Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Material:

Charged Nanoplastics Differentially Affects the Conjugative Transfer of Antibiotic Resistance Genes

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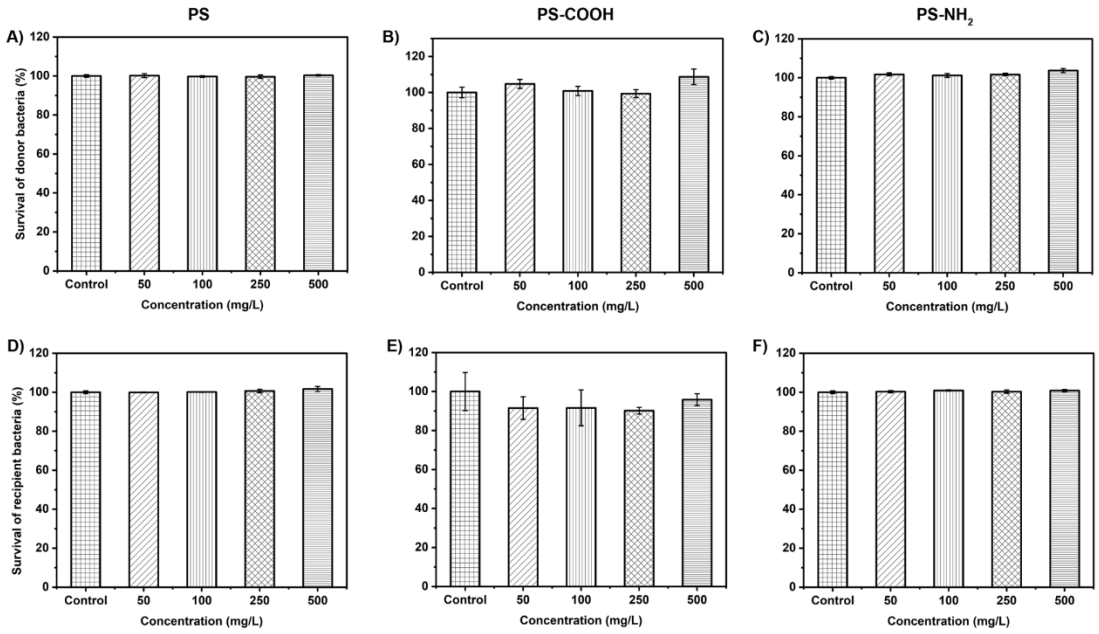

Figure S1 Relative viability of *E. coli* DH5α (top) and *E. coli* K12 (bottom) after incubating with different amounts of PS (A, D), PS-COOH (B, E), and PS-NH_2 (C, F). Number of experiments N ≥ 3.

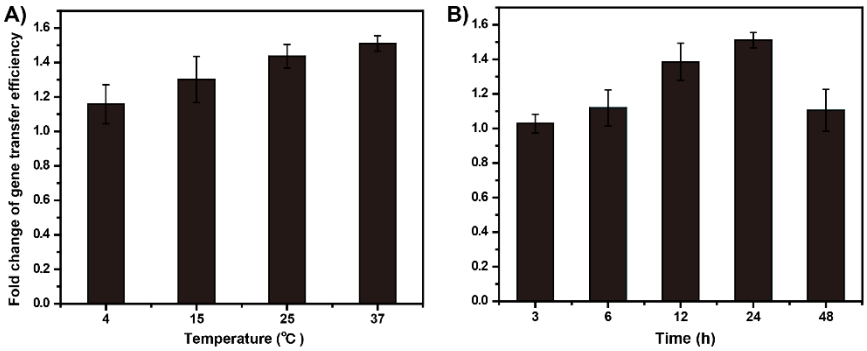

Figure S2 Effects of mating temperature (A) and times (B) on the conjugative transfer of RP4. The concentration of bacteria is OD_{600} = 0.06. All values are represented as mean ± SD. Number of experiments N ≥ 3.

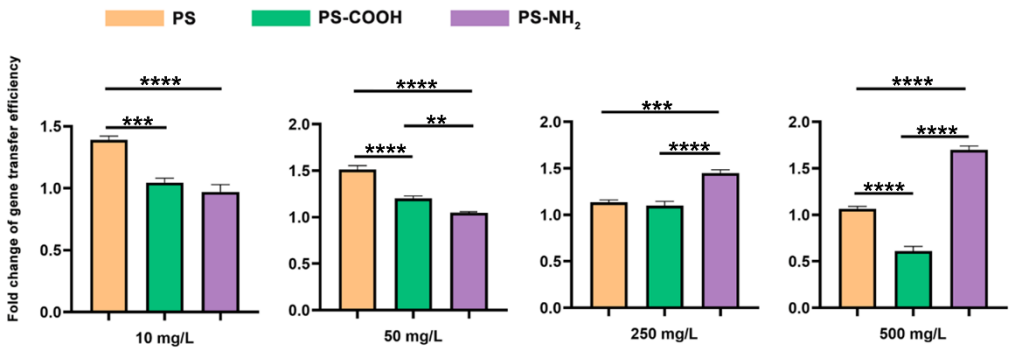

Figure S3 Significant differences between groups (shown in Figure 2) that tested with independent-samples t-test (** represents p < 0.01, *** represents p < 0.001 and **** represents p < 0.0001).

