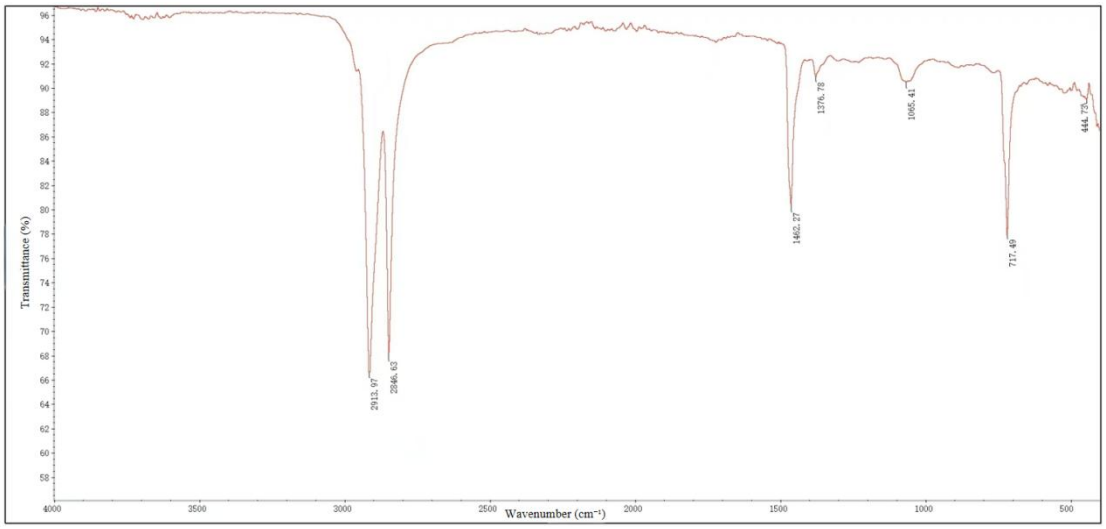
Supplementary Information



Supplementary Figure S1. Fourier-transform infrared spectroscopy (FTIR) spectrum of the polyethylene microplastics used in this study. Characteristic absorption bands at 2914, 2845, 1462, and 717 cm^{-1} confirmed the polymer identity of the particles.

Supplementary Table S1. NCBI accession numbers for the 16S rRNA gene amplicon sequencing and shotgun metagenomic sequencing datasets.

Sample ID	Sample type	BioSample accession	16S rRNA gene amplicon Run accession	Shotgun metagenomic Run accession
A_Water_Control	Water-phase control from WWTP-A	SAMN61800735	SRR39711610	SRR39717925
A_PE_Biofilm	Polyethylene-associated biofilm from WWTP-A	SAMN61800736	SRR39711609	SRR39717924
B_Water_Control	Water-phase control from WWTP-B	SAMN61800737	SRR39711608	SRR39717923
B_PE_Biofilm	Polyethylene-associated biofilm from WWTP-B	SAMN61800738	SRR39711607	SRR39717922
C_Water_Control	Water-phase control from WWTP-C	SAMN61800739	SRR39711606	SRR39717921
C_PE_Biofilm	Polyethylene-associated biofilm from WWTP-C	SAMN61800740	SRR39711605	SRR39717920
D_Water_Control	Water-phase control from WWTP-D	SAMN61800741	SRR39711604	SRR39717919
D_PE_Biofilm	Polyethylene-associated biofilm from WWTP-D	SAMN61800742	SRR39711603	SRR39717918

Supplementary Table S2. Primers used for quantitative PCR quantification of ARG and MGE

markers.

Target gene	Primer	Sequence (5′–3′)
16S rRNA gene	16S-F	ACTCCTACGGGAGGCAGCAG
	16S-R	ATTACCGCGGCTGCTGG
int02	int02-F	CCGTGGGTGGAGATGAAGT
	int02-R	TACATGATGGCCGACTCCG
tet(39)	tet(39)-F	GGAACTTACATGCACCGTTC
	tet(39)-R	CTCCTGAACCTTCTCAGTCA
floR	floR-F	GCGATATTCATTACTTTGGC
	floR-R	TAGGATGAAGGTGAGGAATG

Supplementary Table S3. Ct values obtained from qPCR quantification of 16S rRNA gene, ARGs, and MGE markers in paired water-phase and PE biofilm samples.

