

Contents lists available at ScienceDirect

# Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv



# Review Selection for antimicrobial resistance in the plastisphere

Check for updates

# Emily M. Stevenson<sup>a,b,c</sup>, Angus Buckling<sup>c</sup>, Matthew Cole<sup>b</sup>, Penelope K. Lindeque<sup>b,c</sup>, Aimee K. Murray<sup>a,\*</sup>

<sup>a</sup> European Centre for Environment and Human Health, Environment and Sustainability Institute, University of Exeter Medical School, Faculty of Health and Life Sciences, Penryn Campus, Cornwall, UK

<sup>b</sup> Marine Ecology & Biodiversity, Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

<sup>c</sup> Faculty of Environment, Science and Economy, University of Exeter, Penryn Campus, Cornwall TR10 9FE, UK

## HIGHLIGHTS

#### GRAPHICAL ABSTRACT

- The Plastisphere refers to microbial communities that colonise plastics.
- This review highlights three mechanisms by which AMR evolution may be driven in the Plastisphere:
- Horizontal gene transfer of ARGs may increase in the Plastisphere.
- AMR selective or co-selective compounds adsorb to microplastics.
- AMR selective or co-selective chemicals are impregnated within the plastic matrix.

#### ARTICLE INFO

Editor: Yi Yang

Keywords: Microplastic Biofilm Plastisphere Antimicrobial resistance Pollution Evolution



# ABSTRACT

Microplastics and antimicrobials are widespread contaminants that threaten global systems and frequently coexist in the presence of human or animal pathogens. Whilst the impact of each of these contaminants has been studied in isolation, the influence of this co-occurrence in driving antimicrobial resistance (AMR)<sup>1</sup> in microplastic-adhered microbial communities, known as 'the Plastisphere', is not well understood. This review proposes the mechanisms by which interactions between antimicrobials and microplastics may drive selection for AMR in the Plastisphere. These include: 1) increased rates of horizontal gene transfer in the Plastisphere compared with free-living counterparts and natural substrate controls due to the proximity of cells, cooccurrence of environmental microplastics with AMR selective compounds and the sequestering of extracellular antibiotic resistance genes in the biofilm matrix. 2) An elevated AMR selection pressure in the Plastisphere due to the adsorbing of AMR selective or co-selective compounds to microplastics at concentrations greater than those found in surrounding mediums and potentially those adsorbed to comparator particles. 3) AMR selection pressure may be further elevated in the Plastisphere due to the incorporation of antimicrobial or AMR co-

\* Corresponding author.

E-mail address: a.k.murray@exeter.ac.uk (A.K. Murray).

<sup>1</sup> Abbreviations: AMR: antimicrobial resistance; ARB: antimicrobial resistant bacteria; ARG(s): antimicrobial resistance gene(s); *E. coli: Escherichia coli*; HGT: horizontal gene transfer; MGE(s): mobile genetic element(s); QS: quorum sensing.

#### https://doi.org/10.1016/j.scitotenv.2023.168234

Received 1 September 2023; Received in revised form 20 October 2023; Accepted 29 October 2023 Available online 2 November 2023

0048-9697/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

selective chemicals in the plastic matrix during manufacture. Implications for both ecological functioning and environmental risk assessments are discussed, alongside recommendations for further research.

# 1. Introduction

The increasing threat of anthropogenic stressors to global One Health systems (i.e., humans, animals and the environment (Robinson et al., 2016)) has resulted in the proposal of a new approach to sustainability involving planetary boundaries wherein human society can safely exist and develop (Rockström et al., 2009). One such boundary considers 'novel entities', where safe thresholds can be monitored in terms of the emissions of chemicals, including both antimicrobials and microplastics (Gwenzi, 2022). With over 120 trillion microplastic (sizes 0.1 µm–5 mm (Thompson et al., 2004)) particles estimated to be floating in the global ocean (Lindeque et al., 2020), microplastics are the most prevalent type of marine plastic pollution. Simultaneously, antimicrobials can accumulate in aquatic ecosystems (Hayes et al., 2022), owing to the widespread misuse and overuse of antibiotics. As a result, selection for antimicrobial resistance (AMR) has been documented within environmental settings, leading to the spread of AMR bacteria (ARB) and AMR genes (ARGs) in environments across the globe (Gillings and Stokes, 2012). AMR represents one of the greatest threats to human health, with an estimated 5 million deaths associated with bacterial AMR infections in 2019 alone (Murray et al., 2022).

In many cases, microplastics will co-occur in environments alongside antimicrobials, ARGs and ARB (Fig. 1). Previous research has primarily discussed these contaminants in isolation within singular environments, yet the combined effects of these co-contaminants likely exacerbates the threats they pose individually. A key concern resulting from the coexistence of these contaminants is the role of microplastics as novel ecological habitats, supporting the formation of polymicrobial biofilms. These microplastic-attached communities, referred to as 'the Plastisphere' (Zettler et al., 2013), have not only been found to be distinct from their surrounding environment and other, natural debris, but may also be platforms for the attachment of AMR bacteria and potential human or animal pathogens (Lear et al., 2022; Ormsby et al., 2023). Still, the significance and nature of the unique properties of microplastics (Fig. 2) that shape AMR within the Plastisphere, or in supporting the colonisation of ARB, is not clear. Furthermore, the mechanisms which may drive the emergence of AMR in microplastic-associated communities due to their frequent co-occurrence with antimicrobials and other AMR selective pressures are yet to be understood. This review aims to discuss the potential roles of microplastics in the selection for AMR.



**Fig. 1.** Potential pathways for microplastics, antibiotics and ARGs/ARB to enter the environment. **A**: Domestic/clinical waste - plastics will fragment into secondary microplastics and partially metabolised antibiotics are excreted alongside ARGs and ARB. **B**: Groundwater - antibiotic residues accumulate in groundwater following irrigation of agricultural crops with treated wastewater (Kampouris et al., 2022). Microplastics may be vectors for ARGs from the agroecosystem into groundwater (Lu et al., 2020). **C**: Food systems - widespread use of plastics within veterinary medicines (Eckert et al., 2018; Ma et al., 2020), agriculture e.g. mulching (Shi et al., 2022a; Sun et al., 2018; Steinmetz et al., 2016) and aquaculture (Hou et al., 2021), leading to emission of secondary microplastics. Effect concentrations of antimicrobials are also applied, including agricultural fungicides to crops (Stevenson et al., 2022) and antibiotics used prophylactically in aquaculture (Reverter et al., 2020). **D**: Ice stores - microplastics are stored in pack ice (Peeken et al., 2018), arctic sea ice (Obbard et al., 2014) and mountainous peaks (Napper et al., 2020), where ARGs have also been discovered in ancient permafrost stores (Tuorto et al., 2014; Allen et al., 2009) and arctic soils (McCann et al., 2019). **E**: Coastal recreation – casual littering of plastics emits secondary microplastics, and shedding of ARB can occur from coastal users (Leonard et al., 2018; Gerba, 2000). **F**: Waste streams - including wastewater (Sun et al., 2019; Polanco et al., 2020), sewage sludge (Yang et al., 2019a; Tagg et al., 2022b; Su et al., 2022). **G**: At-sea sources, including fishing (Andrady, 2011), container spills (Redford et al., 1997) or illegal historical dumping at sea (Coyle et al., 2020; Rhodes, 2018). **H**: Biological - microplastics may be ingested and subsequently egested, resulting in the vertical transport of microplastics and associated Plastisphere communities (Cole et al., 2016).

# 2. Plastisphere communities

Although there are many characteristics of microplastics that facilitate and influence the formation of the Plastisphere (Fig. 2), the importance of the microplastic substrate itself in shaping Plastisphere communities is unclear (see meta-analysis from Wright et al. (Wright et al., 2021)). For example, community composition has been found to differ according to plastic polymer (Frère et al., 2018; Hansen et al., 2021; Muthukrishnan et al., 2019; Vaksmaa et al., 2021), particle size (Sun et al., 2022a; Wang et al., 2022a; Wu et al., 2022a; Banerjee et al., 2022; Phothakwanpracha et al., 2021; Liu et al., 2022a; Kim et al., 2022; Wang et al., 2022b; Li et al., 2022a; Zha et al., 2022; Zhao et al., 2023) or even microplastics of different colours (Lear et al., 2022; Wen et al., 2020). On the other hand, previous research has found that abiotic factors, including temperature, nutrient availability, depth and salinity are largely responsible for Plastisphere community profiles (Wright et al., 2021; Amaral-Zettler et al., 2020; Wright et al., 2020; Oberbeckmann et al., 2018), rather than microplastic-dependent features.

One of the barriers thus far to Plastisphere research is the frequent omission of natural substrate controls to compare community diversity and abundance (see Metcalf et al. (Metcalf et al., 2021)). Several studies have recently confirmed that Plastisphere communities are distinct from free-living communities (Zettler et al., 2013; Wen et al., 2020; Oberbeckmann et al., 2018; Bryant et al., 2016; De Tender et al., 2015; Pinnell and Turner, 2019; Pinnell and Turner, 2020; Aguila-Torres et al., 2022; Deng et al., 2022; Yu et al., 2022; Li et al., 2022b; Oberbeckmann et al., 2014; Wang et al., 2020; Hoellein et al., 2017; Amaral-Zettler, 2022), but less commonly whether they are distinct from microbial communities attached to control substrates. So far, Plastisphere communities have been found to differ significantly from steel (Muthukrishnan et al., 2019; Lee et al., 2008; Rogers et al., 1994), the rhizosphere (Shi et al., 2022a), glass (Lee et al., 2008; Vosshage et al., 2018; Ogonowski et al., 2018; Woodall et al., 2018; Kirstein et al., 2018; Pinto et al., 2019; Kirstein et al., 2019), cobblestone (Miao et al., 2019), wood (Muthukrishnan et al., 2019; Oberbeckmann et al., 2018; Miao et al., 2019; Song et al., 2022; Kesy et al., 2019), seston (Hoellein et al., 2017; Kesy et al., 2019; McCormick et al., 2016; McCormick et al., 2014), picoplankton (Bryant et al., 2016), seaweed (Quilliam et al., 2014; Metcalf et al., 2022), sand (Delacuvellerie et al., 2019), gravel (Agostini et al., 2021), leaves (Wu et al., 2019), cellulose (Ogonowski et al., 2018; Tagg et al., 2019), quartzite (Wang et al., 2022c), fabric (Woodall et al., 2018), sponge and fish (Schmidt et al., 2014). In contrast, a core microbiome shared across microplastic and control substrates has been found to occur with glass (Oberbeckmann et al., 2014: Pinto et al., 2019; Parrish and Fahrenfeld, 2019; Richard et al., 2019; Kesy et al., 2016; Dang et al., 2008; Erni-Cassola et al., 2020), bath stone (Kelly et al., 2022), wood (Song et al., 2020; Hu et al., 2021), ceramic (Pinnell and Turner, 2019), and stones (Naz et al., 2016). These comparisons are crucial in developing our understanding of the Plastisphere, as free-living controls can only provide evidence to support the role of surface attachment in selecting for unique, pathogenic or AMR communities, and do not highlight the importance of and risk posed by microplastics over natural substrates.