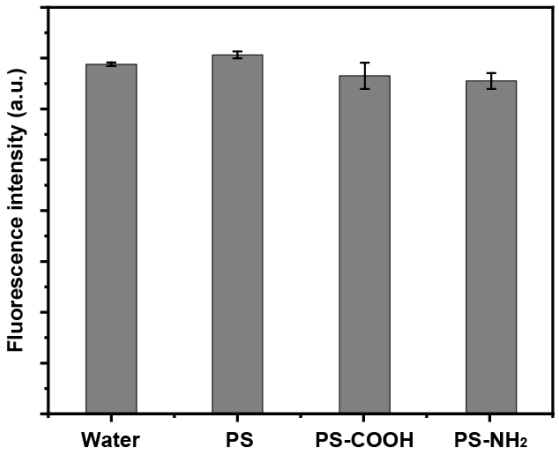

Figure S4 The fluorescence of water, PS, PS-COOH and PS-NH_2 under excitation of 488 nm. The emission at 525 nm was recorded. The concentration of the nanoplastics used is 500 mg/L. Number of experiments $N \geq 3$.

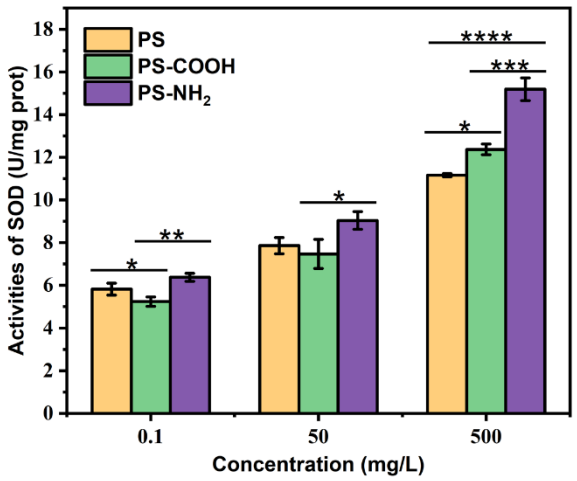

Figure S5 The bacterial SOD activity under exposure of differentially charged nanoplastics. Significant differences between groups (shown in Figure 2) were tested with independent-samples t-test (* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$ and **** represents $p < 0.0001$). Number of experiments $N \geq 3$.

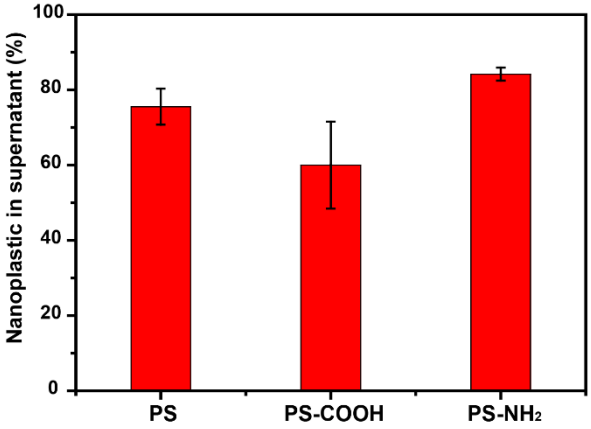

Figure S6 The amounts of nanoplastics in the supernatant. The nanoplastics (500 mg/L) were mixed with the bacteria. After incubation for 1 min, the mixture was centrifugated (8000 rpm, 5 min). The nanoplastics in the supernatant was detected by Pyrolysis-Gas Chromatography/Mass Spectrometry. Without bacteria, negligible amount of nanoplastics were detected in the pellet. Number of experiments $N \geq 3$.

