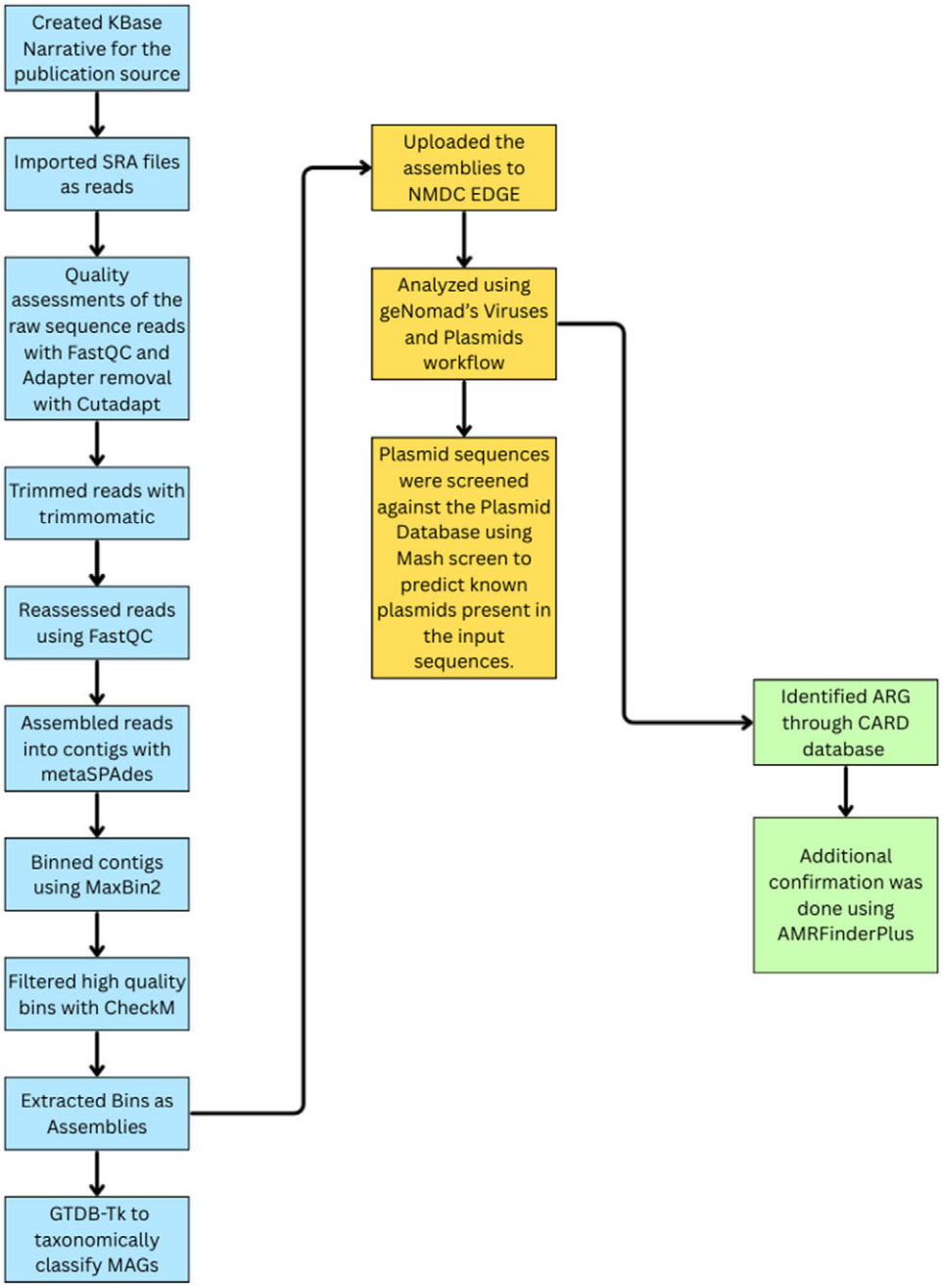


Figure 1. Workflow of metagenomic analysis for ARG and plasmid identification.

MaxBin2 was previously found to assemble and bin >60% of plasmids (with >50% coverage) (Maguire et al., 2020). As binning tools rely on GC content and coverage to link contigs together (and plasmids often diverge from their host genome in both these metrics), plasmid reconstruction from metagenomic assemblies remains inherently constrained, as plasmids frequently undergo recombination and share conserved backbones while differing in accessory structures. This complicates de Bruijn graph-based assemblers, which can fragment sequences that are enriched in repeats or exhibit high microdiversity (Rodríguez-Beltrán et al., 2015; Piera Líndez et al., 2026). As a result, small or low-abundance plasmids may be underrepresented in the reconstructed dataset. We relied on other tools to infer the host phylogeny of the plasmids recovered from bins, discussed later. Medium- and high-quality bins were then filtered using CheckM (v1.0.18) (Parks et al., 2015) with thresholds of ≥50% completeness and ≤ 10% contamination. Medium and high-quality bins were further processed to extract individual assemblies using the "Extract Bins as Assemblies" tool in KBase. The resulting assemblies were combined into a single file per study to streamline downstream analyses. Metagenomic reads from each sequencing library were mapped to the assembled MAGs using Bowtie2 (v2.3.2) (Langmead and Salzberg, 2012), and the abundance of each MAG was calculated as the proportion of reads mapping to the MAG relative to the total number of reads in the corresponding library. Taxonomic classification of the MAGs was performed using GTDB-Tk (v2.3.2) (Chaumeil et al., 2019), which

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assigns taxonomy based on the Genome Taxonomy Database. Phylum (or genus)-level relative abundances were obtained by summing the abundances of all MAGs assigned to each phylum (or genus) and expressing the resulting values as percentages of the total microbial community.

ARGs associated with the *Vibrio* MAGs were identified using the Resistance Gene Identifier (RGI, v6.0.5) tool against the Comprehensive Antibiotic Resistance Database (CARD v4.0.1) (Alcock et al., 2023) using the settings: *Select Criteria: Perfect and Strict hits only; ≥ 95% identity; Loose hits to Strict: Exclude nudge; and Sequence Quality: Low quality/coverage*. VFanalyzer was used to screen for *Vibrio*-specific virulence factors against the Virulence Factor Database (VFDB) (Zhou et al., 2025) (Liu et al., 2019; Zhou et al., 2025).

Plasmid identification and analysis: The combined sequence files were then uploaded into the DOE National Microbiome Data Collaborative (NMDC)-developed Empowering the Development of Genomics Expertise (EDGE) platform (Kelliher et al., 2024) and analyzed using the Viruses and Plasmids workflow, which utilizes geNomad (v4.1.0) for mobile genetic element identification (Camargo et al., 2024). This workflow enabled the identification and characterization of plasmid-associated sequences within the MAGs. Plasmid host inference was subsequently performed by linking plasmid-containing contigs to the MAGs from which they were recovered, followed by the use of the GTDB-Tk-derived taxonomy of each corresponding MAG to infer the most likely bacterial host. Plasmid-associated ARGs were identified using the Resistance Gene Identifier (RGI, v6.0.5) tool against the Comprehensive Antibiotic Resistance Database (CARD v4.0.1) (Alcock et al., 2023). Consistent with previously published metagenomic studies employing CARD and RGI (Jankowski et al., 2022; Ortiz-Severín et al., 2025), perfect, strict and loose hits were retained, while nudge adjustments converting ≥95% identity loose hits to strict were disabled. Sequence quality was set to *High quality/coverage*, which includes complete genomes, plasmids or high-quality assemblies (contigs >20,000 bp) and excludes predictions of partial genes. Additional confirmation was done using AMRFinderPlus (v2024-01-3.1.1) (Feldgarden et al., 2021) using default parameters (minimum reference protein coverage threshold of 0.5 and minimum identity threshold of -1), which applies database-specific cut-offs when available and a default amino acid identity threshold of 0.9 otherwise.