	A-PE	A-Water	B-PE	B-Water	C-PE	C-Water	D-PE	D-Water
16S	16.02	17.51	14.92	15.26	14.26	16.32	14.91	16.53
int02	24.29	24.19	21.74	22.35	21.47	23.31	22.45	23.66
tet(39)	28.59	32.75	29.33	32.67	29.8	32.46	24.53	33.94
floR	26.54	29.19	25.48	26.93	24.82	27.71	25.82	29.6

Ct values represent the mean values of technical qPCR replicates.

Supplementary Table S4. Correspondence between abbreviated mobile genetic element (MGE) marker labels and original annotation identifiers used in ARG–MGE co-variation analysis.

Original feature annotation	Figure_label	Database identifier	MGE	Reference sequence description
IS elements|ISCsp4	ISCsp4	ISCsp4	IS elements	Comamonas sp,Comamonas sp plasmid
IS elements|ISPa38,TnPa38	ISPa38,TnPa38	ISPa38,TnPa38	IS elements	Pseudomonas aeruginosa DK2
Plasmid|NZ_LN868939.1	P11	NZ_LN868939.1	Plasmid	Nocardia farcinica strain NCTC11134 plasmid 2, complete sequence
Integron|CP020917.1	Int07	CP020917.1	Integron	Achromobacter denitrificans
Plasmid|NZ_CP070344.1	P08	NZ_CP070344.1	Plasmid	Variovorax sp. PDNC026 plasmid unnamed, complete sequence
Plasmid|NZ_CP025986.1	P03	NZ_CP025986.1	Plasmid	Ralstonia solanacearum strain RSCM plasmid p-unname2, complete sequence
Plasmid|NZ_CP068286.1	P02	NZ_CP068286.1	Plasmid	Ralstonia syzygii subsp.

Plasmid|NZ_CP063167.1	P07	NZ_CP063167.1	Plasmid	celebesensis strain UGMSS_Db01 plasmid pRscUGMSS_Db01 Variovorax paradoxus strain VAI-C plasmid unnamed, complete sequence
Integron|CP069810.1	Int06	CP069810.1	Integron	Cupriavidus oxalaticus
Integron|CP018839.1	Int04	CP018839.1	Integron	Thauera chlorobenzoica
Integron|CP014646.1	Int05	CP014646.1	Integron	Thauera humireducens
IS elements|ISPxasp1	ISPxasp1	ISPxasp1	IS elements	Pseudoxanthomonas sp.,Pseudoxanthomonas sp. SE1
IS elements|TnAzs17,ISAzs17	TnAzs17,ISAzs17	TnAzs17,ISAzs17	IS elements	Azospirillum sp. B510
Plasmid|NZ_CP043441.1	P12	NZ_CP043441.1	Plasmid	Cupriavidus campinensis strain MJ1 plasmid unnamed1, complete sequence
Plasmid|NZ_CP076061.1	P10	NZ_CP076061.1	Plasmid	Cupriavidus sp. EM10 plasmid p352-1, complete sequence
Integron|CP027667.1	Int01	CP027667.1	Integron	Melaminivora sp. SC2-9
Plasmid|NZ_CP010410.1	P04	NZ_CP010410.1	Plasmid	Xanthomonas sacchari strain R1 plasmid unnamed, complete sequence
Integron|CP000744.1	Int12	CP000744.1	Integron	Pseudomonas aeruginosa PA7
Integron|AP023189.1	Int08	AP023189.1	Integron	Pseudomonas sp. TUM18999
Integron|CP023641.1	Int11	CP023641.1	Integron	Pseudomonas mendocina
Integron|AP024354.1	Int09	AP024354.1	Integron	Pseudomonas alcaligenes
IS elements|TnAs2	TnAs2	TnAs2	IS elements	Aeromonas salmonicida,Aeromonas salmonicida subsp. pectinolytica 34mel
Integron|CP017311.1	Int02	CP017311.1	Integron	Hydrogenophaga sp. PBC
IS elements|ISAba33	ISAba33	ISAba33	IS elements	Acinetobacter baumannii pMMD,Acinetobacter baumannii isolate BAL_056; ENA accession