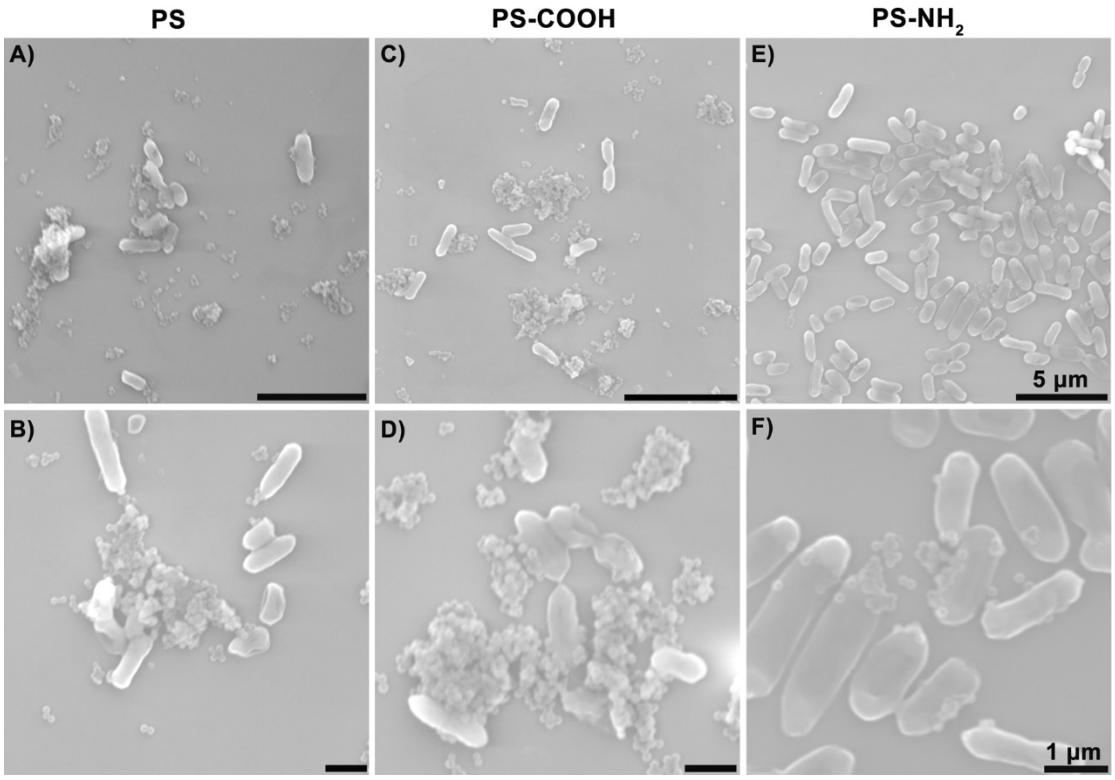

Figure S7 SEM images of bacteria treated with 500 mg/L PS (A and B), PS-COOH (C and D) and PS-NH_2 (E and F).

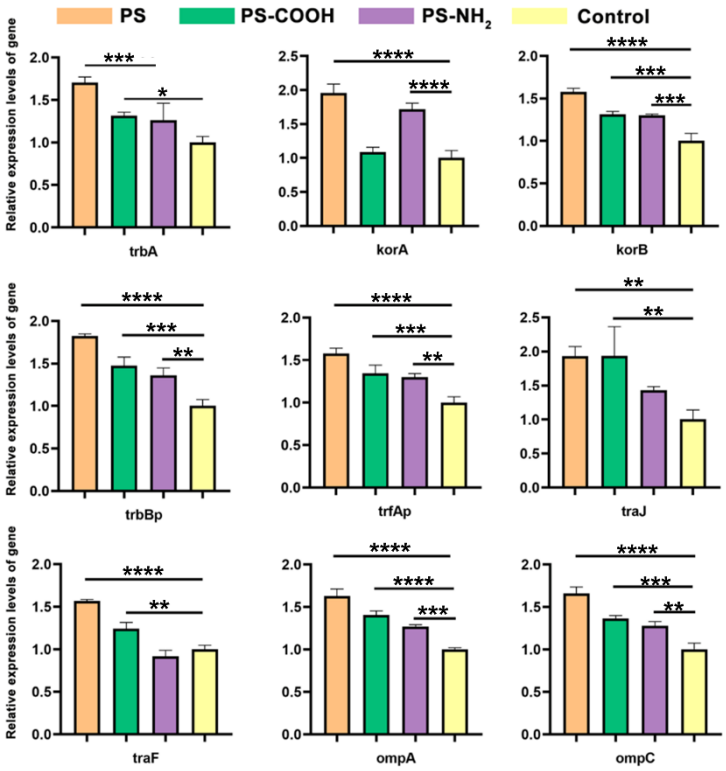

Figure S8 Significant differences between groups (shown in Figure 7) that tested with independent-samples t-test (* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$ and **** represents $p < 0.0001$).