#### 3. Microplastics and the selection of AMR

Regardless of whether the Plastisphere is distinct to communities occupying surrounding environments or neighbouring surfaces,



Fig. 2. Key features of the Plastisphere. Top: Plastisphere-exploited advantages conferred by microplastic attachment – universal to all biofilms. Bottom: microplastic-dependent characteristics potentially influencing Plastisphere communities. (Created with BioRender.com.)

microplastics possess greater persistence and dispersal capabilities than other materials due to their recalcitrant surface, ever-increasing volume, and incomparable transport abilities (Wright et al., 2021). Likewise, if microplastics do harbour unique communities, it could also be suggested that microplastics may select for particular traits within those communities, including AMR. Therefore, it is crucial to determine whether Plastisphere communities have the potential to enrich AMR and human or animal pathogens over natural material controls.

Our understanding of the evolution of AMR is informed largely by studies adopting clinically relevant concentrations of antimicrobials in vitro. However, recent research has uncovered that selection for AMR can occur at very low antibiotic concentrations, similar to those found in wastewater or even surface waters contaminated with antibiotic residues (Fig. 1) (Murray et al., 2018). One of the mechanisms driving the evolution of AMR in natural bacterial communities is horizontal gene transfer (HGT): the transmission of ARGs via mobile genetic elements (MGEs) due to conjugation, transformation or transduction between the same or even phylogenetically distinct species. Selection for de novo mutations, clonal expansion of resistant cells or HGT of ARGs typically increases under an elevated selective pressure, including the presence of selective (e.g., antibiotics) or co-selective (e.g., heavy metals) compounds. Co-selection is the indirect selection for AMR where either a single gene confers for resistance to several compounds (cross-resistance) or where multiple ARGs are harboured on an MGE, such as a plasmid (co-resistance) (Murray et al., 2019).

AMR bacteria have been found to be selectively enriched on microplastics over free-living counterparts (Yang et al., 2019b), and metatranscriptomic evidence has recently identified that, not only were ARGs present in sampled polyvinyl chloride and polylactic acid Plastisphere communities, but they were actively expressed to a greater extent than planktonic water communities (Wu et al., 2022b). Additional interactions between microplastics and AMR have also been investigated, including the increased persistence of ARGs in microplastic biofilms (Yang et al., 2022) and, to a lesser extent, the role of microplastics as vectors of AMR within the food chain (additional summary of relevant literature can be found in Supplementary Material, Table 1). However, in recent years, researchers have begun to elucidate the importance of the Plastisphere in the selection for and emergence of AMR (Table 1).

Importantly, these potential mechanisms driving the selection for AMR within the Plastisphere (Table 1) have not yet been critically synthesised and the importance of microplastics in facilitating this at a greater extent than biofilms associated with natural controls is inconclusive, with much remaining to be understood. Here, we address possible reasons for why microplastics may promote both ecological (between taxa) and evolutionary (within taxa) selection of AMR.

We suggest three mechanisms by which microplastics may promote selection for AMR within the Plastisphere (Fig. 3):

- Due to the proximity of cells in the Plastisphere, co-occurrence of environmental microplastics and selective or co-selective compounds, and the sequestering of extracellular ARGs in the biofilm matrix, rates of HGT are higher in the Plastisphere than in free-living counterparts.
- 2) Microplastics are known to adsorb both AMR selective or co-selective compounds at concentrations greater than those found in surrounding mediums, thus increasing selection or co-selection pressure for AMR in the Plastisphere if bioavailable.
- 3) During the manufacture of plastic polymers, AMR selective or coselective compounds are often incorporated into the plastic matrix, leading to an increased selection or co-selection pressure for AMR local to the Plastisphere if bioavailable.

Though the literature used to support these hypotheses were not gathered in a fully systematic way, we have provided our literature search methodology, search terms and a detailed database of relevant literature in the Supplementary Material.

# 3.1. Horizontal gene transfer

Biofilms in general are associated with increased AMR due to the closer proximity of individual bacteria, promoting the HGT of MGEs bearing ARGs (Arias-Andres et al., 2018). The same is proposed for microplastic-associated biofilms, with biofilm-inducing quorum sensing (QS) systems facilitating cell-to-cell communication, the release of eDNA (Zhang et al., 2022b) and induction of type IV secretion systems (Amaral-Zettler et al., 2020; Kaur et al., 2021; Abe et al., 2020).

Using laboratory microcosm assays, Zhang et al. (Zhang et al., 2022a) reported that the conjugative antibiotic-resistance plasmid RP4 in Escherichia coli (E. coli) promoted the irreversible colonisation of polystyrene microplastics, which was speculatively suggested to be caused by the expression of conjugative pili. This suggests that HGT of ARGs, particularly via conjugation, may increase due to the nature of surface attachment, inducing the expression of HGT factors. In addition to this, Liu et al. (Liu et al., 2023a) found that exposure to smaller fractions of polystyrene microplastics (nanoplastics) enhanced the conjugative transfer frequency of RP4 in E. coli. When investigating the mechanisms behind this, it was revealed that the nanoplastic exposure induced reactive oxygen species and oxygen stress, which subsequently increased cell permeability of both donor and recipient cells, and upregulated the expression of mating pair formation genes and DNA transfer or replication genes. Microplastics themselves could therefore be described as co-selective agents for AMR, as the attachment to microplastics induces HGT machinery, or exposure to micro/nanoplastics results in the upregulation of conjugative transfer-related genes, thus indirectly selecting for an increased spread and genome incorporation of ARGs within Plastisphere communities. Furthermore, genetic linkage of AMR and virulence genes on multidrug resistant plasmids and virulence factors could also lead to the hitchhiking of AMR, due to the benefit conferred by the expression of virulence factors that support attachment.

Arias-Andres et al. (Arias-Andres et al., 2018) performed the first AMR experimental evolution study in the Plastisphere, conducting conjugation experiments on Plastisphere communities versus free-living cells and bacterial communities present on natural aggregates. Biofilms incubated in lake water were also evaluated for plasmid permissiveness using fluorescence-activated cell sorting. Plasmid transfer rates were three orders of magnitude higher on microplastics than those of bacteria in suspension, and the permissiveness of microplastic-associated communities was two orders of magnitude greater than bacteria in the surrounding water and natural aggregates (Arias-Andres et al., 2018). These results clearly demonstrate the elevated HGT potential of the Plastisphere compared with the aqueous phase, and begins to uncover distinctiveness to natural aggregates in the Plastisphere community's ability to receive and maintain AMR plasmids (Moradigaravand et al., 2022). Further to this, Feng et al. (Feng et al., 2023) found that conjugation rates of ARGs were significantly greater in the Plastisphere than compared with control wastewater communities, and that these ARGs could also be transferred horizontally to the free-living cells surrounding the microplastics. This postulates an additional concern where, not only may we see significantly greater HGT rates on microplastics, but this may also result in increased AMR in surrounding environments, including surface waters, if ARGs are indeed transferred from microplastics to the ambient, free-living community.

In addition to conjugation-based assays, the influence of microplastics and nanoplastics on the transformation frequency of *E. coli* was also recently investigated by Wang et al. (Wang et al., 2022e), revealing that the presence of polystyrene nanoplastics increased transformation frequency significantly, but that microplastics had no significant impact. However, this study exposed *E. coli* to these particles and did not investigate transformation frequency within Plastisphere communities (see also Hu et al. (Hu et al., 2022b)). To the best of our knowledge, transformation within the Plastisphere had not been investigated until Wang et al. (Wang et al., 2023) recently investigated and compared Studies investigating selection for antimicrobial resi