Plasmid sequences obtained from geNomad were further screened against the Plasmid Database (PLSDB, v2024) using Mash screen with parameters (max. p-value = 0.1, min. identity = 0.80) to predict known plasmids present in the input sequences (Molano et al., 2025).

Statistical analyses

All statistical analyses were performed using R software v4.3.0. The alpha diversity of microbial communities (using the read-based genus assignments) was computed using the *vegan* (v2.7-2) package (Oksanen et al., 2007) in RStudio. Statistical differences in means were assessed using the Kruskal–Wallis test. To assess the effects of polymer type and geographic effects on plastisphere composition, a distance-based redundancy analysis (dbRDA) was performed using the *vegan* (v2.7-2) package based on the Bray-Curtis dissimilarity test. The read-based genus assignments were used for this analysis. Polymer type and sampling location were included as explanatory variables, and significance was assessed using permutation-based ANOVA with 999 permutations (considered significant at $p < 0.05$). Also, figures, including stacked bar charts, heatmaps and network visualizations, were generated using *ggplot2* (v4.0.1) (Wickham, 2011), *pheatmap* (v1.0.13) (Kolde and Kolde, 2015), *networkD3* (v0.4.1) (Allaire et al., 2017) and supporting R packages including *dplyr* (v1.1.4), *tidyr* (v1.3.1), *RColorBrewer* (v1.1-3) and *viridis* (v0.6.5).

Results

Microplastic-associated bacterial communities

We compiled metagenomic datasets from six independent studies investigating microplastic-associated microbial communities. Collectively, the datasets encompassed samples from marine (n = 2 studies), estuarine/coastal (n = 3 studies) and freshwater (n = 1 study) environments. Geographically, the studies spanned three continents, with datasets originating from Europe (n = 3 studies: Baltic Sea/Warnow Estuary, Tyrrhenian Sea, Mediterranean Sea), Asia (n = 1 study: Haihe River, China), Australia (n = 1 study: Lake Macquarie Estuary, Australia) and the open ocean of the North Pacific Gyre (n = 1 study) (Figure 2). In total, the combined dataset included 50 metagenomic read libraries (Supplementary Table S1). Polymer types associated with the microplastic biofilms were determined using metadata for the sequencing libraries retrieved from the SRA database. Each sequencing library was categorized according to the reported polymer type of the microplastic sample from which it originated, as described in the original studies (Supplementary Table S1). In total, six distinct polymer types were identified across all samples, with polypropylene (PP) and polyethylene (PE) being the most reported, both representing 16.0% of the total (n = 8/50 metagenomic read libraries each). This was followed by polystyrene (PS) and polyvinyl chloride (PVC), accounting for 14.0% (n = 7/50) and 12.0% (n = 6/50), respectively. The full distribution of microplastic types across the datasets is shown in Figure 3A.

Alpha diversity of bacterial communities across polymer types was quantified using the Shannon diversity index based on genus-level profiles derived from metagenomic reads (Supplementary Figure S1). Differences among plastics were then evaluated using a Kruskal–Wallis test. No significant differences in bacterial diversity were observed among polymer types (Kruskal–Wallis $\chi^2 = 7.29$, $p = 0.295$). Additionally, a dbRDA analysis was performed on the genus-level community profiles to assess the influence of both location (*i.e.*, country of origin or the North Pacific Gyre) and polymer type on community structure. Polymer categories exclusive to a single sampling location (polycaprolactone [PCL] and polylactic acid [PLA]) as well as mixed polymer samples were excluded from this analysis. Sampling location was a significant driver of community structure ($F = 13.49$, $p = 0.001$), whereas polymer type was not ($F = 0.65$, $p = 0.947$), indicating that local aquatic communities exert a stronger influence on plastic colonization than polymer chemistry. The metagenomic reads were then binned and assembled to enable a more sensitive analysis of community composition and plasmid analysis. A total of 1,188 MAGs were binned. Of these, 341 were classified as medium quality (≥ 50% completeness and ≤ 10% contamination), and 105 of those met the criteria for high-quality MAGs (≥ 90% completeness and ≤ 5% contamination) (Supplementary Table S2). Medium- and high-quality MAGs were retained for further analyses.

The MAG-based taxonomic profiles of microbial communities revealed notable compositional differences among samples from different locations (Figure 3B), consistent with our dbRDA analysis

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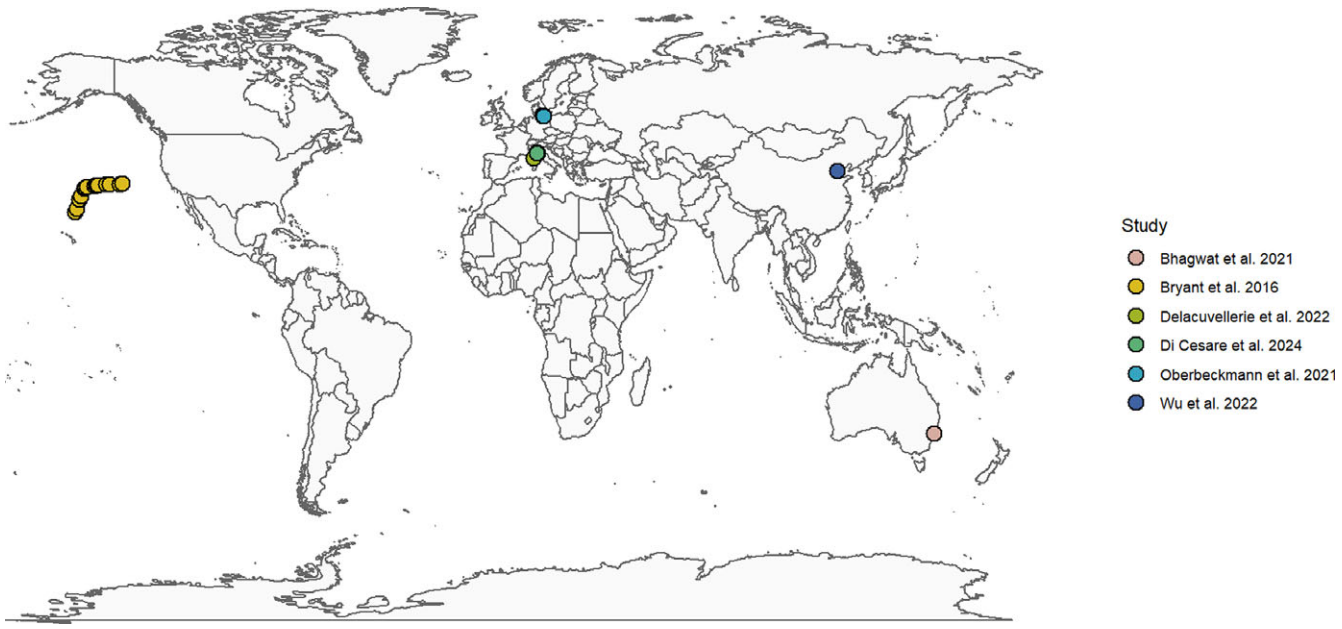


Figure 2. Map showing the geographical distribution of the metagenomic datasets included in this analysis. Sampling sites spanned Europe (Baltic Sea, Tyrrhenian Sea, Mediterranean Sea), Asia (Haihe River, China), Australia (Lake Macquarie Estuary) and North Pacific Gyre.