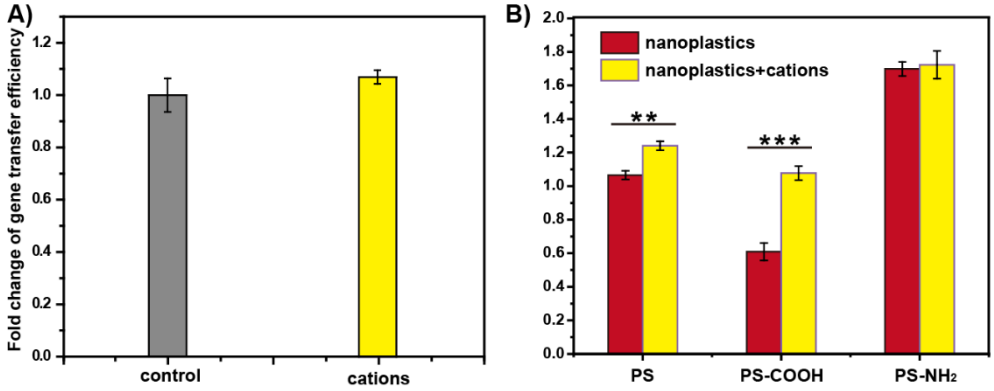

Figure S9 Fold changes of gene transfer efficiency under exposure of cations (A) and cations+ nanoplastics (B) when compared to the control. Ca^{2+} (10 mM) and Mg^{2+} (10 mM) ions were mixed and used as cation solution in the experiment. The concentrations of nanoplastics used was 500 mg/L. Significant differences between groups were tested with independent-samples t-test (** represents $p < 0.01$ and *** represents $p < 0.001$). Number of experiments $N \geq 3$.

Table S1 Primer sequences used in this study.

Gene	primer	Sequence (5'-3')	Length of product (bp)
16S rRNA	*16S rRNA-F*	CCTACGGGAGGCAGCAG	194
	16S rRNA-R	ATTACCGCGGCTGCTGG	
trbA	*trbA-F*	TGGAAACTCCCCTACCTCTT	120
	trbA-R	CCACACTGATGCGTTCGTAT	
korA	*korA-F*	TCGGGCAAGTTCTTGTCC	147
	korA-R	GCAGCAGACCATCGAGATA	
korB	*korB-F*	CTGGTCGGCTTCGTTGTA	149
	korB-R	TGAAGTCACCCATTTCGGT	
trbBp	*trbBp-F*	CGCGGTCGCCATCTTCACG	131
	trbBp-R	TGCCCGAGCCAGTACCGCCAATG	
traJ	*traJ-F*	GCCCGTGATTTTGTAGCCC	151
	traJ-R	TGAAACCAAGCCAACCAGGAA	
traF	*traF-F*	GGCAACCTCGTCGCCTTTA	118
	traF-R	GCAAGTCGGCGTGTTTTCG	
trfAp	*trfAp-F*	GAAGCCCATCGCCGTCGCCTGTAG	183
	trfAp-R	GCCGACGATGACGAACTGGTGTGG	
ompA	*ompA-F*	TGAGCCTGGGTGTTTCCTA	161
	ompA-R	CAGAGCAGCCTGACCTTCC	
ompC	*ompC -F*	AAGTAGTAGGTAGCACCAACATCA	163
	ompC-R	GGGCGAACAAAGCACAGAA	

Table S2 The physical properties of nanoplastics used in the study.

	TEM size (nm)±SD	DLS (nm)±SD	ζ –potential (mV) ±SD
PS	139.04±16.5	155.74±8.73	-26.32±0.63
PS-COOH	126.51±7.78	146.25±8.11	-37.65±1.11
PS-NH_2	129.63±5.78	144.18±5.29	21.07±0.4

Table S3 Descriptive statistics of *16S rRNA* genes based on the crossing point (CP) of control, PS, PS-COOH and PS-NH_2 samples. The analysis was carried out with BestKeeper software.

Factor	*16S rRNA*
N	4
GM [CP]	3.49
AM [CP]	3.50
Min [CP]	3.18
Max [CP]	3.77
SD [+/- CP]	0.27
CV [%CP]	7.64
Min [x-fold]	-1.24
Max [x-fold]	1.21
SD [+/- x-fold]	1.20

Abbreviations: N: number of samples; GM [CP]: the geometric mean of CP; AM [CP]: the arithmetic mean of CP; Min [CP] and Max [CP]: the extreme values of CP; SD [± CP]: the standard deviation of the CP; CV [%CP]: the coefficient of variance expressed as a percentage on the CP level; Min [x-fold] and Max [x-fold]: the extreme values of expression levels expressed as an absolute x-fold over- or under-regulation coefficient; SD [± x-fold]: standard deviation of the absolute regulation coefficients.

Gene with the SD higher than 1 can be considered inconsistent.[1] According to BestKeeper, the lower the SD, the greater the stability of gene expression. Thus the *16S rRNA* was considered to be a stably expressed gene.

References

1. M. W. Pfaffl, A. Tichopad, C. Prgomet, T. P. Neuvians, Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–Excel-based tool using pair-wise correlations. *Biotechnol. Lett.*, 2004, **26**, 509.