Supporting Information (SI)

(Nano)microplastics promote the propagation of antibiotic resistance genes in landfill leachate

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Table S1 Characteristics of original leachate used in (nano)microplastics exposure experiment.

Parameters	Original leachate
pH	7.9 ± 0.1
Conductivity (ms/cm)	19.2 ± 0.4
DOC (mg/L)	5395 ± 172
DON (mg/L)	2538 ± 145
$NH4^+$-N (mg/L)	1304 ± 86
NO_3^--N (mg/L)	21.5 ± 2.8

DOC: Dissolved organic carbon; DON: Dissolved organic nitrogen; $NH4^+$-N: Ammonia nitrogen; NO_3^--N: nitrate nitrogen.

Table S2. Hydraulic diameters of the different polystyrene beads.

Polystyrene beads	Particle Size
50-100 nm	72±6 nm
200-500 nm	315±28 nm
9.0-9.9 μm	9.3±0.2 μm

The landfill leachate as a special substrate contains high organic carbon and other nutrients. In this study, the content of dissolved organic carbon (DOC) and nitrogen (DON) in the original leachate was 5395 mg/L and 1938 mg/L, respectively. After 60 days, the content of DOC in the control and N/MP-exposed groups were 2487 mg/L and 2897-3260 mg/L, and the content of DON were 1032 mg/L and 1169-1374 mg/L, respectively. Besides, according to our previous experiments (Shi et al., 2019), the content of dissolved organic carbon in leachate with longer landfill age was ~ 1500mg/L and decreased gradually over a period of nearly two months, indicating that it was available to microbes. Thus, microbes were not suffered from the limitation of carbon and nitrogen sources in this study during the 60 days incubation periods.

Parameters	Leachate after 60 days			
	Control	50-100 nm	200-500 nm	9.0-9.9 μm
DOC (mg/L)	2487 ± 56	2943 ± 137	3260 ± 37	2897 ± 195
DON (mg/L)	1032 ± 98	1169 ± 174	1374 ± 135	1205 ± 204

Table S3 Primers and PCR conditions for gene analyses.

Targeted genes	Amplicon length (bp)	Primer sequences (5' – 3')	Annealing temperatures (℃)
*sul*1	172	FW CACCGGAAACATCGCTGCA RV AAGTTCCGCCGCAAGGCT	55
*sul*2	165	FW CTCCGATGGAGGCCGGTAT RV GGGAATGCCATCTGCCTTGA	55
*aad*A1	195	FW AGCTAAGCGCGAACTGCAAT RV TGGCTCGAAGATACCTGCAA	55
*str*B	185	FW GCTCGGTCGTGAGAACAATCT RV CAATTTCGGTCGCCTGGTAGT	55
*erm*B	139	FW AAAACTTACCCGCCATAC CA RV TTTGGCGTGTTTCATTGC TT	60
*mef*A	186	FW ATACCCCAG CACTCAATTCG RV CAATCACAGCACCCA ATACG	59
bla_{TEM}	211	FW ATGTGCAGYACCAGTAARGTKATGGC RV ATCACKCGGRTCGCCNGGRAT	57.5
bla_{OXA}	195	FW CGGATGGTTTGAAGGGTTTATTAT RV TCTTGGCTTTTATGCTTGATGTTAA	57
intl1	190	FW GGCTTCGTGATGCCTGCTT RV CATTCCTGGCCGTGGTTCT	57
intl2	143	FW GTTATTTTATTGCTGGGATTAGGC RV TTTTACGCTGCTGTATGGTGC	57
traA	272	FW AAGTGTTCAGGGTGCTTCTGCGC RV GTCATGTACATGATGACCAAAA	62
trbC	255	FW CGGYATWCCGSCSACRCTGCG RV GCCACCTGYSBGCAGTCMCC	60
16S rRNA	193	FW CCTACGGGAGGCAGCAG RV TTACCGCGGCTGCTGGCAC	55

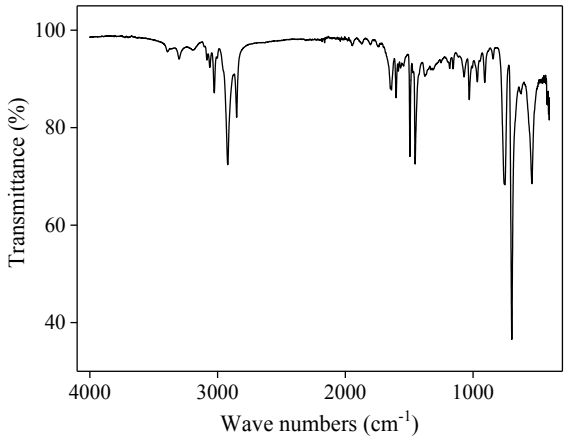

Fig. S1 The FTIR spectrum of polystyrene beads.