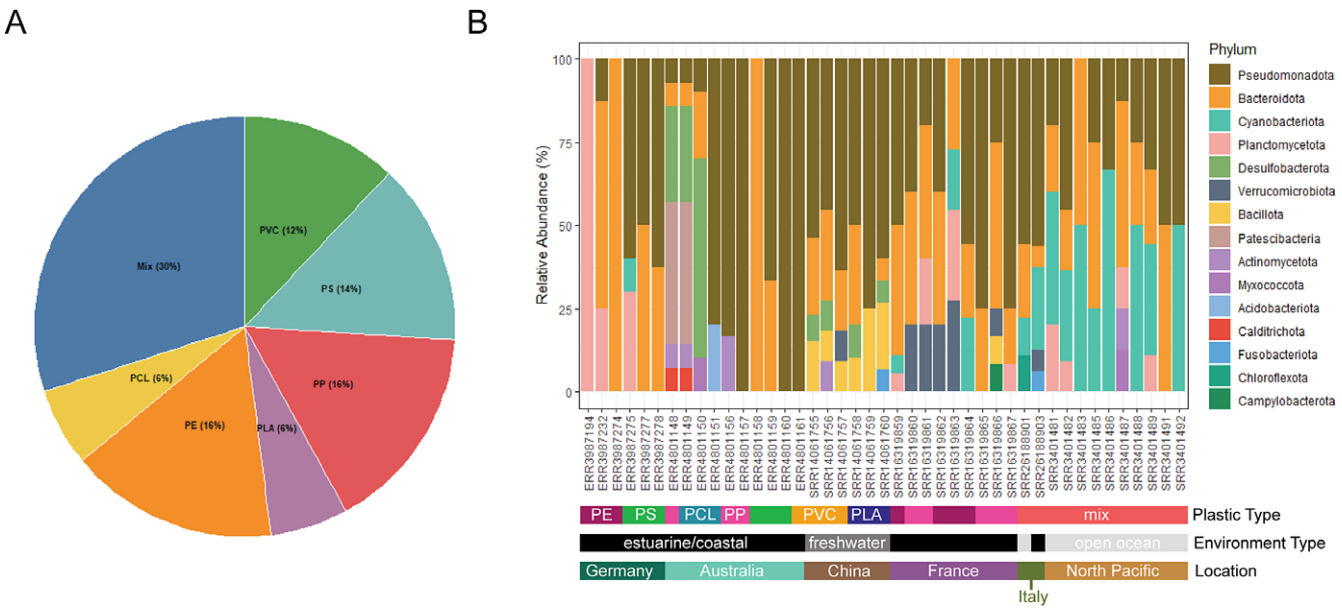


Figure 3. (A) Distribution of microplastic types in the metagenomic dataset. Mix = a mixture of different plastic types, PVC = polyvinyl chloride, PS = polystyrene, PP = polypropylene, PLA = polylactic acid, PE = polyethylene, PCL = polycaprolactone. (B) Relative MAG-based abundance and phylum-level taxonomic composition of microbial communities across the samples. These relative abundances represent the proportion of reads mapped to the MAGs assigned to each bacterial phylum within the microbial communities from each sample. Only the samples where MAGs were recovered are shown. At the bottom are overlaid sample metadata.

of the metagenomic reads. However, with few exceptions, the phylum *Pseudomonadota* dominated the communities, including previously described species such as *Pseudoalteromonas atlantica, Pseudoalteromonas shioyasakiensis, Vibrio lentus, Vibrio coralliirubri, Azospirillum cavernae, Cochlodiniinecator piscidefendens, Sphingorhabdus lacus, Azonexus hydrophilus* and *Cobetia amphilecti* as well as novel species of genera such as *Erythrobacter, Allorhizobium, Henriciella, Tolumonas, Ketobacter, Gemmobacter, Yoonia* and *Tateyamaria* (Supplementary Table S3; Figure S2). *Bacteroidota* MAGs were also common across most of the samples, including novel representatives of genera such as *Tunicamonas, Muricauda, Lewinella, Flavobacterium* and *Croceitalea* (Supplementary Table S3). Several genera identified among the MAGs are known to harbor pathogenic or opportunistic bacteria associated with both marine organisms and humans (Supplementary Table S3; Figure S2). Across the MAG dataset,

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Vibrio (Baker-Austin et al., 2018; Marques et al., 2022) and *Pseudoalteromonas* (Pujalte et al., 2007) were among the most abundant genera detected across multiple polymer types (Supplementary Figure S2). In addition, MAGs assigned to several other genera associated with infectious disease in human or aquatic animal hosts were identified at lower abundance, including *Tenacibaculum* (Mabrok et al., 2022), *Psychromonas* (Wei et al., 2024), *Flavobacterium* (Schiff et al., 1961; Loch and Faisal, 2015; Zurbuchen et al., 2023) and *Shewanella* (Paździor, 2016; Yu et al., 2022) (Supplementary Table S3).

Core plastisphere microbiome

We next asked whether a "core" plastic-associated microbiome is shared across polymers from different locations. Despite its higher taxonomic accuracy and resolution, the genome-resolved analyses can miss low-abundance community members. To enable a presence–absence-based core microbiome analysis, we therefore relied on the unassembled metagenomic reads. At the genus level, a strict core of 12 genera (≥90% prevalence; 12/2,211 genera detected; 0.54%) was identified (Figure 4). Of these, five genera of the *Pseudomonadota* – *Paracoccus, Pseudomonas, Sphingomonas, Sulfitobacter* and *Vibrio* – were present in all samples, with *Vibrio* among the most abundant core members. Our genome-resolved analyses further supported a prominent role for *Vibrio*, with the recovery of MAGs representing three *Vibrio coralliirubri* strains (Bin.001.fasta_SRR26188903, Bin.002.fasta_SRR26188901 and Bin.004.fasta_SRR26188901); *Vibrio lentus* (Bin.001.fasta_SRR16319865); and two novel *Vibrio* species (Bin.004.fasta_SRR16319867 and Bin.014.fasta_SRR16319867). *V. lentus* (Farto et al., 2003; Schuh et al. 2019) and *V. coralliirubri* (Dinçtürk et al., 2023) have previously documented associations with disease in marine organisms. Consistent with this pathogenic potential, these MAGs encoded multiple virulence-associated traits (Supplementary Table S4), including factors for attachment and biofilm formation (mannose-sensitive hemagglutinin in Bin.001.fasta_SRR16319865, Bin.002.fasta_SRR2618890 and Bin.004.fasta_SRR26188901; autoinducer-2 in Bin.001.fasta_SRR26188903, Bin.002.fasta_SRR26188901, Bin.004.fasta_SRR16319867 and Bin.004.fasta_SRR26188901); secretion systems (T2SS in all MAGs, T6SS in Bin.004.fasta_SRR16319867); and toxins (RTX toxin in Bin.001.fasta_SRR16319865 and thermolabile hemolysin in Bin.001.fasta_SRR16319865, Bin.004.fasta_SRR26188901, Bin.014.fasta_SRR16319867) (Lin et al., 1999; Watnick et al., 1999; Ali and Benitez, 2009; Zhang et al., 2023). Notably, several of these *Vibrio* MAGs (Bin.001.fasta_SRR26188903, Bin.004.fasta_SRR26188901, Bin.001.fasta_SRR16319865) also encoded the quinolone resistance determinant *qnrS2*, a gene commonly associated with mobile genetic elements in *Vibrio* (Cattoir et al., 2007; Xu et al., 2023) (Supplementary Table S5).