istance in the Plastisphere.								
Methods	Key Finding							

Environment (in vitro)	Plastic	Control	Methods	Key Finding	Ref
Microcosm	PP, PE & PS	Quartz sand & free-living community	Transformation assay & 16s rRNA sequencing	Transformation frequency of ARGs in the Plastisphere was up to 1000-fold more than controls	(Wang et al., 2023)
Microcosm	PE, PP & PS	Landfill leachate	qPCR & 16s rRNA sequencing	Zinc oxide and zinc ion exposure significantly increased abundances of ARGs in the Plastisphere over control.	(Shi et al., 2023)
Batch reactor system	PS	Without PS exposure	Metagenomics & HT-qPCR	PS exposure significantly increased absolute abundance of <i>int11</i> and other ARGs.	(Ren et al., 2023)
Microcosm	PS nano- plastics	Without PS exposure	Conjugation assay & RT-qPCR	Smaller PS significantly enhanced conjugative transfer frequency.	(Liu et al., 2023a)
Aerobic and anaerobic wastewater	PE, PP-PE, PC, PET & PS	Wastewater community	16s rRNA sequencing, shotgun metagenomics & conjugation assay	Rate of HGT was significantly greater on microplastics than control.	(Feng et al., 2023)
Microcosm	PE	Without PE exposure	Conjugation assay & RT-qPCR	PE exposure significantly enhanced conjugative transfer rate.	(Yu et al., 2023)
Activated sludge	Virgin or aged PVC	Without tetracycline exposure	16s rRNA sequencing & qPCR	Increased adsorption of tetracycline on aged PVC and ARGs were significantly enriched on tetracycline-adsorbed PVC compared to controls.	(Tian et al., 2023)
Anaerobic digestor	PE	Without PE exposure	16s rRNA sequencing & metagenomics	Concentration of PE was significantly correlated with MGE and ARG abundance.	(Wang et al., 2022d)
Microcosm	PVC	Without PVC leachate exposure	Metagenomics	PVC leachate exposure increased ARG relative abundance compared with control.	(Vlaanderen et al., 2023)
Cropland soil	PP	Soil	16 rRNA sequencing & qPCR	Doxycycline exposure increased abundance of ARGs on PP.	(Liu et al., 2023b)
Microcosm	PS nano- plastics	Bio-degradable nanoplastics (PLA & PHA) & without nanoplastic exposure	Conjugation assay	MGE transfer frequency increased following exposure to biodegradable nanoplastics more than PS exposure.	(Liu et al., 2023c)
Activated sludge	PE & PVC	Without microplastic exposure	Metagenomics	Microplastic exposure increased ARG abundance and HGT.	(Luo et al., 2023)
Coastal sedimentary sludge	PE, PET & PVC	Without microplastic exposure	16s rRNA sequencing & metagenomics	Microplastics promoted HGT of ARGs.	(Zeng et al., 2023)
River (in situ)	LDPE, PET, PP & PVC (macro- plastic)	Glass, rock & free-living community	qPCR	Relative abundance of ARGs was greatest in free- living community over time, except <i>ermF</i> , which was greatest on plastic in later incubation periods.	(Martínez- Campos et al., 2023)
Microcosm	HDPE	Glass & free-living community	Flow cytometry (fluorescence)	AMR, measured using fluorescence, was significantly higher in biofilms than free-living communities, but more on glass than HDPE.	(Hu et al., 2022a)
Microcosm	PS nano- plastics	Without PS nanoplastic exposure	qPCR, SEM of conjugative transfer system & conjugation assav	Exposure to PS increased expression of conjugative transfer genes, transfer efficiency and production of EPS.	(Liu et al., 2022b)
Agricultural soil	PE	Without PE exposure	Metagenomic next-generation sequencing & disk diffusion	Greater prevalence of AMR in plastic-degrading bacteria on PE than non-plastic degraders in control.	(Edet et al., 2022)
Synthetic wastewater effluent and tap water	PS	Free-living community	Cultivation, 16s rRNA sequencing & qPCR	Significantly higher ARGs and MGEs on PS than control & with antibiotic pressure, compared to without antibiotic pressure.	(Perveen et al., 2022)
Activated sludge	PVC	Sludge	Metagenomics	PVC had more potential ARG hosts, stronger correlation with ARGs and enriched resistance mechanisms compared to control.	(Li et al., 2022c)
Microcosm	PS	Without plasmid	Conjugation assay, PCR & qPCR	AMR plasmid promoted irreversible colonisation of ARB on PS.	(Zhang et al., 2022a)
Microcosm	PE	Free-living community, sediment & quartz sand	qPCR & 16s rRNA sequencing	Antibiotics and metals significantly increased transfer of ARGs on PE, compared to controls.	(Liu et al., 2022c)
Wastewater	PE, PP, PS & recycled PE	Without microplastic exposure	Conjugation/transformation assays & qPCR	Exposure to microplastics enhanced HGT of ARGs.	(Cheng et al., 2022)
Lake water	PE, PVC & PET	Free-living community	qPCR & 16s rRNA sequencing	Intracellular ARGs and extracellular ARGs were selectively enriched on microplastics.	(Zhang et al., 2022b)
Microcosm	PS	Without microplastic exposure	Conjugation assay & qPCR	Conjugation of ARGs was found to depend on PS size.	(Zha et al., 2022)
Microcosm	PS (aged vs virgin)	No	Conjugation assay, qPCR & transcriptomics	UV-ageing & PS leachates enhanced HGT.	(Yuan et al., 2022)
Soil	HDPE	Without HDPE phthalate exposure	Metagenomics	Phthalates released from HDPE enhanced soil ARG abundance, but effect of phthalates on ARGs was greater than that of HDPE.	(Lu and Chen, 2022)
Surface water	PET	Free-living community & bio-degradable microplastics (PBAT)	qPCR & 16s rRNA sequencing	Transfer of ARGs between microplastics and receiving waters occurred.	(Zhou et al., 2022a)
Microcosm	PS	No	Transformation assay & PCR	Smaller PS particles influenced the transformation of ARGs in <i>E. coli</i> .	(Hu et al., 2022b)

(continued on next page)

#### Table 1 (continued)

Environment (in vitro)	Plastic	Control	Methods	Key Finding	Ref
Microcosm	PS nano-	Without microplastic	Transformation assay	PS nanoplastics significantly enhanced	(Wang et al.,
	plastics &	exposure		transformation of an exogenous ARG into E. coli,	2022e)
	micro-plastics			whilst PS microplastics exerted no influence.	
Soil	PE	Soil	qPCR & 16s rRNA	Increase in total relative abundance of ARGs on	(Wang et al.,
				PE was higher than that in soil.	2021a)
Activated sludge	PE & PS	Fine sand	16s rRNA sequencing & qPCR	PE and PS enriched ARGs in comparison with	(Pham et al.,
				fine sand.	2021)
Sewage	PVC	Sewage	HT-qPCR & 16s rRNA	Only tetracycline, ampicillin or zinc spiking	(Zhao et al.,
			sequencing	resulted in higher ARG abundances on PVC than	2021)
				in sewage.	
Lake water	PS	Free-living community &	Fluorescence activated cell	Increased frequency of plasmid transfer on PS	(Arias-Andres
simulation		natural aggregates	sorting, flow cytometry & 16s	compared to bacteria that are free-living or on	et al., 2018)
		0	rRNA sequencing	natural aggregates.	

AMR: antimicrobial resistance, ARB: antimicrobial resistant bacteria, ARG: antimicrobial resistance gene, *E. coli: Escherichia coli*, EPS: extracellular polymeric substance, HDPE: high-density polyethylene, HGT: horizontal gene transfer, HT-qPCR: high-throughput-qPCR, LDPE: low-density polyethylene, MGE: mobile genetic element, PBAT: polybutylene adipate terephthalate, PC: polycarbonate, PCR: polymerase chain reaction, PE: polyethylene, PET: polyethylene terephthalate, PHA: polyhydroxyalkanoate, PLA: polylactic acid, PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride, qPCR: quantitative PCR, rRNA: ribosomal ribonucleic acid, RT-qPCR: reverse transcription-qPCR, SEM: scanning electron microscopy, UV: ultraviolet.



Fig. 3. Schematic diagram summarising the three proposed AMR selection mechanisms. Left: horizontal gene transfer, Bottom: adsorbed co/selective agents, Right: co/selective agents incorporated in plastic matrix. (Created with BioRender.com.)

transformation frequency of ARGs in the Plastisphere, quartz sand biofilms and free-living counterparts. This work provides some of the first evidence to support a unique role of the Plastisphere in enhanced transformation-dependent HGT compared with natural substrate controls and free-living communities, finding that transformation frequency was significantly greater in the Plastisphere compared with both freeliving and natural particle controls. The authors suggest that enhanced transformation in the Plastisphere was observed due to correlations between transformation frequency and bacterial density, extracellular polymeric substance content (including eDNA (Cheng et al., 2022; Zhang et al., 2022b)), expression of biofilm formation or DNA uptake genes (e.g. flagella motility and bacterial adhesion) and, at the community-wide level, greater colonisation of transformants. Furthermore, cell lysis of biofilm-housed cells has previously been observed as a mechanism to strengthen the structure of the biofilm, which suggests a further source of eDNA available for transformation (Abe et al., 2020).

To date, we are unaware of any investigation of transduction within Plastisphere communities, even though there is reason to suggest that elevated rates of this HGT mechanism would also be observed, due to the binding of bacteriophage proteins to plastics such as polystyrene (Bakhshinejad and Sadeghizadeh, 2016).

Given that many of these HGT mechanisms are experienced universally in biofilms, further research is required to identify the importance of microplastic substrates in comparison to natural materials. However, even with the current lack of studies adopting appropriate natural substrate controls, the uniqueness and potential increased risk of microplastics can still be proposed, given the ubiquity or longevity of microplastic particles in comparison with natural materials, cooccurrence with selective chemicals (Fig. 1) (Bydalek et al., 2023) or additional microplastic-dependent drivers (Fig. 2).

# 3.2. Adsorbed selective and co-selective compounds

Microplastics display elevated adsorptive capabilities compared to other natural debris owing to higher surface-to-volume ratios, and can achieve chemical loads over 100-fold greater than the surrounding water (Imran et al., 2019). Many pollutants found to accumulate on microplastics are responsible for selection or co-selection of AMR, including antibiotics, pesticides, biocides, heavy metals and other xenobiotics (Laganà et al., 2019; Rillig et al., 2018). Whilst the surface properties of microplastics (Fig. 2) or environmental conditions (e.g. salinity) partly facilitate the adsorption of these compounds, ageing (i.e. weathering, ultraviolet degradation of surface, biofilm formation) of microplastic particles has also been found to increase adsorption of pollutants (Bhatt et al., 2021). Though chemical contaminants can also adsorb to natural materials (Cederlund et al., 2016), it could be argued that microplastics more frequently come into contact with such pollutants given co-occurrences (Fig. 1), especially in environments polluted with sewage or landfill leachate (Shen et al., 2022).

Antibiotics are extremely susceptible to microplastic adsorption, which may result in the enhanced emergence, recruitment and fixation of ARGs in Plastisphere communities if these compounds are bioavailable (Du et al., 2022). To support this, a recent study found antibiotics (enoxacine, norfloxacin, ofloxacin and enroflocaxin) and various heavy metals adsorbed to macroplastic facemasks at significantly greater concentrations than stone controls (Liu et al., 2023d). Following further analysis, this work found that the selective pressure of the adsorbed cocontaminants led to the greater enrichment of ARGs in the Plastisphere compared to the biofilms attached to the natural, stone controls. Further examples of antibiotics shown to adsorb to microplastics include ciprofloxacin (Atugoda et al., 2020), sulfamethoxazole (Wang et al., 2022f), oxytetracycline (Zhang et al., 2018), ampicillin (Zhao et al., 2021; Wang et al., 2021b), tetracycline (Ma et al., 2020), trimethoprim (Li et al., 2018), chloramphenicol (Wang et al., 2020) and roxithromycin (Zhang et al., 2022c). Yu et al. (Yu et al., 2022), for example, detected relatively high concentrations of antibiotics adsorbed to microplastics, including nearly 26 ng/g of the macrolide antibiotic spiramycin. Likewise, heavy metals have also been found to have a high affinity for microplastics, with concentrations reaching more than 600 times those present in surrounding waters (Yang et al., 2019b). Examples of heavy metals that have been previously documented to adsorb to microplastics include lead, cadmium, copper, zinc, chromium, iron, arsenic, silver and mercury (Khalid et al., 2021).

