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

Supporting Information (SI)

How does zinc oxide and zero valent iron nanoparticles impact the occurrence of antibiotic resistance genes in landfill leachate?

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Table S1 Characteristics of leachate used in NPs exposure studies

	Leachate	
	M	**O**
PH	8.7(0.08)	9.0 (0.12)
EC (ms/cm)	28.3(0.6)	15.1(0.2)
DOC	9667(447)	1417(57)
TN (mg/L)	5805(145)	4943(206)
NH_4^+-N (mg/L)	3040(158)	1304(49)
TP(mg/L)	43.9 (9.6)	20.6 (6.7)
Zn^{2+}(mg/L)	0.13(0.01)	0.04(0.01)
Fe^{2+}(mg/L)	0.68(0.05)	0.64(0.04)

The letters "M" and "O" represented the middle-aged and old-aged leachate, respectively. Values in the brackets represent standard deviations of triplicate tests.

Table S2 Primers and PCR conditions for ARG analyses

Targeted genes	Amplicon length (bp)	Primer sequences (5' – 3')	Linearity (R_2) Efficiency (%)
*sul*1	172	FW CACCGGAAACATCGCTGCA RV AAGTTCCGCCGCAAGGCT	0.99 94
*sul*2	165	FW CTCCGATGGAGGCCGGTAT RV GGGAATGCCATCTGCCTTGA	0.99 92
*aad*A1	195	FW AGCTAAGCGCGAACTGCAAT RV TGGCTCGAAGATACCTGCAA	0.99 96
*str*B	185	FW GCTCGGTCGTGAGAACAATCT RV CAATTTCGGTCGCCTGGTAGT	0.99 99
*erm*B	139	FW AAAACTTACCCGCCATAC CA RV TTTGGCGTGTTTCATTGC TT	0.99 97
*mef*A	186	FW ATACCCCAG CACTCAATTCG RV CAATCACAGCACCCA ATACG	0.99 95
*tet*M	205	FW CCGTTGGGAAGTGGAATGC RV TCCGAAAATCTGCTGGGGTA	0.99 91
*tet*Q	196	FW AGAATCTGCTGTTTGCCAGTG RV CGGAGTGTCAATGATATTGCA	0.99 95
$bla_{\text{CTX-M}}$	211	FW ATGTGCAGYACCAGTAARGTKATGGC RV ATCACKCGGRTCGCCNGGRAT	0.99 105
bla_{OXA}	195	FW CGGATGGTTTGAAGGGTTTATTAT RV TCTTGGCTTTTATGCTTGATGTTAA	0.99 95
*Intl*1	190	FW GGCTTCGTGATGCCTGCTT RV CATTCCTGGCCGTGGTTCT	0.99 105
*Intl*2	143	FW GTTATTTTATTGCTGGGATTAGGC RV TTTTACGCTGCTGTATGGTGC	0.99 97