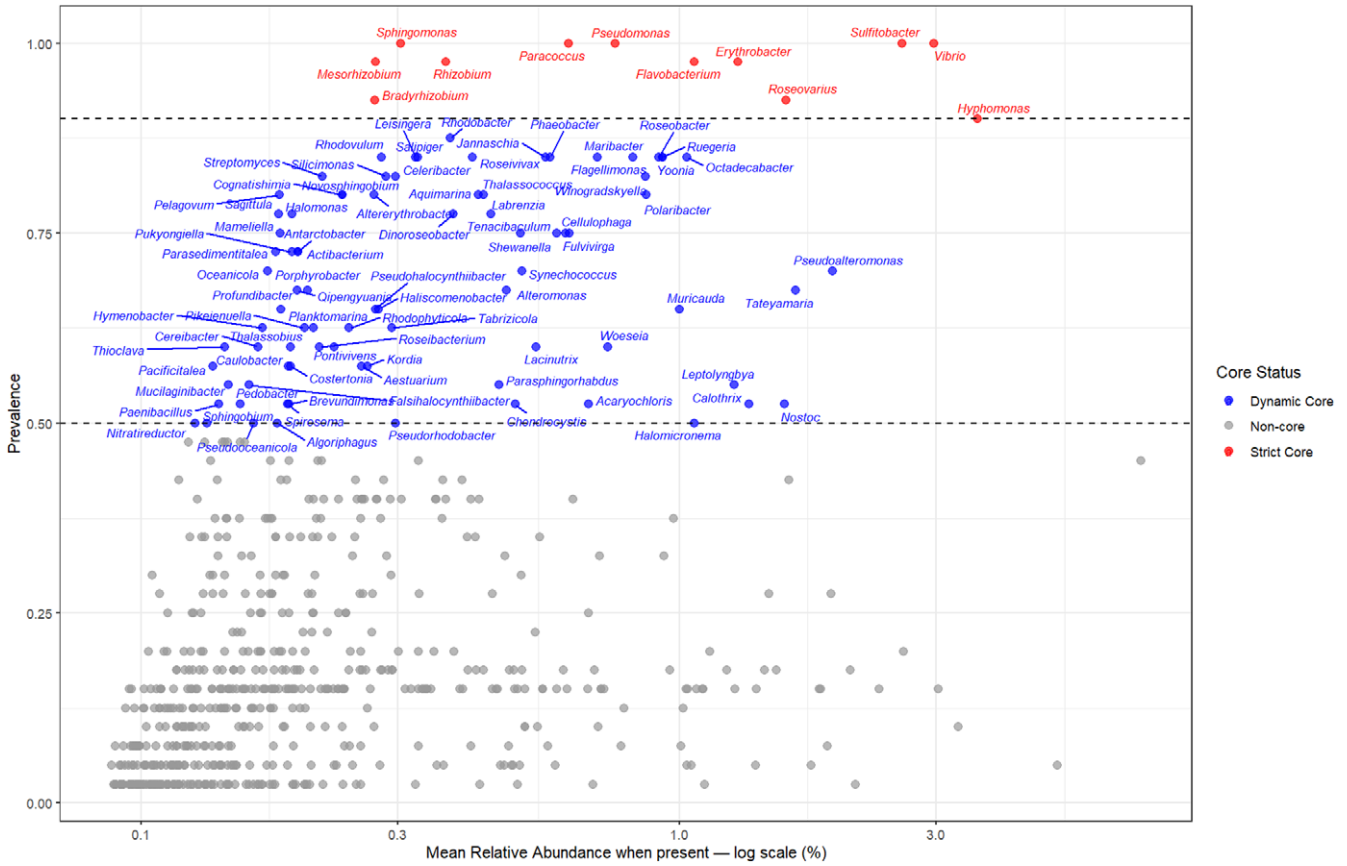


Figure 4. Core plastisphere microbiome identified across all samples using the unassembled metagenomic reads. Scatter plot showing the prevalence against the mean relative abundance of all bacterial genera detected across the plastisphere dataset. Each point represents one genus (n = 2,211). Prevalence was calculated as the proportion of samples in which a genus was detected above a relative abundance threshold of 0.1%. Mean relative abundance was calculated exclusively across samples in which each genus was detected above this threshold. Dashed horizontal lines indicate the thresholds used to define the strict core (≥90% prevalence) and dynamic core (≥50% prevalence). Genera are colored according to core status: strict core (red; n = 12), dynamic core (blue; n = 83) and non-core (gray; n = 2,116). Genus names are shown for all strict and dynamic core members.

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The remaining members of the strict core microbiome were *Mesorhizobium, Rhizobium, Bradyrhizobium, Flavobacterium, Roseovarius* and *Hyphomonas*. *Flavobacterium* is a common fish pathogen that has previously been reported to cause opportunistic infection in humans (Loch and Faisal, 2015; Zurbuchen et al., 2023). All the *Flavobacterium* MAGs represented novel species (Bin.022.fasta_SRR14061755, Bin.022.fasta_SRR14061756, Bin.025.fasta_SRR14061756, Bin.041.fasta_SRR14061758, Bin.046.fasta_SRR14061758, Bin.052.fasta_SRR14061755). Also, at a ≥ 50% dynamic core threshold, 83 additional genera were identified (n = 83/2,211; 3.75%). These include potentially pathogenic genera such as *Shewanella* (Yu et al., 2022), *Pseudoalteromonas* (Pujalte et al., 2007) and *Tenacibaculum* (Mabrok et al., 2022). The remaining 2,116 genera (n = 2,116/2,211; 95.7%) were classified as non-core, as they were detected in fewer than 50% of samples.

Distribution of predicted plasmid mobility across microplastic types

To further assess the potential for plasmid-mediated dissemination of ARGs within microplastic biofilms, we examined plasmids predicted from the binned contigs. An analysis of the predicted mobility of these plasmids revealed varied patterns across the six microplastic types (Figure 5; Supplementary Table S6). A total of 871 replicons were predicted in the plasmid sequences from the geNomad predictions, with varying numbers across the six studies (Supplementary Table S7). Overall, conjugative plasmids, which have a complete set of genes required for self-mediated cell-to-cell transfer, were the most commonly recovered plasmid type in most microplastic-associated communities, accounting for over half of the plasmid content in PE (63%, n = 206/325), PVC (55%, n = 117/211) and PP (53%, n = 55/103). Mobilizable plasmids, lacking a full conjugation system but that can be transferred in the presence of co-resident conjugative elements, were frequently observed on PS, accounting for 72% (n = 57/79) of the recovered plasmids. PLA-associated communities harbored the highest fraction of non-mobilizable plasmids, which lack mobility and transmission genes (65%, n = 73/113), with notable numbers also recovered from PP (40%, n = 41/103) and PE (36%, n = 117/325).