Very low, sub-inhibitory concentrations similar to those found adsorbed to microplastics have been previously shown to have AMR selective effects, including a study finding the minimal selective concentration for ciprofloxacin to be 100 pg/ml (Murray et al., 2018), suggesting that even low concentrations of antimicrobials present on microplastics may have a selective effect if they are bioavailable. In a study performed by Li et al. (Li et al., 2022c), polyvinyl chloride microplastics were pre-adsorbed with copper and/or tetracycline and incubated in an artificial activated sludge system. ARG diversity and abundance on microplastics and in sludge were then evaluated using metagenomics. The antimicrobials were found to distinctively enrich ARGs in the Plastisphere and surrounding sludge, particularly tetracycline resistance genes, supporting the role of microplastics in exerting a selective pressure due to adsorbed antimicrobials.

However, the role of selective concentrations of antimicrobial contaminants adsorbed to microplastics or the co-occurrence of antibiotic and metal resistance genes (Pal et al., 2015) driving co-selection for AMR on microplastics are yet to be fully understood, and the AMR selective risk posed by this is largely dependent on the bioavailability of these chemicals once adsorbed. Bioavailability of compounds adsorbed to microplastics has been previously contested. For example, the bioavailability of organic pollutants to copepods was higher in the dissolved phase than when adsorbed to microplastics (Sørensen et al., 2020). However, this study did not account for the potential influence microbes within the Plastisphere may have in increasing bioavailability of microplastic-associated chemicals through biodegradation, which is a potentially crucial yet understudied concern, highlighted below (Section 3.3).

# 3.3. Selective and co-selective compounds in the plastic matrix

Chemical additives are incorporated into the plastic matrix during the manufacturing process (Li et al., 2022c), with nearly 1000 different compounds currently associated with plastic polymers (Wright et al., 2020). Examples of some of these compounds include heavy metals like arsenic, cadmium, chromium and lead (Turner and Filella, 2021) or known antibacterial/antifungal agents, such as triclosan (Junker and Hay, 2004). Some of the services provided by these additives include performance enhancement, increased durability, plasticisers, antioxidants, antifoulants, antimicrobials, ultraviolet and thermal stabilisers, colour pigments and flame retardants (Richard et al., 2019; Massos and Turner, 2017; Hansen et al., 2013).

Though many of these chemicals are not chemically bound and leach from plastics due to environmental ageing (Hahladakis et al., 2018), it is unknown what proportion of these compounds are bioavailable to Plastisphere communities, and if so, whether they increase selection or co-selection for AMR. For the most part, the wider biological effects of these additives are also unknown. However, a recent study found that polyvinyl chloride leachate exposure significantly increased ARG and virulence gene relative abundance in a marine bacterial community, compared to the no-plastic leachate control (Vlaanderen et al., 2023). In addition, Lu and Chen (Lu and Chen, 2022) exposed a farmland soil community to high density polyethylene microplastics containing phthalates, and used metagenomics to explore the shifts in community composition and relative abundances of ARGs. Leaching phthalates from microplastics had a greater influence on ARG prevalence than microplastic exposure alone (e.g., particles physically causing changes to soil porosity), demonstrating that plastic additives can drive the evolution of AMR.

Notably however, these studies did not investigate the impact of additives on AMR in Plastisphere communities and focussed only on impacts to the surrounding microbiome and resistome. Further research should thus target understanding the impacts of plastic additives specifically within the Plastisphere and the importance of these unique, microplastic-dependent interactions in driving AMR. For example, the physical, chemical or biological degradation of microplastics may result in the increased bioavailability of additives to the Plastisphere. Indeed, the Plastisphere itself may increase additive leaching through the enzymatic hydrolysis of microplastic hydrocarbon bonds. Bacteria capable of producing plastic-degrading enzymes, also known as hydrocarbonoclastic bacteria, have been previously documented in the Plastisphere (Kelly et al., 2022; Du et al., 2022; Liu et al., 2022d). The subsequent release of additives as a result of biodegradation has not yet been investigated, but it has been noted that microplastics may be more likely to release these compounds than macroplastics, due to shorter diffusion path lengths (Liu et al., 2022d; Paluselli et al., 2018; Luo et al., 2020; Bandow et al., 2017).

Furthermore, some compounds included as plastic additives are known co-selective agents, including heavy metals. Turner et al. (Turner et al., 2019) found that concentrations of bromine, cadmium, chromium and lead on ~10 % of beached microplastics were 'non-compliant' or 'potentially non-compliant' under the present regulations on hazardous plastic waste. Similar findings were observed in beached microplastics, where bioavailable concentrations of cadmium, lead and bromine exceeded those estimated as acceptable in the local seabird diet (Massos and Turner, 2017). It was proposed that the origin of bromine was likely due to the addition of brominated flame retardants. Crucially, a simulated avian digestive tract study revealed that heavy metal additives were bioavailable from microplastics during seabirds' digestion (Turner, 2017). This is of particular concern as seabirds are renowned for their

ingestion of plastic debris, and release of additives could co-select for AMR in seabird gut microbiomes. In addition, seabirds are already recognised as important sentinels of AMR in the environment and are also used as microplastic biosentinels (Morrison et al., 2014; Van Franeker et al., 2011).

Crucially, if microplastic leachates are bioavailable and exert an AMR selective pressure, this is a mechanism unique to microplastics and should be seriously considered when addressing ecotoxicological risk and the AMR co-selective nature of microplastics in environmental compartments.

## 4. Pathogens and the plastisphere

In addition to AMR, it is important to understand the role of the Plastisphere in supporting the adhesion of pathogenic bacteria, given the threat of AMR pathogens to both human and animal health. This has recently been reviewed (Kaur et al., 2021; Junaid et al., 2022; Bowley et al., 2021; Bowley et al., 2022), where the capability for microplastics to harbour a variety of potential pathogens was reported.

Biofilms in general play a role in bacterial pathogenesis (Parsek and Singh, 2003). For example, the inducing of attachment phenotypes may increase infectivity of microbes, due to an increase in functional diversity or metabolic responses and pathways (Lyons et al., 2010; Sun et al., 2020). As previously discussed, QS is known to be an important driver of biofilm formation and cell-to-cell communication (Kaur et al., 2021), and it is proposed that the change from planktonic to biofilm lifestyle is governed by QS systems (Salta et al., 2013). QS is also responsible for the control of virulence factors (e.g. Lami (Lami, 2019)), which suggests a direct link between biofilm formation and the expression of virulence. Virulence genes can also be associated with adhesion factors, leading to the expression of virulence proteins as a result of surface attachment (Kaur et al., 2021; Radisic et al., 2020). Inversely, whilst existing as sessile cells, biofilms do not express invasion or motility machinery (Feltman et al., 2001; Mahenthiralingam et al., 1994), which may reduce colonisation potential and subsequent infection of the host.

Some of the clearest evidence for pathogen enrichment on marine microplastics involves the colonisation of Vibrio species (Zettler et al., 2013; Kirstein et al., 2016; Laverty et al., 2016; Rodrigues et al., 2019; Curren and Leong, 2019; Foulon et al., 2016; Silva et al., 2019; Sun et al., 2022b; Tan et al., 2022; Lai et al., 2022). Using next-generation sequencing, Frère et al. (Frère et al., 2018) detected members of the Vibrio genus on nearly 80 % of their microplastic samples, yet not all Vibrio spp. are pathogenic and Vibrio biofilms have also been identified on various natural debris, including wood and glass (Bowley et al., 2021). Other studies show the Plastisphere can not only harbour potential human pathogens, including faecal indicator organism E. coli (Metcalf et al., 2022; Rodrigues et al., 2019), but also potent animal pathogens, such as the fish pathogen Aeromonas salmonicida (Lai et al., 2022; Viršek et al., 2017). Strikingly, within the terrestrial environment, potential pathogens were found to be 12.4 times higher in Plastisphere communities than in the ambient soil (Zhu et al., 2022). If Plastisphere communities do indeed select for pathogens as detailed in these studies, the need for continued exploration of microplastic-associated AMR is even greater, in order to reduce the risks posed by microplasticassociated, multi-drug resistant pathogens on public health and important food systems (e.g., aquaculture).

# 5. Ecological implications

Microplastic ingestion has been reported in over 200 species, with biofilm formation thought to increase palatability and thus the likelihood of consumer ingestion (Reisser et al., 2014; Hodgson et al., 2018). If colonised microplastics are ingested, associated ARB, ARGs or adsorbed antimicrobials could detach, transmit or desorb into the host microbiome. Additionally, following the egestion of microplastics,

members of the gut microbiota may be incorporated into Plastisphere communities and integrated into microplastic-laden faecal pellets (Cole et al., 2016).

There is currently no information available on whether gut microbiota are incorporated into the excreted biofilm, how long they can survive after egestion, or the role of subsequent ingestion of contaminated faecal pellets. However, studies have started to elucidate the impact of microplastic exposure on gut microbiomes in vivo, where perturbed microbiomes can have critical negative impacts upon the host. Xu and Yu (Xu and Yu, 2021) exposed earthworms to polystyrene nanoplastics and microplastics, finding higher abundances of ARGs present in earthworm guts treated with microplastics in comparison to nanoplastics. Furthermore, a freshwater fish model (Carassius auratus) was used to assess the co-influence of the antibiotic roxithromycin and microplastics on ARG distribution on the fish gut microbiome and found a near 70 % increase in the ARG sul1 from the combined exposure to the antibiotic and microplastics, which was a marked increase compared with the single microplastic treatment and roxithromycin alone (Zhang et al., 2022c).

These results suggest that the co-occurrence of microplastics and antimicrobial residues in environmental compartments may risk biosecurity by increasing ARG prevalence in reared food exposed to both pollutants, such as fish (Zhang et al., 2022c). This could result in higher risk of treatment failure in the reared organisms, in addition to the potential increased risk of transmission to humans through occupational exposure or via the food chain.

Finally, when considering the ecological impacts of adsorbed or incorporated antimicrobial compounds and microplastics, there are some critical points that must be considered. For example, adsorption kinetics of antimicrobial compounds, the degradation rate of antimicrobials in water/sediment versus those associated with microplastics, and the impact of microplastic biofilms in influencing these two processes. Previous works (Tian et al., 2023; Wang et al., 2022g; Fan et al., 2021; Sun et al., 2023; Zhou et al., 2022b; Shi et al., 2022c; Tang et al., 2022) have begun to elucidate these interactions, for example, Sun et al. (Sun et al., 2023) found an increased adsorption of oxytetracycline to biodegradable microplastics when a biofilm was present, in comparison to virgin microplastics. In addition, Shi et al. (Shi et al., 2022c) report that the main adsorption mechanisms of microplastics rely on hydrophobicity interactions, hydrogen bonding and electrostatic interactions, and ageing increases heavy metal adsorption to both non-biodegradable and biodegradable microplastics. Further work is now required, especially when comparing microplastics to natural controls and adopting environmentally realistic conditions and concentrations, to fully understand these mechanisms and the unique risk posed by microplastics.