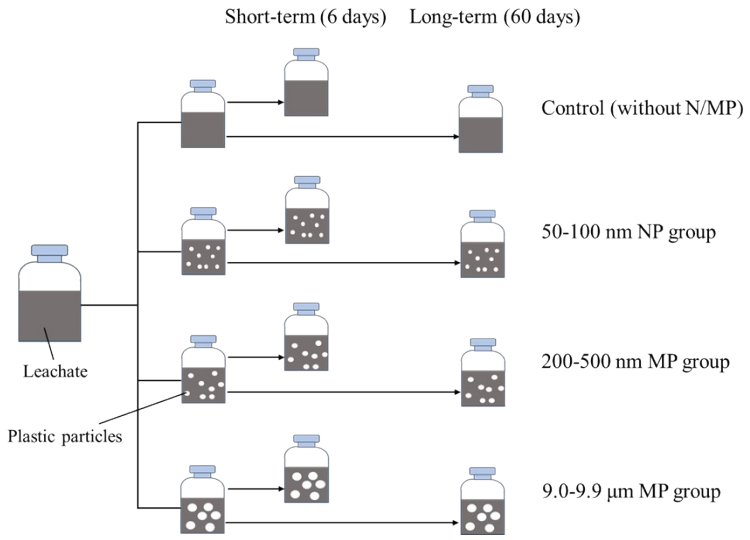

Fig. S2 The schematic diagram showing the experimental set-up of N/MPs exposure.

Intracellular ROS production measurement

The mixtures, drawn from every sample bottle, were centrifuged at 10000 rpm for 10 min. The supernatant was removed and the pellets were washed with 0.1 M phosphate buffer (pH 7.4) for 3 times. Subsequently, the pellets were resuspended in 0.1 M phosphate buffer containing 50 μM 2, 7-Dichlorodi-hydrofluorescein diacetate (DCFH-DA) and incubated at 30 ± 1 °C in dark for 30 min. After removing the phosphate buffer containing DCFH-DA, the pellets were washed three times with 0.1 M phosphate buffer. Finally, the pellets were resuspended in 0.1 M phosphate buffer and plated into a 96-well plate. The fluorescence intensity was monitored using a microplate reader (BioTek, USA) with 485 nm excitation and 525 nm emission filter.

Lactate dehydrogenase release assay

The LDH activity were assayed using LDH release assay kit (Roche Applied Science) according to the manufacturer's instructions. Briefly, the leachate, drawn from every sample bottle, were centrifuged at 5500 rpm for 3 min. Then the supernatants were seeded on a 96-well plate, followed by the addition of 50 μL of substrate mix (Roche Applied Science). After 30 min of incubation at room temperature in dark, 50 μL of stop solution (Roche Applied Science) was added to each well. The absorbance was monitored at 490 nm using a microplate reader (BioTek, USA).

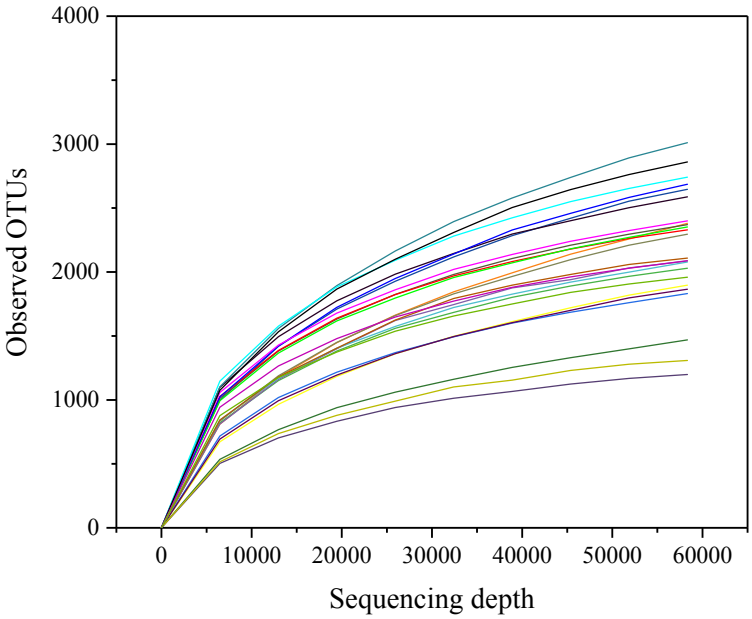

Fig. S3 Rarefaction curves of all the samples.

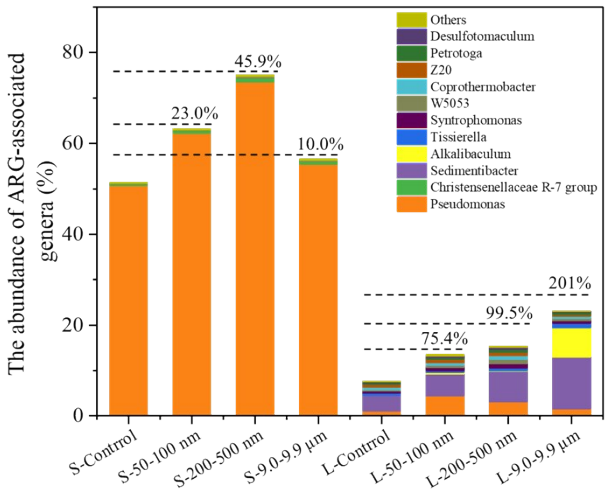

Fig. S4 The relative abundance of ARG-associated genera based on total 16S rRNA gene sequences for each presented taxa. The number denotes that the increased percentage of total ARG-associated genera abundances in each N/MP group compared to the control.