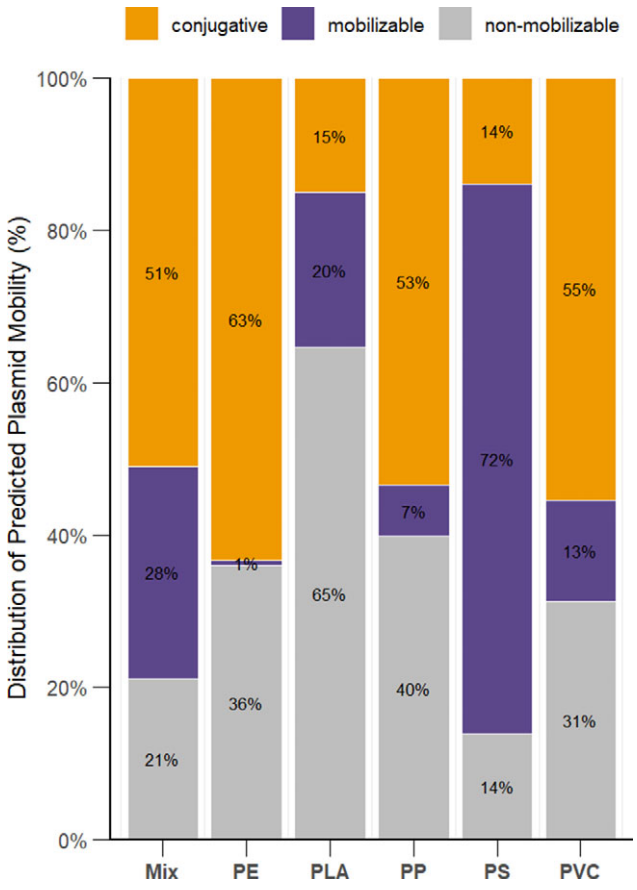


Figure 5. Distribution of predicted plasmid mobility types across different plastic types. Plasmid contigs were identified from MAGs using the NMDC viruses and plasmid workflow, and mobility classification was performed using the Plasmid Database (PLSDB). Each bar represents the relative proportion of predicted plasmids categorized as conjugative (orange), mobilizable (purple), or non-mobilizable (gray) for each plastic type. Percentages are based on the total number of plasmids identified per plastic type.

Co-occurrence of antibiotic resistance genes, plasmid replicons and mobility types

A total of 284 ARGs were identified among the predicted plasmids (Supplementary Table S8). These ARGs were classified into 24 different antibiotic resistance classes (Figure 6A, Supplementary Table S9). The classes with the highest number of ARGs were beta-lactam (n = 82) and aminoglycoside (n = 56) resistance genes, followed by fluoroquinolone (n = 21) and tetracycline (n = 19) resistance genes (Figure 6A). Additional ARG classes, including phenicol and macrolide resistance genes, were also detected but at lower frequencies (Supplementary Table S9). Analysis of plasmids and ARGs co-occurrence revealed a diverse distribution of resistance determinants across different plasmid replicons (Figure 6B, Supplementary Table S10). Among the detected plasmids, members of the IncF family were the most observed, co-occurring with genes conferring resistance to multiple antibiotic classes, such as aminoglycosides and beta-lactams.

Figure 7 illustrates the connectivity between plasmid mobility categories, the top 25 most observed plasmid replicons, and their associated antimicrobial drug classes. Most ARGs were observed in contigs containing conjugative plasmid markers (153 ARGs across 46 plasmids), followed by non-mobilizable (135 ARGs across 26 plasmids) and mobilizable plasmids (15 ARGs across 10 plasmids), reflecting their differing capacities for HGT. Within the replicon layer, the well-characterized incompatibility replicons of the Inc. plasmid family (IncFIB, IncFII, IncC, IncFIC, IncFIA) dominated the dataset. In addition, less-characterized replicons, the rep_cluster groups (*e.g.*, rep_cluster_1068, rep_cluster_1418, rep_cluster_2183), were also frequently observed. Across drug classes, beta-lactams, macrolides, aminoglycosides, fluoroquinolones and tetracyclines were the most widely represented and associated with the Inc replicon types.

The analysis showing the relationships between bacterial phyla, plasmid replicon types and antimicrobial resistance classes (Figure 8) revealed that *Pseudomonadota* contributed the largest proportion of plasmid-borne AMR genes, followed by *Bacteroidota*, *Actinomycetota*, *Bacillota*, *Desulfobacterota* and *Cyanobacteriota*. Among plasmid types, IncF and rep-cluster replicons were the most frequently associated with AMR genes. Beta-lactam, aminoglycoside and tetracycline resistance were the most dominant AMR classes, largely carried on plasmids from *Pseudomonadota* and *Bacteroidota*.

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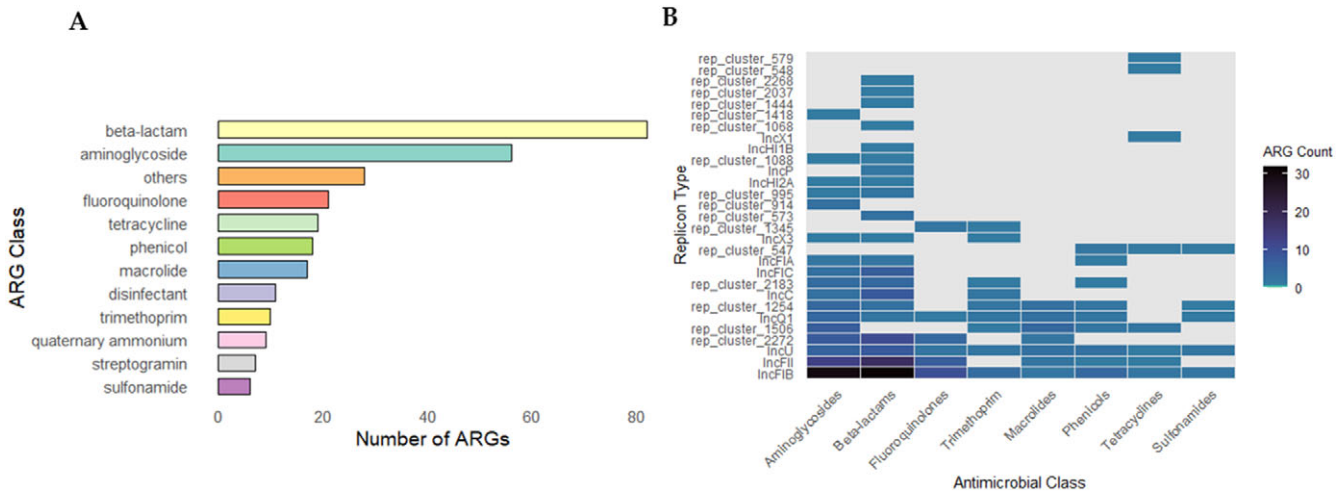


Figure 6. (A) Distribution of ARG classes detected in the predicted plasmids. Bars represent the number of ARGs assigned to each drug class based on identification with the RGI tool on the CARD database and AMRFinderPlus. ARG classes with fewer than six genes are not shown. The complete list of ARGs is provided in Supplementary Table S9. (B) Heatmap showing the number of plasmid replicon types associated with different antimicrobial classes. The rows represent the plasmid replicons identified using PLSDB, while the columns represent the most frequently observed ARG classes (those with ≥5 ARGs detected across plasmid sequences). Color intensity reflects the count of plasmid-ARG associations, with darker shades indicating higher co-occurrence. Counts for each co-occurrence are listed in Supplementary Table S10.