IS elements|ISDev1	ISDev1	ISDev1	IS elements	ERR190414,Acinetobacter haemolyticus DNA Devosia sp. I507,Aquamicrobium sp.
IS elements|ISSphsp2	ISSphsp2	ISSphsp2	IS elements	Sphingobium sp.,Sphingobium sp. SA2 Sphingopyxis sp.
IS elements|ISSphsp7	ISSphsp7	ISSphsp7	IS elements	SE2,Sphingobium sp.,Sphingobium sp. SA2,Sphingobium yanoikuyae
Integron|CP001220.2	Int10	CP001220.2	Integron	SK-NIH.Env6_1116,Sphingopyxis sp. Comamonas testosteroni CNB-2
Plasmid|NC_017958.1	P05	NC_017958.1	Plasmid	Tistrella mobilis KA081020-065 plasmid pTM3, complete sequence
IS elements|TnAs3	TnAs3	TnAs3	IS elements	Aeromonas salmonicida subsp. salmonicida A449 plasmid 4
IS elements|ISSpma2	ISSpma2	ISSpma2	IS elements	Sphingopyxis macrogoltabida
IS elements|ISR1	ISR1	ISR1	IS elements	Rhizobium lupini class IV plasmid pRP4
Plasmid|NZ_CP022367.1	P09	NZ_CP022367.1	Plasmid	Azospirillum sp. TSH58 plasmid TSH58_p02, complete sequence
Plasmid|NZ_CP069303.1	P01	NZ_CP069303.1	Plasmid	Shinella sp. PSBB067 plasmid unnamed1, complete sequence
Integron|CP017781.1	Int03	CP017781.1	Integron	Rhodobacter sp. LPB0142
Plasmid|NZ_AP014705.1	P06	NZ_AP014705.1	Plasmid	Methylobacterium aquaticum strain MA-22A plasmid pMaq22A_1p, complete sequence

Supplementary Methods

1. qPCR normalization and PE-associated enrichment calculation

The qPCR data were analyzed using a 16S rRNA gene-normalized approach to compare the relative abundance of selected antibiotic resistance genes (ARGs) and mobile genetic element

(MGE) markers between polyethylene (PE) biofilm and paired water-phase samples.

For each sample, the relative abundance of target genes was first normalized to the bacterial 16S rRNA gene using the threshold cycle (Ct) values. The relative abundance of target genes was normalized against the bacterial 16S rRNA gene based on the difference between the Ct values of target genes and the 16S rRNA gene:

$$\Delta C_t = C_{t,target} - C_{t,16S}$$

For each wastewater treatment plant (WWTP), PE biofilm samples were directly compared with their corresponding water-phase controls. The relative difference between the two habitats was calculated as:

$$\Delta\Delta C_t = \Delta C_{t,PE} - \Delta C_{t,Water}$$

To facilitate interpretation of habitat-associated tendencies, the value of $-\Delta\Delta$Ct was used as the relative PE-associated enrichment index. Positive values indicate that the target marker showed a higher 16S-normalized abundance in PE biofilms, whereas negative values indicate preferential occurrence in water-phase samples.

2. ARG–MGE network construction and feature selection

ARG–MGE co-variation analysis was performed using representative ARG subtypes and MGE markers detected in the metagenomic annotation results.

Only ARG subtypes and MGE markers with detectable signals across the analyzed samples were included for network construction. Pairwise Spearman correlation coefficients were calculated between ARG and MGE features across the eight paired samples, including water-phase and PE biofilm samples from four WWTPs.

Positive associations with Spearman's rho ≥ 0.70 were retained as co-variation links and visualized in the ARG–MGE network. MGE markers were classified according to their annotation categories, including plasmid-associated elements, integron-associated elements, and insertion sequence (IS)-associated elements.