# 6. Environmental risk assessment implications

The universal framework for determining the environmental risk of pollutants (including both microplastics and antimicrobial chemicals), is to perform an 'environmental risk assessment'. This typically includes an exposure assessment, effect assessment and risk characterisation (Everaert et al., 2018). Traditionally, effect assessments will quantify 'safe' concentrations of a pollutant using ecotoxicological tests, with endpoints including reduced survival, reproduction, or growth. The concentration at which no adverse effect on a test organism is observed is known as the no observed effect concentration. A no observed effect concentration can then be used to generate a predicted no effect concentration through application of an assessment factor, which takes into account extrapolating data from lab to field, and the breadth, depth and quality of effect data available (CHMP, 2006). Ultimately, environmental risk assessments produce a risk quotient or risk characterisation ratio by dividing the predicted or measured environmental concentration by the predicted no effect concentration. Generally, if the risk quotient or risk characterisation ratio exceeds 1, then the risk of that pollutant is considered high under European Union legislation (EU,

#### 2006).

There are areas for improving environmental risk assessment of antimicrobials, discussed elsewhere, e.g. Murray et al. (Murray et al., 2021) and Alejandre et al. (Alejandre et al., 2023). However, the monitoring, legislation and mitigation of antimicrobials in the environment seems to be adopted more widely by both industry and government than those for microplastics. For example, concentrations of chemicals (including antimicrobials) in wastewater across the UK are currently being monitored by the UK Water Industry Research and Environment Agency's Chemicals Investigation Programme (UKWIR, 2020), and predicted no effect concentrations for both ecotoxicity and AMR selection risk are used to inform the EU Commission Water Framework Directory's Watch List of potentially hazardous substances (Gomez Cortes et al., 2022).

On the other hand, the first environmental risk assessment performed on microplastics was conducted in 2018 (Everaert et al., 2018), followed by Burns and Boxall (Burns and Boxall, 2018) and then for the first time with measured environmental concentrations in 2021 (Adam et al., 2021). All three of these global environmental risk assessments found that the concentrations of microplastics detected in the environment were orders of magnitude lower than those reported to have adverse effects on test organisms. However, these assessments did not include the risk posed by microplastics as vectors for chemicals in the environment or the effect of plastic additives. Endpoints that are typically adopted in microplastic ecotoxicological tests include reduced feeding, reproduction and growth, or tissue inflammation and mortality (Burns and Boxall, 2018), and test species are varied, including arthropods (e.g. *Calanus* sp. (Cole et al., 2015)) and molluscs (e.g. *Mytilus* sp.) (Adam et al., 2021).

Interestingly, one issue that has previously been highlighted for antimicrobial environmental risk assessments is the lack of testing against target microorganisms. Yet, ecotoxicological effects of microplastic exposure have recently been tested against the bacterium *Vibrio angiillarium*, where the bacterial culture was exposed to increasing concentrations of polystyrene microplastics, and a no observed effect concentration was generated at the concentration of microplastics that significantly reduced culturability of the bacteria (Gambardella et al., 2018). However, this does not consider any impact of microplastics on microbes associated with the Plastisphere.

This review has discussed some potential interactions between microplastics and antimicrobials in the environment that may exacerbate the existing ecological threats of these pollutants. These interactions are not currently considered in either antimicrobial or microplastic environmental risk assessment frameworks and so we recommend the following:

- a. To consider the role of microplastics as vectors for chemicals and the effects of plastic additives.
- b. To consider the mixture effects of antimicrobials and other potentially co-selective pollutants (e.g. microplastics).
- c. To improve the monitoring of antimicrobials by considering those incorporated into plastics or adsorbed to the plastic surface.
- d. To consider that antimicrobials may persist on microplastics (Liu et al., 2018), increasing half-life durations and potentially altering where and when risks are present.

#### 7. Concluding remarks

This review highlights three potential drivers of AMR selection within the Plastisphere, including increased HGT and the elevated AMR selection or co-selection pressures due to the presence of adsorbed or impregnated compounds. Though the ability of microplastics to enrich AMR communities relative to other substrates remains inconclusive, the persistence and transportation potential of these vastly abundant particles which co-exist with existing AMR selective pressures is evident. Therefore, it is imperative to develop our understanding of AMR within the Plastisphere so that combined threats of microplastics, ARB, ARGs and antimicrobial pollutants across One Health sectors can be fully assessed.

# 8. Outstanding research questions

- Biofilm-forming species are inherently resistant to antibiotic chemotherapy, due to reduced antibiotic penetration and slower growth rates. Also, large numbers of antibiotic-susceptible cheats may be enriched in the Plastisphere if antibiotic inactivating enzymes, e.g., beta-lactamases, are secreted into the extracellular polymeric substance. What is the role of the Plastisphere in the reduction of antibiotic efficacy and in the survival of susceptible and resistant human or animal pathogens?
- Are existing laboratory-based findings reproducible in more realistic environments with less optimal growth conditions?
- Is there greater acquisition of ARGs or de novo mutations in the Plastisphere than free-living phases, or natural substrate controls?
- Do nanoplastics have a role in supporting attachment of AMR bacteria, or the increased transmission of ARGs?
- Antimicrobials exist in complex mixtures in the environment. Do these behave synergistically or antagonistically in terms of driving AMR in the Plastisphere?
- Does transduction play a role in HGT of ARGs within the Plastisphere, as well as conjugation and transformation?
- What is the role of microplastics in the increased transport of AMR bacteria between different environments, owing to increased protection and thus survival within the extracellular polymeric matrix?
- If colonised microplastics are ingested, could associated ARBs, ARGs or adsorbed antimicrobials detach/desorb and affect the host microbiome?
- Following the egestion of microplastics: are gut microbiota incorporated into the excreted Plastisphere, how long do these bacteria survive after egestion, and what is the role of subsequent ingestion of contaminated faecal pellets?
- Are fungi and viruses important members of the Plastisphere?

# CRediT authorship contribution statement

EMS wrote the main manuscript text and prepared the figures, and all authors reviewed them. All authors conceptualized the project, reviewed the manuscript and led the funding acquisition. AKM was responsible for project administration. AB, MC, PKL and AKM supervised the project.

# 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.

#### Data availability

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

#### Acknowledgments

We acknowledge support and give thanks to Rosanne Bostock, Melissa Murdoch, the Barnsbury Trust and Beach Guardian. Thank you to Emma Lamb for thorough proofreading and grammatical corrections of this manuscript. For the purpose of open access, the authors have applied a Creative Commons Attribution (CC BY) to any Author Accepted Manuscript version arising. This work was funded by the generous philanthropic donations made from Melissa Murdoch, the Barnsbury Trust and Beach Guardian. Thank you also to the University of Exeter and Plymouth Marine Laboratory for providing funding for this research. AB acknowledges support from NERC and BBSRC. AKM acknowledges support from NERC (NE/W006251/1). PKL and MC acknowledge support from NERC (NE/V007351/1).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168234.

#### References

- Abe, K., et al., 2020. Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. FEMS Microbiol. Ecol. 96, fiaa031.
- Adam, V., et al., 2021. Probabilistic environmental risk assessment of microplastics in marine habitats. Aquat. Toxicol. 230, 105689.
- Agostini, L., et al., 2021. Deep-sea plastisphere: long-term colonization by plasticassociated bacterial and archaeal communities in the Southwest Atlantic Ocean. Sci. Total Environ. 793, 148335.
- Aguila-Torres, P., et al., 2022. Associations between bacterial communities and microplastics from surface seawater of the Northern Patagonian area of Chile. Environ. Pollut. 306, 119313.
- Alejandre, J.C., et al., 2023. Eco-directed and Sustainable Prescribing of Pharmaceuticals in the United Kingdom. Prepared by the Office of Baroness Bennett of Manor Castle, House of Lords, Parliament of the United Kingdom.
- Allen, H.K., et al., 2009. Functional metagenomics reveals diverse  $\beta$ -lactamases in a remote Alaskan soil. ISME J. 3, 243–251.
- Amaral-Zettler, L.A., 2022. Colonization of Plastic Marine Debris: The Known, the Unknown, and the Unknowable. Plastics and the Ocean: Origin, Characterization, Fate, and Impacts, pp. 301–316.
- Amaral-Zettler, L.A., et al., 2020. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151.
- Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
- Arias-Andres, M., et al., 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. Environ. Pollut. 237, 253–261.
- Atugoda, T., et al., 2020. Adsorptive interaction of antibiotic ciprofloxacin on polyethylene microplastics: implications for vector transport in water. Environ. Technol. Innov. 19, 100971.
- Bakhshinejad, B., Sadeghizadeh, M., 2016. A polystyrene binding target-unrelated peptide isolated in the screening of phage display library. Anal. Biochem. 512, 120–128.
- Bandow, N., et al., 2017. Contaminant release from aged microplastic. Environ. Chem. 14, 394–405.
- Banerjee, A., et al., 2022. Effects of polystyrene micro/nanoplastics on liver cells based on particle size, surface functionalization, concentration and exposure period. Sci. Total Environ. 836, 155621.
- Bhatt, P., et al., 2021. Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies. Environ. Res. 200, 111762.
- Bowley, J., et al., 2021. Oceanic hitchhikers-assessing pathogen risks from marine microplastic. Trends Microbiol. 29, 107–116.
- Bowley, J., et al., 2022. Pathogens transported by plastic debris: does this vector pose a risk to aquatic organisms? Emerging Top. Life Sci. 6, 349–358.
- Bryant, J.A., et al., 2016. Diversity and activity of communities inhabiting plastic debris in the North Pacific Gyre. MSystems 1, e00024-00016.
- Burns, E.E., Boxall, A.B., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796.
- Bydalek, F., et al., 2023. Microplastic biofilm, associated pathogen and antimicrobial resistance dynamics through a wastewater treatment process incorporating a constructed wetland. Water Res. 235, 119936.
- Cederlund, H., et al., 2016. Adsorption of pesticides with different chemical properties to a wood biochar treated with heat and iron. Water Air Soil Pollut. 227, 1–12.
- Cheng, Y., et al., 2022. Enhanced propagation of intracellular and extracellular antibiotic resistance genes in municipal wastewater by microplastics. Environ. Pollut. 292, 118284.
- CHMP, 2006. Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. European Medicines Agency EMEA/CHMP/SWP/4447/00.
- Cole, M., et al., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137.
- Cole, M., et al., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. Environ. Sci. Technol. 50, 3239–3246.
- Coyle, R., et al., 2020. Microplastics in the marine environment: a review of their sources, distribution processes, uptake and exchange in ecosystems. Case Stud. Chem. Environ. Eng. 2, 100010.
- Curren, E., Leong, S.C.Y., 2019. Profiles of bacterial assemblages from microplastics of tropical coastal environments. Sci. Total Environ. 655, 313–320.
- Dang, H., et al., 2008. Cross-ocean distribution of Rhodobacterales bacteria as primary surface colonizers in temperate coastal marine waters. Appl. Environ. Microbiol. 74, 52–60.