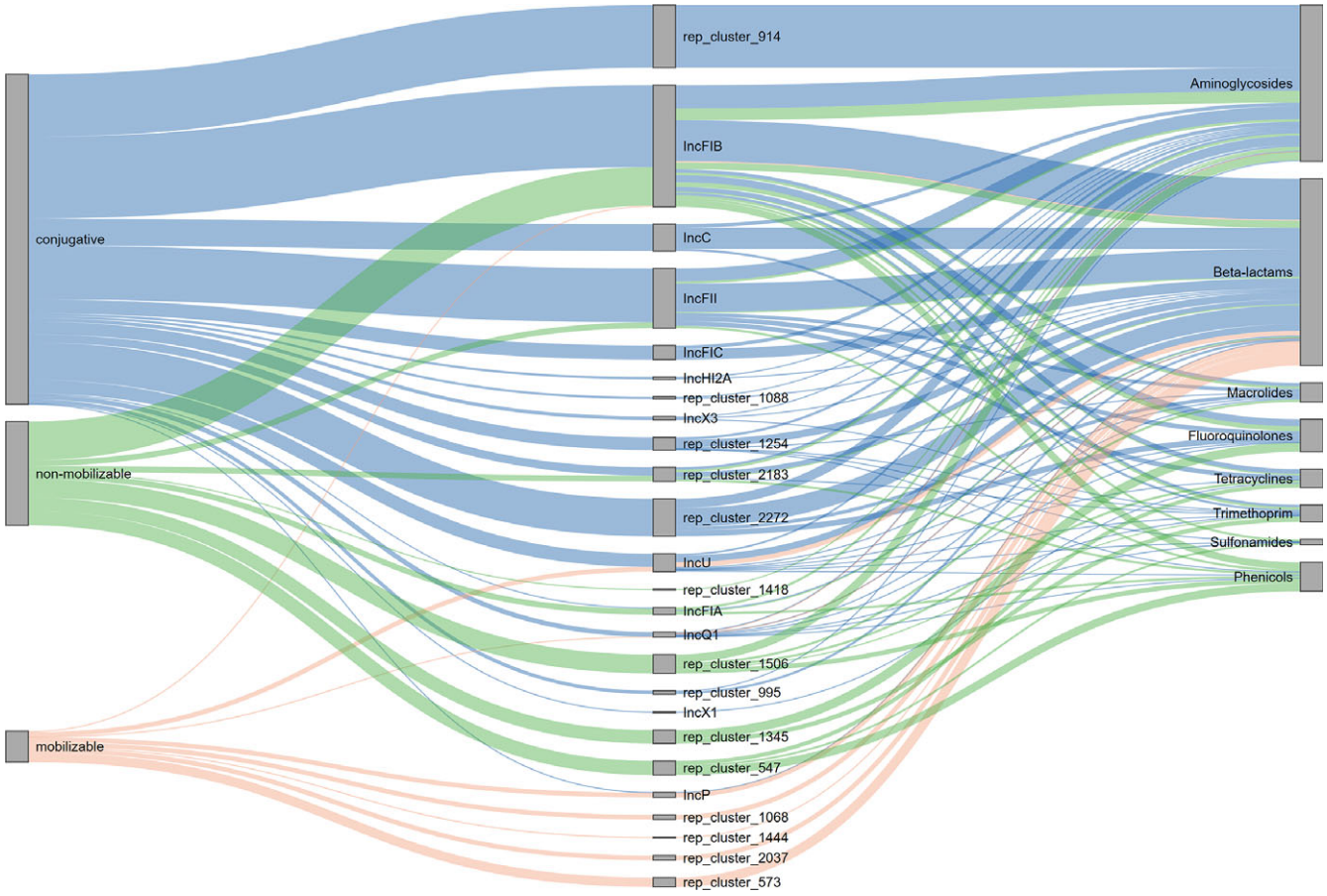


Figure 7. Sankey diagram illustrating the connections between plasmid replicon types, their predicted mobility classifications and their associated ARG classes. The diagram links (left) plasmid mobility types, (center) the top 25 most common plasmid replicons within the dataset and (right) ARG classes co-occurring with the plasmids. Mobility groups are categorized as conjugative, mobilizable or non-mobilizable, and the ribbon colors reflect the mobility classification of the plasmid, tracing the flow from mobility type through replicon to ARG class. The most frequently observed ARG classes (those with ≥5 ARGs detected across plasmid sequences) are displayed.

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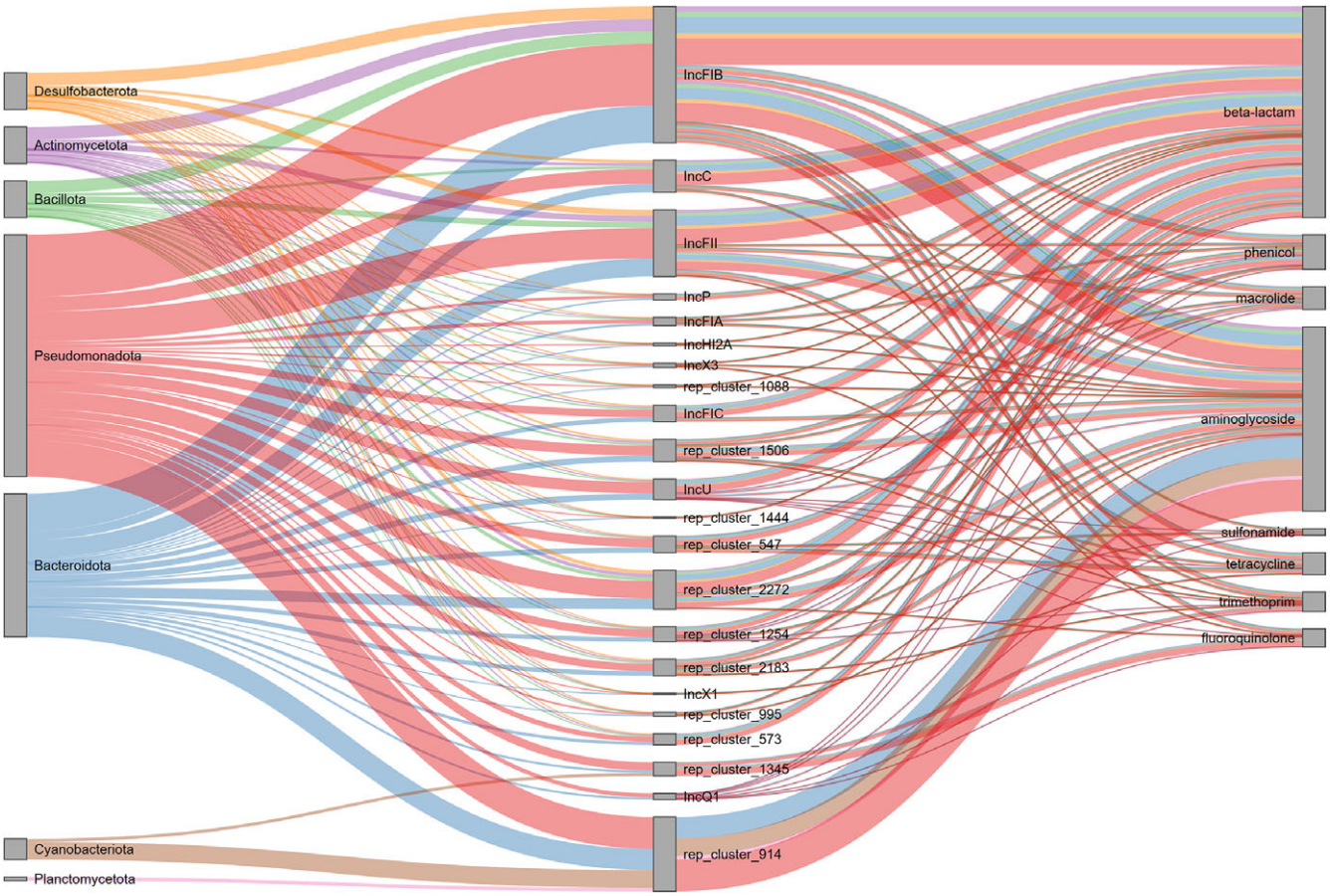