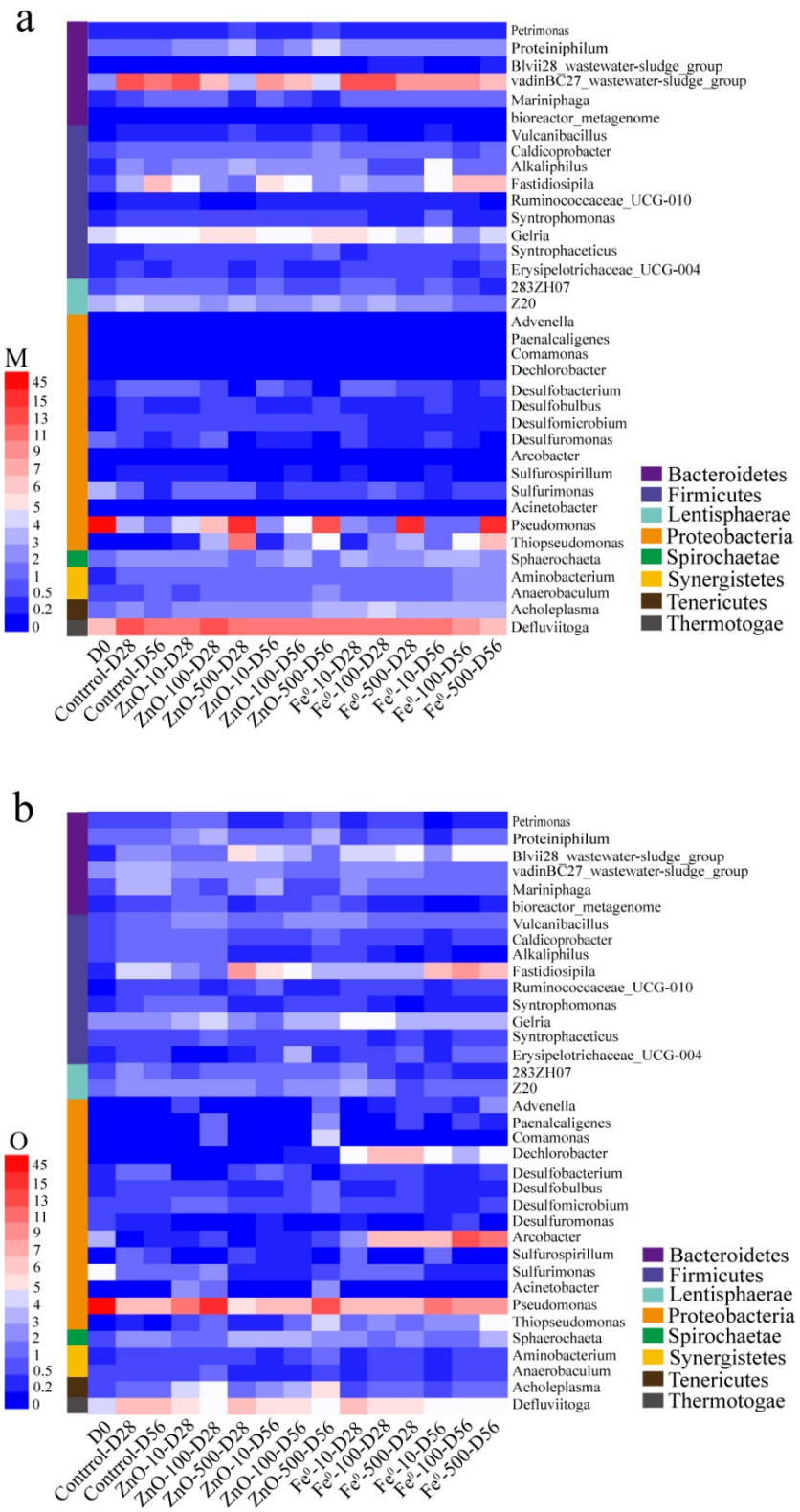


Figure S1 Relative abundance of bacteria at genera level in middle-aged (a) and old-aged (b) leachate with ZnO and Fe^0 NPs. Genera of any one sample with greater than 1% abundance are listed.

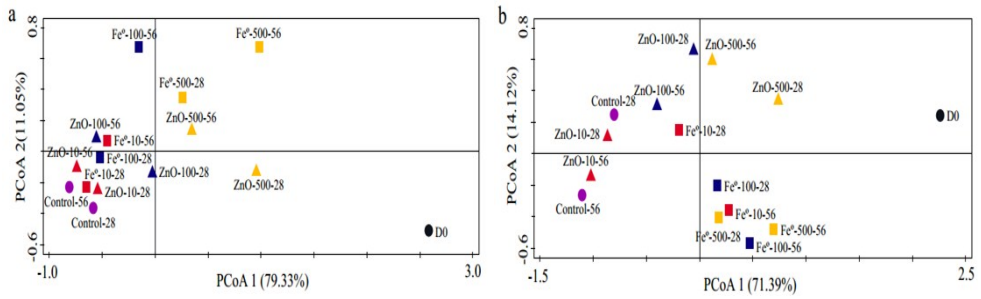


Figure S2 Principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the overall distribution of bacterial community composition in leachate with ZnO NPs (a) and Fe^0 NPs (b).

Table S3 Spearman correlations between absolute ARG and MGE abundances in leachate with ZnO and Fe^0 NPs.

	ZnO NPs		Fe^0 NPs	
	*intI*1	*intI*2	*intI*1	*intI*2
*sul*1	0.623**	0.626**	0.724**	0.650**
*sul*2	0.768**	0.359**	0.790**	0.435**
*aad*A1	0.775**	0.652**	0.781**	0.530**
*str*B	0.707**	0.498**	0.686**	0.609**
*erm*B	0.032	0.360**	0.025	0.385**
*mef*A	-0.027	0.602**	-0.051	0.683**
*tet*M	0.006	0.258*	0.006	0.594**
*tet*Q	0.052	0.310*	0.126	0.726**
bla_{OXA}	0.366*	-0.407**	0.417**	-0.533**
$bla_{CTX\text{-}M}$	-0.092	0.235*	-0.172	0.484**

Values represent the correlation coefficient *R*-values derived from the Spearman correlation analysis. Significance levels are indicated: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