- De Tender, C.A., et al., 2015. Bacterial community profiling of plastic litter in the Belgian part of the North Sea. Environ. Sci. Technol. 49, 9629–9638.
- Delacuvellerie, A., et al., 2019. The plastisphere in marine ecosystem hosts potential specific microbial degraders including Alcanivorax borkumensis as a key player for the low-density polyethylene degradation. J. Hazard. Mater. 380, 120899.
- Deng, H., et al., 2022. Bacterial communities on polyethylene microplastics in mangrove ecosystems as a function of exposure sites: compositions and ecological functions. J. Environ. Chem. Eng. 107924.
- Du, Y., et al., 2022. A review on marine plastisphere: biodiversity, formation, and role in degradation. Comput. Struct. Biotechnol. J. 20, 975–988.
- Eckert, E.M., et al., 2018. Persistence of antibiotic resistance genes in large subalpine lakes: the role of anthropogenic pollution and ecological interactions. Hydrobiologia 824, 93–108.
- Edet, U.O., et al., 2022. Impact of "sachet water" microplastic on agricultural soil physicochemistry, antibiotics resistance, bacteria diversity and function. SN Appl. Sci. 4, 323.
- Erni-Cassola, G., et al., 2020. Early colonization of weathered polyethylene by distinct bacteria in marine coastal seawater. Microb. Ecol. 79, 517–526.
- EU, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
- Everaert, G., et al., 2018. Risk assessment of microplastics in the ocean: modelling approach and first conclusions. Environ. Pollut. 242, 1930–1938.
- Fan, X., et al., 2021. Adsorption and desorption behaviors of antibiotics by tire wear particles and polyethylene microplastics with or without aging processes. Sci. Total Environ. 771, 145451.
- Feltman, H., et al., 2001. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. Microbiology 147, 2659–2669.
- Feng, Y., et al., 2023. Microplastics exhibit accumulation and horizontal transfer of antibiotic resistance genes. J. Environ. Manag. 336, 117632.
- Foulon, V., et al., 2016. Colonization of polystyrene microparticles by Vibrio crassostreae: light and electron microscopic investigation. Environ. Sci. Technol. 50, 10988–10996.
- Frère, L., et al., 2018. Microplastic bacterial communities in the bay of Brest: influence of polymer type and size. Environ. Pollut. 242, 614–625.
- Gambardella, C., et al., 2018. Ecotoxicological effects of polystyrene microbeads in a battery of marine organisms belonging to different trophic levels. Mar. Environ. Res. 141, 313–321.

Gerba, C.P., 2000. Assessment of enteric pathogen shedding by bathers during

- recreational activity and its impact on water quality. Quant. Microbiol. 2, 55–68. Gillings, M.R., Stokes, H., 2012. Are humans increasing bacterial evolvability? Trends Ecol. Evol. 27, 346–352.
- Gomez Cortes, L., et al., 2022. Selection of substances for the 4th Watch List under the Water Framework Directive. Publications Office of the European Union, Luxembourg. https://doi.org/10.2760/909608. EUR 31148 EN (JRC130252).
- Gwenzi, W., 2022. Ten (10) key research questions on emerging contaminants and novel entities, and their health risks. In: Emerging Contaminants in the Terrestrial-Aquatic-Atmosphere Continuum. Elsevier, pp. 383–394.
- Hahladakis, J.N., et al., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199.
- Hansen, E., et al., 2013. Hazardous Substances in Plastic Materials. COWI in cooperation with Danish Technological Institute, pp. 7–8.
- Hansen, J., et al., 2021. Effect of polymer type on the colonization of plastic pellets by marine bacteria. FEMS Microbiol. Lett. 368, fnab026.
- Hayes, A., et al., 2022. Predicting selection for antimicrobial resistance in UK wastewater and aquatic environments: ciprofloxacin poses a significant risk. Environ. Int. 169, 107488.
- Hodgson, D., et al., 2018. Ingestion and fragmentation of plastic carrier bags by the amphipod Orchestia gammarellus: effects of plastic type and fouling load. Mar. Pollut. Bull. 127, 154–159.
- Hoellein, T.J., et al., 2017. Longitudinal patterns of microplastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. Freshw. Sci. 36, 491–507.
- Hou, D., et al., 2021. Prokaryotic community succession and assembly on different types of microplastics in a mariculture cage. Environ. Pollut. 268, 115756.
- Hu, H., et al., 2021. Distinct profile of bacterial community and antibiotic resistance genes on microplastics in Ganjiang River at the watershed level. Environ. Res. 200, 111363.
- Hu, X., et al., 2022a. Tetracycline accumulation in biofilms enhances the selection pressure on *Escherichia coli* for expression of antibiotic resistance. Sci. Total Environ. 159441.
- Hu, X., et al., 2022b. Impact of plastic particles on the horizontal transfer of antibiotic resistance genes to bacterium: dependent on particle sizes and antibiotic resistance gene vector replication capacities. Environ. Sci. Technol. 56 (21), 14948–14959.
- Imran, M., et al., 2019. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: an emerging health threat. Chemosphere 215, 846–857.
- Junaid, M., et al., 2022. Enrichment and dissemination of bacterial pathogens by microplastics in the aquatic environment. Sci. Total Environ. 154720.
- Junker, L.M., Hay, A.G., 2004. Effects of triclosan incorporation into ABS plastic on biofilm communities. J. Antimicrob. Chemother. 53, 989–996.
- Kampouris, I.D., et al., 2022. Elevated levels of antibiotic resistance in groundwater during treated wastewater irrigation associated with infiltration and accumulation of antibiotic residues. J. Hazard. Mater. 423, 127155.

#### E.M. Stevenson et al.

Kaur, K., et al., 2021. Microplastic-associated pathogens and antimicrobial resistance in environment. Chemosphere 133005.

Kelly, M.R., et al., 2022. Bacterial colonisation of plastic in the Rockall Trough, North-East Atlantic: an improved understanding of the deep-sea plastisphere. Environ. Pollut. 305, 119314.

- Kesy, K., et al., 2016. Polystyrene influences bacterial assemblages in *Arenicola marina*populated aquatic environments in vitro. Environ. Pollut. 219, 219–227.
- Kesy, K., et al., 2019. Spatial environmental heterogeneity determines young biofilm assemblages on microplastics in Baltic Sea mesocosms. Front. Microbiol. 10, 1665. Khalid, N., et al., 2021. Interactions and effects of microplastics with heavy metals in
- aquatic and terrestrial environments. Environ. Pollut. 290, 118104. Kim, S.Y., et al., 2022. Interactions between bacteria and nano (micro)-sized polystyrene particles. Chemosphere 135584.
- Kirstein, I.V., et al., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Mar. Environ. Res. 120, 1–8.
- Kirstein, I.V., et al., 2018. Mature biofilm communities on synthetic polymers in seawater-specific or general? Mar. Environ. Res. 142, 147–154.
- Kirstein, I.V., et al., 2019. The plastisphere–uncovering tightly attached plastic "specific" microorganisms. PLoS One 14, e0215859.
- Laganà, P., et al., 2019. Do plastics serve as a possible vector for the spread of antibiotic resistance? First insights from bacteria associated to a polystyrene piece from King George Island (Antarctica). Int. J. Hyg. Environ. Health 222, 89–100.
- Lai, K.P., et al., 2022. Microplastics act as a carrier for wastewater-borne pathogenic bacteria in sewage. Chemosphere 301, 134692.
- Lami, R., 2019. Quorum sensing in marine biofilms and environments. Quorum Sens. 55–96.
- Laverty, A., et al., 2016. Abundance and antibiotic susceptibility of Vibrio spp. isolated from microplastics. Am. Geophys. Union 2016, MM24C-0461.
- Lear, L., et al., 2022. Bacterial colonisation dynamics of household plastics in a coastal environment. Sci. Total Environ. 156199.
- Lee, J.-W., et al., 2008. Bacterial communities in the initial stage of marine biofilm formation on artificial surfaces. J. Microbiol. 46, 174–182.
- Leonard, A.F., et al., 2018. Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). Environ. Int. 114, 326–333.
- Li, H., et al., 2022b. Watershed urbanization enhances the enrichment of pathogenic bacteria and antibiotic resistance genes on microplastics in the water environment. Environ. Pollut. 120185.
- Li, J., et al., 2018. Adsorption of antibiotics on microplastics. Environ. Pollut. 237, 460–467.
- Li, Q., et al., 2022c. Plastisphere showing unique microbiome and resistome different from activated sludge. Sci. Total Environ. 158330.
- Li, Y., et al., 2022a. Size-dependent effects of polystyrene microplastics on anaerobic digestion performance of food waste: focusing on oxidative stress, microbial community, key metabolic functions. J. Hazard. Mater. 129493.
- Lindeque, P.K., et al., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environ. Pollut. 265, 114721.
- Liu, J., et al., 2018. Polystyrene nanoplastics-enhanced contaminant transport: role of irreversible adsorption in glassy polymeric domain. Environ. Sci. Technol. 52, 2677–2685.
- Liu, S., et al., 2022a. Integrated effects of polymer type, size and shape on the sinking dynamics of biofouled microplastics. Water Res. 118656.
- Liu, S., et al., 2023b. Doxycycline combined manure microbes to enhances biofilm formation of the soil plastisphere and increases the surface bio-risk of microplastics vehicle. Chem. Eng. J. 454, 140530.
- Liu, X., et al., 2022b. Nano-and microplastics aided by extracellular polymeric substances facilitate the conjugative transfer of antibiotic resistance genes in bacteria. ACS ES&T Water 2, 2528–2537.
- Liu, X., et al., 2022c. Do microplastic biofilms promote the evolution and co-selection of antibiotic and metal resistance genes and their associations with bacterial communities under antibiotic and metal pressures? J. Hazard. Mater. 424, 127285.
- Liu, X., et al., 2022d. Microplastics aging in wastewater treatment plants: focusing on physicochemical characteristics changes and corresponding environmental risks. Water Res. 118780.
- Liu, X., et al., 2023a. Nanoplastics promote the dissemination of antibiotic resistance through conjugative gene transfer: implications from oxidative stress and gene expression. Environ. Sci. Nano 10 (5), 1329–1340.
- Liu, X., et al., 2023c. Effects comparison between the secondary nanoplastics released from biodegradable and conventional plastics on the transfer of antibiotic resistance genes between bacteria. Environ. Pollut. 317, 120680.
- Liu, Y.-J., et al., 2023d. Antibiotic resistomes in face-mask biofilm along an urban river: multiple drivers and co-occurrence with human opportunistic pathogens. J. Hazard. Mater. 455, 131587.
- Lu, X.-M., Chen, Y.-L., 2022. Varying characteristics and driving mechanisms of antibiotic resistance genes in farmland soil amended with high-density polyethylene microplastics. J. Hazard. Mater. 428, 128196.
- Lu, X.-M., et al., 2020. Fate and abundance of antibiotic resistance genes on microplastics in facility vegetable soil. Sci. Total Environ. 709, 136276.
- Luo, H., et al., 2020. Effects of accelerated aging on characteristics, leaching, and toxicity of commercial lead chromate pigmented microplastics. Environ. Pollut. 257, 113475.
- Luo, T., et al., 2023. Different microplastics distinctively enriched the antibiotic resistance genes in anaerobic sludge digestion through shifting specific hosts and promoting horizontal gene flow. Water Res. 228, 119356.