Figure 8. Sankey diagram showing associations among bacterial phyla, plasmid replicon types and antimicrobial resistance drug classes. The Sankey diagram illustrates the relationships between bacterial phyla, the top 25 most common plasmid replicon types and the most frequently observed antimicrobial resistance classes (those with ≥5 ARGs detected across plasmid sequences). The flow of the ribbon proceeds from the bacterial phyla (left nodes) to plasmid replicon types (middle nodes) and to the AMR drug classes (right nodes).

Discussion

The composition of microplastics analyzed in this study (Figure 3A) reflects the diverse range of plastic polymers commonly detected in aquatic environments, with PP as the most frequently reported. This is consistent with previous findings that have identified PP, PS and PE as the most abundant plastic types in aquatic environments (Eriksen et al., 2013; Matsuguma et al., 2017; Di and Wang, 2018) due to their widespread use in consumer packaging, cosmetics and care products (Fendall and Sewell, 2009). Our analyses indicate that microplastic microbial communities comprise both a shared core present across many polymer types and polymer-specific, non-core microbiomes that likely reflect the surrounding aquatic community. There is uncertainty in the literature regarding the extent to which polymer type and geography control the assembly of plastisphere microbial communities (Jacquin et al., 2019; Bhagwat et al., 2021; Miao et al., 2023). While certain studies report distinct community profiles for specific plastics (McCormick et al., 2016; Mughini-Gras et al., 2021), others find that communities frequently converge despite variations in polymer properties (Delacuvellerie et al., 2022; Sérvulo et al., 2023; Wallbank et al., 2025). This inconsistency may reflect the smaller sample sizes and narrower geographic scope of prior studies, thus highlighting the strength of our larger-scale integrative analysis, which found geography to be the major driver of beta diversity between samples.

Many genera within the *Pseudomonadata* were observed in both the strict and dynamic core microbiomes (Figures 3B, 4), albeit with varying relative abundances. This aligns with multiple prior studies reporting that *Pseudomonadota* are among the most frequently recovered phyla from microplastic surfaces (Wu et al., 2019; Wang et al., 2020; Witsø et al., 2025). A key factor contributing to their dominance is the ability of many *Pseudomonadota* taxa to produce large quantities of extracellular polymeric substances and surface-adhesive structures, which enhance initial attachment and biofilm maturation on hydrophobic substrates. These traits likely provide a competitive advantage on plastic surfaces, enabling *Pseudomonadota* to establish and persist as core members of plastisphere communities (Tang, 2024). For example, several members of the strict core microbiome are well-documented biofilm-formers within the *Pseudomonadota*, including *Paracoccus* (Morinaga et al., 2020), *Pseudomonas* (Oliveira et al., 2021), *Sphingomonas* (Czieborowski et al., 2020), *Sulfitobacter* (Cui et al., 2024), *Rhizobium* (Fujishige et al., 2006), *Mesorhizobium* (Das et al., 2017), *Bradyrhizobium* (Bogino et al., 2015), *Hyphomonas* (Abraham, 2020), *Roseovarius* (Doghri et al., 2015) and *Vibrio* (Decho and Gutierrez, 2017). We speculate that this same selective filter may, incidentally, favor organisms with traits associated with colonization of mucosal surfaces, including certain pathogens.

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Across all samples, we also commonly observed members of the *Bacteroidota* (*e.g.*, the strict core member *Flavobacterium* and dynamic core members *Polaribacter, Winogradskyella, Cellulophaga, Maribacter, Muricauda, Aquimarina, Kordia* and *Tenacibaculum*) and *Cyanobacteriota* (*e.g.*, dynamic core members *Synechococcus, Nostoc* and *Leptolyngbya*) in the core and dynamic core microbiomes (Figure 4). The presence of *Bacteroidota*, known for their role in the degradation of organic matter, is consistent with prior observations of their enrichment in microplastic-associated biofilms, particularly in nutrient-rich or anthropogenically impacted waters (Ventura et al., 2024; Fortin et al., 2025). *Cyanobacteriota*, on the other hand, have been noted in plastisphere studies from both freshwater and marine systems, likely favored by the hydrophobic surfaces and light availability in surface waters (de Oliveira et al., 2020; Zhai et al., 2023).

The potential hazards associated with microplastics are threefold: (1) the inherent toxicity of the plastic particles, (2) the toxicity of adsorbed chemicals and (3) the colonization by pathogenic microorganisms (Auta et al., 2017; Noventa et al., 2021). In this study, microplastics hosted several taxa with known pathogenic potential in both aquatic organisms and humans, including *Vibrio, Pseudoalteromonas, Tenacibaculum, Psychromonas, Flavobacterium* and *Shewanella* (Farto et al., 2003, 2006; Richards et al., 2008; Rodgers et al., 2014; Loch and Faisal, 2015; Johnson et al., 2025). Microplastics originate largely from terrestrial sources and are transported through riverine networks into coastal and open-ocean systems (Jambeck et al., 2015; Lebreton et al., 2017), where they accumulate in long-lived environmental reservoirs such as coastal margins, oceanic gyres and sediments (Eriksen et al., 2014; Brandon et al., 2019; Amenábar et al., 2024). Fragmentation into smaller particles increases their mobility and bioavailability (Shamskhany et al., 2021; Onink et al., 2022). These microplastic particles can enter aquatic food webs via ingestion, followed by trophic transfer from primary consumers to higher trophic levels (Diepens and Koelmans, 2018; Farrell and Nelson, 2013, 2013). Although evidence for pathogen transmission *via* microplastics remains limited, a recent study found that PVC microplastics served as vectors for the transmission of *Vibrio parahaemolyticus* from the surrounding water to shrimp (Li et al., 2025). As the gastrointestinal tract is a primary route of bacterial infection in fish and aquatic invertebrates, we speculate that ingestion of contaminated microplastics may increase exposure risk and facilitate the spread of infections in natural and aquaculture systems (Ghosh, 2025). Notably, several detected genera in our study, particularly *Vibrio* and *Shewanella*, also cause opportunistic infections in humans, particularly in immunocompromised individuals. These genera have been implicated in skin and soft tissue infections, including cellulitis and wound-associated infections, as well as infections acquired through seafood consumption or direct contact with marine environments (Di Bartolomeo et al., 2025). Considering these findings, we speculate that plastic-associated biofilms may function as environmental reservoirs that facilitate the dissemination of human and aquatic animal pathogens.