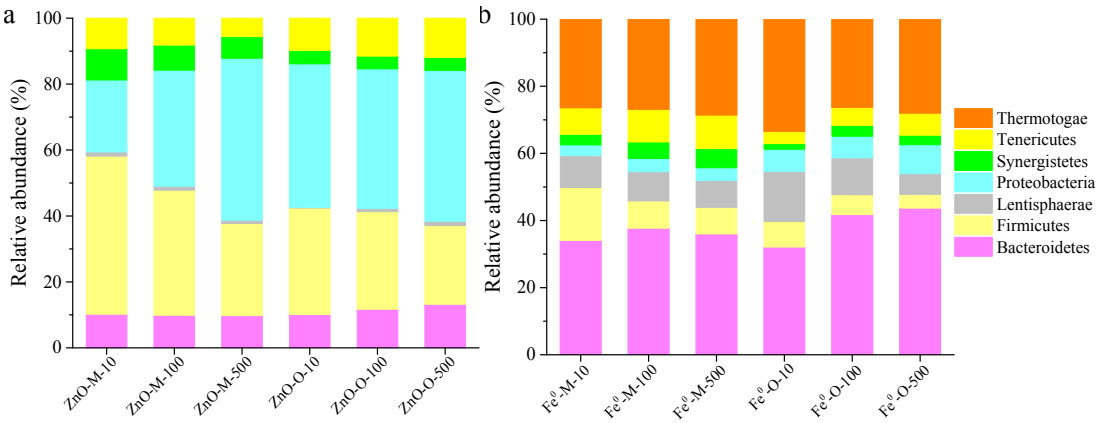


Figure S3 The relative abundances of potential ARG-associated host bacteria at phylum level in middle-aged (M) and old-aged (O) leachate with ZnO NPs (a) and Fe^0 NPs (b).