- Lyons, M., et al., 2010. Theory of island biogeography on a microscopic scale: organic aggregates as islands for aquatic pathogens. Aquat. Microb. Ecol. 60, 1–13.
- Ma, J., et al., 2020. Microplastics combined with tetracycline in soils facilitate the formation of antibiotic resistance in the *Enchytraeus crypticus* microbiome. Environ. Pollut. 264, 114689.
- Mahenthiralingam, E., et al., 1994. Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. Infect. Immun. 62, 596–605.
- Martínez-Campos, S., et al., 2023. Time-course biofilm formation and presence of antibiotic resistance genes on everyday plastic items deployed in river waters. J. Hazard. Mater. 443, 130271.
- Massos, A., Turner, A., 2017. Cadmium, lead and bromine in beached micro- plastics. Environ. Pollut. 227, 139–145.
- McCann, C.M., et al., 2019. Understanding drivers of antibiotic resistance genes in high Arctic soil ecosystems. Environ. Int. 125, 497–504.
- McCormick, A., et al., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48, 11863–11871.
- McCormick, A.R., et al., 2016. Microplastic in surface waters of urban rivers:
- concentration, sources, and associated bacterial assemblages. Ecosphere 7, e01556. Metcalf, R., et al., 2021. Quantifying the importance of plastic pollution for the
- dissemination of human pathogens: the challenges of choosing an appropriate 'control' material. Sci. Total Environ. 152292.
- Metcalf, R., et al., 2022. Sewage-associated plastic waste washed up on beaches can act as a reservoir for faecal bacteria, potential human pathogens, and genes for antimicrobial resistance. Mar. Pollut. Bull. 180, 113766.
- Miao, L., et al., 2019. Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci. Total Environ. 650, 2395–2402.
- Moradigaravand, D., et al., 2022. Plasmid Permissiveness of Wastewater Microbiomes Can Be Predicted From 16S rDNA Sequences by Machine Learning. bioRxiv (2022.2007. 2009.499415).
- Morrison, A., et al., 2014. Gulls as carriers of antibiotic resistance in Northern Ireland. In: Northern Ireland Seabird Report 2014.
- Murray, A.K., et al., 2018. Novel insights into selection for antibiotic resistance in complex microbial communities. MBio 9, e00969-00918.
- Murray, A.K., et al., 2019. Comparing the selective and co-selective effects of different antimicrobials in bacterial communities. Int. J. Antimicrob. Agents 53, 767–773.
- Murray, A.K., et al., 2021. Dawning of a new ERA: environmental risk assessment of antibiotics and their potential to select for antimicrobial resistance. Water Res. 200, 117233.
- Murray, C.J., et al., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399, 629–655.
- Muthukrishnan, T., et al., 2019. Fouling microbial communities on plastics compared with wood and steel: are they substrate-or location-specific? Microb. Ecol. 78, 361–374.
- Napper, I.E., et al., 2020. Reaching new heights in plastic pollution—preliminary findings of microplastics on Mount Everest. One Earth 3, 621–630.
- Naz, I., et al., 2016. Effect of the chemical composition of filter media on the microbial community in wastewater biofilms at different temperatures. RSC Adv. 6, 104345–104353.
- Obbard, R.W., et al., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future 2, 315–320.
- Oberbeckmann, S., et al., 2014. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. FEMS Microbiol. Ecol. 90, 478–492.
- Oberbeckmann, S., et al., 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. Front. Microbiol. 8, 2709.
- Ogonowski, M., et al., 2018. Evidence for selective bacterial community structuring on microplastics. Environ. Microbiol. 20, 2796–2808.
- Ormsby, M.J., et al., 2023. Clinically important *E. coli* strains can persist, and retain their pathogenicity, on environmental plastic and fabric waste. Environ. Pollut. 326, 121466.
- Pal, C., et al., 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16, 1–14.

Paluselli, A., et al., 2018. Phthalate release from plastic fragments and degradation in seawater. Environ. Sci. Technol. 53, 166–175.

- Parrish, K., Fahrenfeld, N., 2019. Microplastic biofilm in fresh-and wastewater as a function of microparticle type and size class. Environ. Sci.: Water Res. Technol. 5, 495–505.
- Parsek, M.R., Singh, P.K., 2003. Bacterial biofilms: an emerging link to disease pathogensis. Annu. Rev. Microbiol. 57, 677.
- Peeken, I., et al., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9, 1505.
- Perveen, S., et al., 2022. Growth and prevalence of antibiotic-resistant bacteria in microplastic biofilm from wastewater treatment plant effluents. Sci. Total Environ. 159024.
- Pham, D.N., et al., 2021. Microplastics as hubs enriching antibiotic-resistant bacteria and pathogens in municipal activated sludge. J. Hazard. Mater. Lett. 2, 100014.
- Phothakwanpracha, J., et al., 2021. Effects of sizes and concentrations of different types of microplastics on bioaccumulation and lethality rate in the green mussel, *Perna viridis*. Mar. Pollut. Bull. 173, 112954.
- Pinnell, L.J., Turner, J.W., 2019. Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. Front. Microbiol. 1252.
- Pinnell, L.J., Turner, J.W., 2020. Temporal changes in water temperature and salinity drive the formation of a reversible plastic-specific microbial community. FEMS Microbiol. Ecol. 96, fiaa230.

#### Science of the Total Environment 908 (2024) 168234

#### E.M. Stevenson et al.

Pinto, M., et al., 2019. The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. PLoS One 14, e0217165.

- Polanco, H., et al., 2020. The presence and significance of microplastics in surface water in the Lower Hudson River Estuary 2016–2019: a research note. Mar. Pollut. Bull. 161, 111702.
- Quilliam, R.S., et al., 2014. Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments. Mar. Pollut. Bull. 84, 201–207.
- Radisic, V., et al., 2020. Marine plastics from Norwegian west coast carry potentially virulent fish pathogens and opportunistic human pathogens harboring new variants of antibiotic resistance genes. Microorganisms 8, 1200.
- Redford, D.P., et al., 1997. Sources of plastic pellets in the aquatic environment. In: Marine Debris. Springer, pp. 335–343.
- Reisser, J., et al., 2014. Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. PLoS One 9, e100289.
- Ren, Z., et al., 2023. P, N, and C-related functional genes in SBR system promoted antibiotics resistance gene transmission under polystyrene microplastics stress. Water Res. 235, 119884.
- Reverter, M., et al., 2020. Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat. Commun. 11, 1–8.
- Rhodes, C.J., 2018. Plastic pollution and potential solutions. Sci. Prog. 101, 207–260. Richard, H., et al., 2019. Biofilm facilitates metal accumulation onto microplastics in
- estuarine waters. Sci. Total Environ. 683, 600–608. Rillig, M.C., et al., 2018. Evolutionary implications of microplastics for soil biota. Environ. Chem. 16, 3–7.
- Robinson, T.P., et al., 2016. Antibiotic resistance is the quintessential One Health issue. Trans. R. Soc. Trop. Med. Hyg. 110, 377–380.
- Rockström, J., et al., 2009. Planetary boundaries: exploring the safe operating space for humanity. Ecol. Soc. 14.
- Rodrigues, A., et al., 2019. Colonisation of plastic pellets (nurdles) by *E. coli* at public bathing beaches. Mar. Pollut. Bull. 139, 376–380.
- Rogers, J., et al., 1994. Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. Appl. Environ. Microbiol. 60, 1842–1851.
- Salta, M., et al., 2013. Marine biofilms on artificial surfaces: structure and dynamics. Environ. Microbiol. 15, 2879–2893.
- Schmidt, V.T., et al., 2014. Oligotyping reveals community level habitat selection within the genus Vibrio. Front. Microbiol. 5, 563.
- Shen, M., et al., 2022. Microplastics in landfill and leachate: occurrence, environmental behavior and removal strategies. Chemosphere 135325.
- Shi, J., et al., 2022b. Distinguishing removal and regrowth potential of antibiotic resistance genes and antibiotic resistant bacteria on microplastics and in leachate after chlorination or Fenton oxidation. J. Hazard. Mater. 430, 128432.
- Shi, J., et al., 2023. Insight into the responses of antibiotic resistance genes in microplastic biofilms to zinc oxide nanoparticles and zinc ions pressures in landfill leachate. J. Hazard. Mater. 132096.
- Shi, K., et al., 2022c. Adsorption behaviors of triclosan by non-biodegradable and
- biodegradable microplastics: kinetics and mechanism. Sci. Total Environ. 156832. Shi, Z., et al., 2022a. Alteration of bacterial communities and co-occurrence networks as a legacy effect upon exposure to polyethylene residues under field environment.
- J. Hazard. Mater. 426, 128126. Silva, M.M., et al., 2019. Dispersal of potentially pathogenic bacteria by plastic debris in
- Guanabara Bay, RJ, Brazil. Mar. Pollut. Bull. 141, 561–568. Song, J., et al., 2020. The travelling particles: investigating microplastics as possible
- transport vectors for multidurg resistant *E. coli* in the Weser estuary (Germany). Sci. Total Environ. 720, 137603.
- Song, J., et al., 2022. The travelling particles: community dynamics of biofilms on microplastics transferred along a salinity gradient. ISME Commun. 2, 1–12.
- Sørensen, L., et al., 2020. Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co-exposure conditions. Environ. Pollut. 258, 113844.
- Steinmetz, Z., et al., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550, 690–705.
- Stevenson, E.M., et al., 2022. Antifungal exposure and resistance development: defining minimal selective antifungal concentrations and testing methodologies. Front. Fungal Biol. 3.
- Su, Y., et al., 2021. Microplastics act as vectors for antibiotic resistance genes in landfill leachate: the enhanced roles of the long-term aging process. Environ. Pollut. 270, 116278.
- Sun, J., et al., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37.
- Sun, M., et al., 2018. Changes in tetracycline partitioning and bacteria/phagecomediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid. J. Hazard. Mater. 345, 131–139.
- Sun, M., et al., 2022a. Deciphering the role of microplastic size on anaerobic sludge digestion: changes of dissolved organic matter, leaching compounds and microbial community. Environ. Res. 214, 114032.
- Sun, R., et al., 2022b. Impact of the surrounding environment on antibiotic resistance genes carried by microplastics in mangroves. Sci. Total Environ. 837, 155771.
- Sun, X., et al., 2020. Impact of mariculture-derived microplastics on bacterial biofilm formation and their potential threat to mariculture: a case in situ study on the Sungo Bay, China. Environ. Pollut. 262, 114336.
- Sun, Y., et al., 2023. Adsorption and desorption mechanisms of oxytetracycline on poly (butylene adipate-co-terephthalate) microplastics after degradation: the effects of biofilms, Cu (II), water pH, and dissolved organic matter. Sci. Total Environ. 863, 160866.