The dense biofilms that form on microplastics also increase cell-to-cell contact rates, promoting enhanced rates of HGT (Aminov, 2011). The strong representation of IncF and rep_cluster plasmids harboring beta-lactam and aminoglycoside resistance genes, predominantly associated with *Pseudomonadota* and *Bacteroidota* (Figure 8), indicates a substantial potential for HGT within dominant plastisphere taxa. Human pathogens like *Vibrio cholerae* (Lutz et al., 2013), *Vibrio vulnificus* (Takemura et al., 2014), *V. parahaemolyticus* and *Aeromonas hydrophila* (Botero et al., 2023) have natural aquatic reservoirs. In addition, contamination of aquatic systems with gastrointestinal pathogens is an increasingly common concern globally (Sharma et al., 2003). Biofilms within these systems can be hotspots for the dissemination of ARGs (Lupo et al., 2012). In this context, the detection of ARG-carrying mobile genetic elements within plastisphere communities strengthens our hypothesis that microplastics may serve as microenvironments that facilitate HGT, leading to the emergence of multidrug-resistance plasmids and the spread of these plasmids from environmental bacteria to potential pathogens.

Distinct plasmid mobility patterns were also observed across plastic types, with conjugative plasmids commonly recovered from biofilms on PE, PVC and PP – polymers that also hosted taxonomically diverse microbes. Because conjugative plasmids encode self-transfer machinery, their dominance on these surfaces raises the possibility that the mixed-species assemblages associated with these materials may possess an enhanced capacity for genetic exchange. This is ecologically relevant, as conjugative plasmids frequently carry accessory genes linked to stress tolerance, metabolic flexibility and antimicrobial resistance, traits that can enhance bacterial persistence in heterogeneous and dynamic surface-associated environments such as microplastics.

Accordingly, conjugative plasmids were the most frequently observed to co-occur with ARGs (Figure 7), reflecting both their broad host range and established role as major vectors for the horizontal dissemination of clinically important resistance determinants, including to beta-lactams, macrolides and aminoglycosides. Several of the ARGs are of particular concern from a One Health perspective (Supplementary Table S8). These include extended-spectrum and carbapenemase genes such as $bla_{\text{CTX-M-3}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$ and notably $bla_{\text{KPC-2}}$, a globally disseminated carbapenem resistance determinant that severely limits treatment options for Gram-negative infections (Lee et al., 2016). The detection of $bla_{\text{KPC-2}}$ on microplastic-associated plasmids is especially noteworthy given its strong association with healthcare-associated outbreaks and multidrug-resistant *Enterobacterales* (Kerdsin et al., 2019). Resistance determinants targeting fluoroquinolones and aminoglycosides were also prevalent, including *qnrVC4*, *qnrVC5*, *aac(6)-Ib-cr*, *aadA2* and *adeF*, which collectively confer reduced susceptibility to first-line antibiotics commonly used in both clinical and veterinary medicine. In addition, the presence of *dfrA* variants (*dfrA19, dfrA31*), *sul1* and *sul2* highlights resistance to folate pathway inhibitors, drugs that are widely used and frequently detected in aquatic environments (Chaturvedi et al., 2021). The co-occurrence of clinically relevant ARGs with conjugative plasmids on microplastic surfaces suggests that the plastisphere may act as an environmental reservoir in which resistance determinants of direct human health relevance are concentrated within highly mobile genetic platforms.

Within this framework, the incompatibility family replicons, particularly IncFIB and IncFII, emerged as prominent hubs connecting multiple ARG classes across mobility categories. These replicons are well-documented in both environmental and clinical contexts, especially among *Enterobacterales*, where they are frequently associated with multidrug-resistant phenotypes (Carattoli, 2009, 2013; Rozwandowicz et al., 2018). Their strong representation in the plastisphere communities suggests that microplastic-associated resistomes can be shaped by plasmids commonly implicated in both environmental and clinically relevant resistance dissemination, consistent with a One Health perspective in which resistance genes circulate across different ecological compartments. Notably, IncFIB and IncFII plasmids were the replicon types most

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frequently associated with ARGs in this study, with strong associations to beta-lactam and aminoglycoside resistance genes (Figure 5B). IncF plasmids are known for their large, flexible accessory regions, which facilitate the accumulation and maintenance of multiple resistance determinants (Osborn et al., 2000). Hence, their presence suggests that the plastisphere may provide favorable conditions for the persistence of such plasmids, potentially due to prolonged surface attachment and biofilm stability.

In parallel, several less-characterized rep_cluster replicons (*e.g.*, rep_cluster_1068, rep_cluster_1418, rep_cluster_2183) were frequently observed to co-occur with multiple ARG classes, including sulfonamides and tetracyclines. Although the mobility potential and host range of these replicons remain poorly resolved, their repeated occurrence suggests that microplastic-associated biofilms may harbor a diverse pool of environmentally adapted plasmid backbones that can maintain resistance genes. Rather than acting as dominant vectors of horizontal transfer, these less-characterized plasmids may function as stable reservoirs of ARGs within plastisphere communities, highlighting the role of microplastics as sites of resistome accumulation, evolution and persistence.

Conclusions, limitations and future directions

This comparative multi-study, metagenomic analysis demonstrates that, despite originating from independent studies and environmental contexts, plastisphere communities shared common taxonomic and functional features, including the dominance of surface-adapted bacterial phyla and a high prevalence of plasmid-encoded ARGs. Given the strong selection for surface-colonizing bacteria (particularly those with pathogenic potential), future work should examine the mechanisms governing colonization of plastic surfaces relative to other materials. Additionally, our study is strictly bioinformatic and does not assess actual infection risk: future work should evaluate the potential for transfer of these surface-associated bacteria to human and animal hosts.

A key contribution of this study is the characterization of plasmids underlying plastisphere resistomes. Conjugative plasmids formed the primary links between plasmid replicons and clinically relevant ARG classes, highlighting their central role as genetic platforms facilitating the dissemination of resistance within surface-associated microbial communities. Importantly, several ARGs detected in this study, including extended-spectrum and carbapenem-associated beta-lactamases as well as fluoroquinolone resistance genes, are of recognized public health concern. The strong representation of Inc family replicons, particularly IncFIB and IncFII, mirrors well-established patterns in both environmental and clinical microbiology and underscores the relevance of plastisphere-associated plasmids to the broader circulation of resistance genes. At the same time, the frequent detection of less-characterized rep_cluster replicons suggests that microplastic-associated biofilms also harbor diverse, environmentally adapted plasmid backbones that may function as reservoirs for resistance determinants, even in the absence of confirmed mobility. Future work should focus on measuring rates of transfer of different plasmid types within microplastic biofilms.

Despite these insights, a key limitation of this study is the uneven sample sizes across polymer types, studies and environmental contexts, which limits the generalizability of our conclusions. In addition, reconstructing complete plasmid architectures from short-read assemblies remains inherently challenging due to the mosaic and repetitive nature of mobile genetic elements. Long-read sequencing and proximity ligation will be critical for improving plasmid recovery and host assignment from microplastic microbiomes, thereby strengthening the usability of metagenomic data for source tracking and risk assessment.

Open peer review. To view the open peer review materials for this article, please visit http://doi.org/10.1017/plc.2026.10052.

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Data availability statement. The data supporting the findings of this study are available within the article and/or the Supplementary Tables.

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Competing interest. The authors declare none.

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