- Science of the Total Environment 908 (2024) 168234
- Tagg, A.S., et al., 2019. Paint particles are a distinct and variable substrate for marine bacteria. Mar. Pollut. Bull. 146, 117–124.
- Tagg, A.S., et al., 2022. Agricultural application of microplastic-rich sewage sludge leads to further uncontrolled contamination. Sci. Total Environ. 806, 150611.
- Tan, B., et al., 2022. Microplastics accumulation in mangroves increasing the resistance of its colonization Vibrio and Shewanella. Chemosphere 295, 133861.
- Tang, S., et al., 2022. Adsorption mechanisms of metal ions (Pb, Cd, Cu) onto polyamide 6 microplastics: New insight into environmental risks in comparison with natural media in different water matrices. Gondwana Res. 110, 214–225.
- Thompson, R.C., et al., 2004. Lost at sea: where is all the plastic? Science 304, 838.
- Tian, Y., et al., 2023. Photoaging processes of polyvinyl chloride microplastics enhance the adsorption of tetracycline and facilitate the formation of antibiotic resistance. Chemosphere 320, 137820.
- Tuorto, S.J., et al., 2014. Bacterial genome replication at subzero temperatures in permafrost. ISME J. 8, 139–149.
- Turner, A., 2017. Trace elements in fragments of fishing net and other filamentous plastic litter from two beaches in SW England. Environ. Pollut. 224, 722–728.
- Turner, A., Filella, M., 2021. Hazardous metal additives in plastics and their environmental impacts. Environ. Int. 156, 106622.
- Turner, A., et al., 2019. Identification, origin and characteristics of bio-bead microplastics from beaches in western Europe. Sci. Total Environ. 664, 938–947.
- UKWIR, 2020. Chemical Investigations Programme, 2015–2020.
  Vaksmaa, A., et al., 2021. Microbial communities on plastic polymers in the Mediterranean Sea. Front. Microbiol. 12, 673553.
- Van Franeker, J.A., et al., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environ. Pollut. 159, 2609–2615.
- Viršek, M.K., et al., 2017. Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Mar. Pollut. Bull. 125, 301–309.
- Vlaanderen, E.J., et al., 2023. Plastic leachate exposure drives antibiotic resistance and virulence in marine bacterial communities. Environ. Pollut. 327, 121558.
- Vosshage, A.T., et al., 2018. Plastic alters biofilm quality as food resource of the freshwater gastropod Radix balthica. Environ. Sci. Technol. 52, 11387–11393.
- Wang, C., et al., 2022a. Different sizes of polystyrene microplastics induced distinct microbial responses of anaerobic granular sludge. Water Res. 118607.
- Wang, H., et al., 2022g. Adsorption of tetracycline and Cd (II) on polystyrene and polyethylene terephthalate microplastics with ultraviolet and hydrogen peroxide aging treatment. Sci. Total Environ. 157109.
- Wang, H., et al., 2023. Microplastic biofilm: an important microniche that may accelerate the spread of antibiotic resistance genes via natural transformation. J. Hazard. Mater. 132085.
- Wang, J., et al., 2022c. Slower antibiotics degradation and higher resistance genes enrichment in plastisphere. Water Res. 222, 118920.
- Wang, S., et al., 2020. Selectively enrichment of antibiotics and ARGs by microplastics in river, estuary and marine waters. Sci. Total Environ. 708, 134594.
- Wang, S., et al., 2022d. Deciphering the role of polyethylene microplastics on antibiotic resistance genes and mobile genetic elements fate in sludge thermophilic anaerobic digestion process. Chem. Eng. J. 139520.
- Wang, X., et al., 2022e. A neglected risk of nanoplastics as revealed by the promoted transformation of plasmid-borne ampicillin resistance gene by *Escherichia coli*. Environ. Microbiol. 24 (10), 4946–4959.
- Wang, Y., et al., 2021a. Effects of coexistence of tetracycline, copper and microplastics on the fate of antibiotic resistance genes in manured soil. Sci. Total Environ. 790, 148087.
- Wang, Z., et al., 2021b. Plastisphere enrich antibiotic resistance genes and potential pathogenic bacteria in sewage with pharmaceuticals. Sci. Total Environ. 768, 144663

Wang, Z., et al., 2022b. Size-dependent effects of microplastics on antibiotic resistance genes fate in wastewater treatment systems: the role of changed surface property and microbial assemblages in a continuous exposure mode. Sci. Total Environ. 158264.

- Wang, Z., et al., 2022f. Sorption of selected pharmaceutical compounds on polyethy-lene microplastics: roles of pH, aging, and competitive sorption. Chemosphere 135561.
- Wen, B., et al., 2020. Community structure and functional diversity of the plastisphere in aquaculture waters: does plastic color matter? Sci. Total Environ. 740, 140082.
- Woodall, L.C., et al., 2018. Deep-sea anthropogenic macrodebris harbours rich and diverse communities of bacteria and archaea. PLoS One 13, e0206220.
- Wright, R.J., et al., 2020. Marine plastic debris: a new surface for microbial colonization. Environ. Sci. Technol. 54, 11657–11672.
- Wright, R.J., et al., 2021. Food or just a free ride? A meta-analysis reveals the global diversity of the Plastisphere. ISME J. 15, 789–806.
- Wu, C., et al., 2022a. Effect of particle size on the colonization of biofilms and the potential of biofilm-covered microplastics as metal carriers. Sci. Total Environ. 821, 153265.
- Wu, X., et al., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res. 165, 114979.
- Wu, X., et al., 2022b. Integrated metagenomic and metatranscriptomic analysis reveals actively expressed antibiotic resistomes in the plastisphere. J. Hazard. Mater. 430, 128418.
- Xu, G., Yu, Y., 2021. Polystyrene microplastics impact the occurrence of antibiotic resistance genes in earthworms by size-dependent toxic effects. J. Hazard. Mater. 416, 125847.
- Yang, L., et al., 2019a. Removal of microplastics in municipal sewage from China's largest water reclamation plant. Water Res. 155, 175–181.
- Yang, Y., et al., 2019b. Plastics in the marine environment are reservoirs for antibiotic and metal resistance genes. Environ. Int. 123, 79–86.

#### E.M. Stevenson et al.

- Yang, Z., et al., 2022. Alteration in microbial community and antibiotic resistance genes mediated by microplastics during wastewater ultraviolet disinfection. Sci. Total Environ. 825, 153918.
- Yu, X., et al., 2022. Selective adsorption of antibiotics on aged microplastics originating from mariculture benefits the colonization of opportunistic pathogenic bacteria. Environ. Pollut. 120157.
- Yu, X., et al., 2023. Microplastics exacerbate co-occurrence and horizontal transfer of antibiotic resistance genes. J. Hazard. Mater. 451, 131130.
- Yuan, Q., et al., 2022. UV-aging of microplastics increases proximal ARG donor-recipient adsorption and leaching of chemicals that synergistically enhance antibiotic resistance propagation. J. Hazard. Mater. 427, 127895.
- Zeng, Q., et al., 2023. Microplastics affect nitrogen cycling and antibiotic resistance genes transfer of sediment. Chem. Eng. J. 454, 140193.
- Zettler, E.R., et al., 2013. Life in the "plastisphere": microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146.
- Zha, Y., et al., 2022. Size-dependent enhancement on conjugative transfer of antibiotic resistance genes by micro/nanoplastics. J. Hazard. Mater. 431, 128561.
- Zhang, G., et al., 2022a. Conjugative antibiotic-resistant plasmids promote bacterial colonization of microplastics in water environments. J. Hazard. Mater. 430, 128443.
- Zhang, H., et al., 2018. Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: kinetics, isotherms and influencing factors. Environ. Pollut. 243, 1550–1557.

- Zhang, P., et al., 2022c. Metagenomic analysis explores the interaction of aged microplastics and roxithromycin on gut microbiota and antibiotic resistance genes of Carassius auratus. J. Hazard. Mater. 425, 127773.
- Zhang, S., et al., 2022b. Microplastics can selectively enrich intracellular and extracellular antibiotic resistant genes and shape different microbial communities in aquatic systems. Sci. Total Environ. 822, 153488.
- Zhao, Y., et al., 2021. Responses of bacterial communities and resistance genes on microplastics to antibiotics and heavy metals in sewage environment. J. Hazard. Mater. 402, 123550.
- Zhao, Y., et al., 2023. Size-dependent promotion of micro (nano) plastics on the horizontal gene transfer of antibiotic resistance genes in constructed wetlands. Water Res. 244, 120520.
- Zhou, Q., et al., 2022a. Persistent versus transient, and plastic versus bioplastic?—two key questions about microplastic-water exchange of antibiotic resistance genes. Water Res. 118899.
- Zhou, Z., et al., 2022b. Adsorption behavior of Cu (II) and Cr (VI) on aged microplastics in antibiotics-heavy metals coexisting system. Chemosphere 291, 132794.
- Zhu, D., et al., 2022. Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens. ISME J. 16, 521–532.