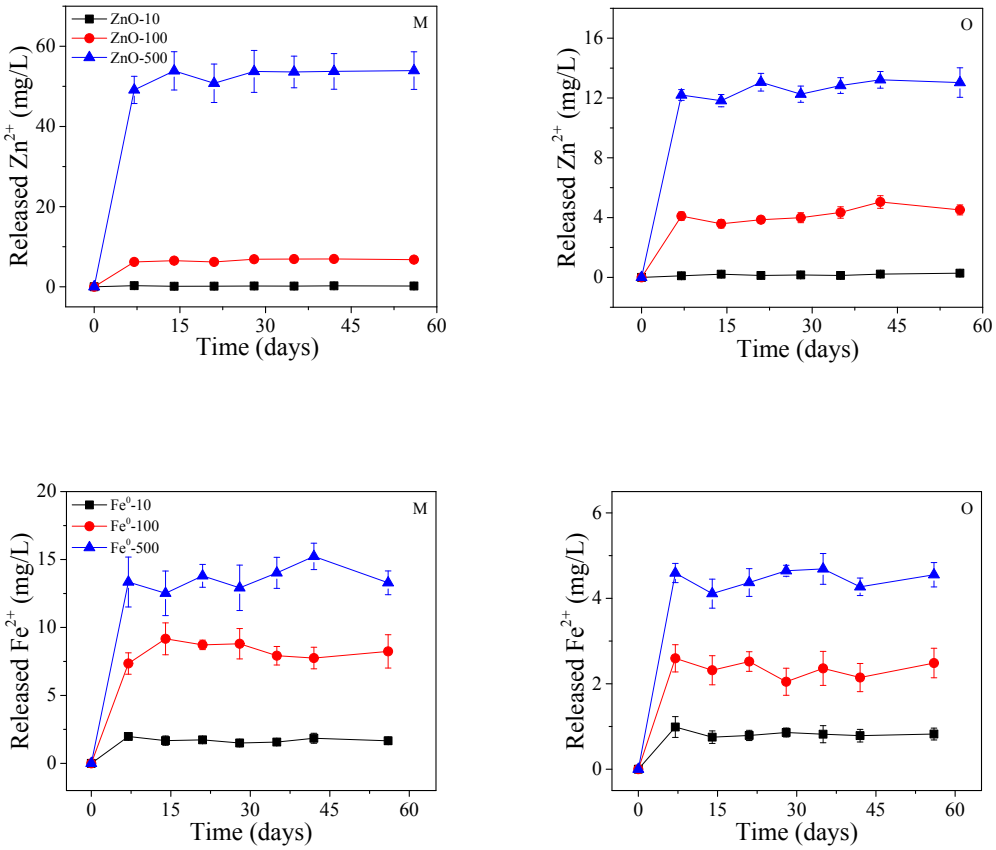
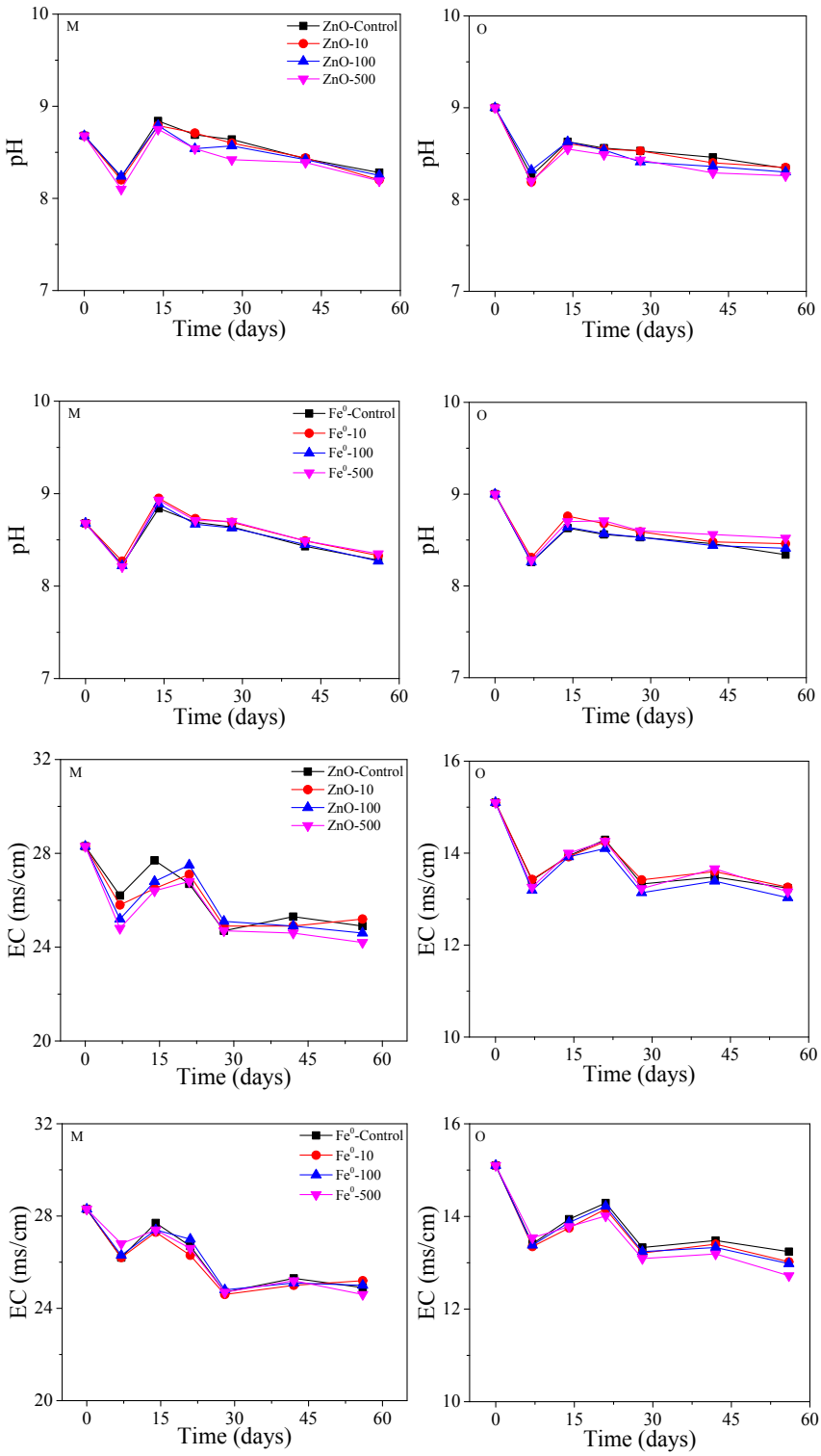


Figure S4 Changes of released ion concentrations from ZnO and Fe^0 NPs in middle-aged (M) and old-aged (O) leachate. Error bars represent one standard deviation of the mean from triplicate measurements.


M
ZnO-Control
ZnO-10
ZnO-100
ZnO-500
pH
Time (days)
O
pH
Time (days)
M
Fe0-Control
Fe0-10
Fe0-100
Fe0-500
pH
Time (days)
O
pH
Time (days)
M
ZnO-Control
ZnO-10
ZnO-100
ZnO-500
EC (ms/cm)
Time (days)
O
EC (ms/cm)
Time (days)
M
Fe0-Control
Fe0-10
Fe0-100
Fe0-500
EC (ms/cm)
Time (days)
O
EC (ms/cm)
Time (days)

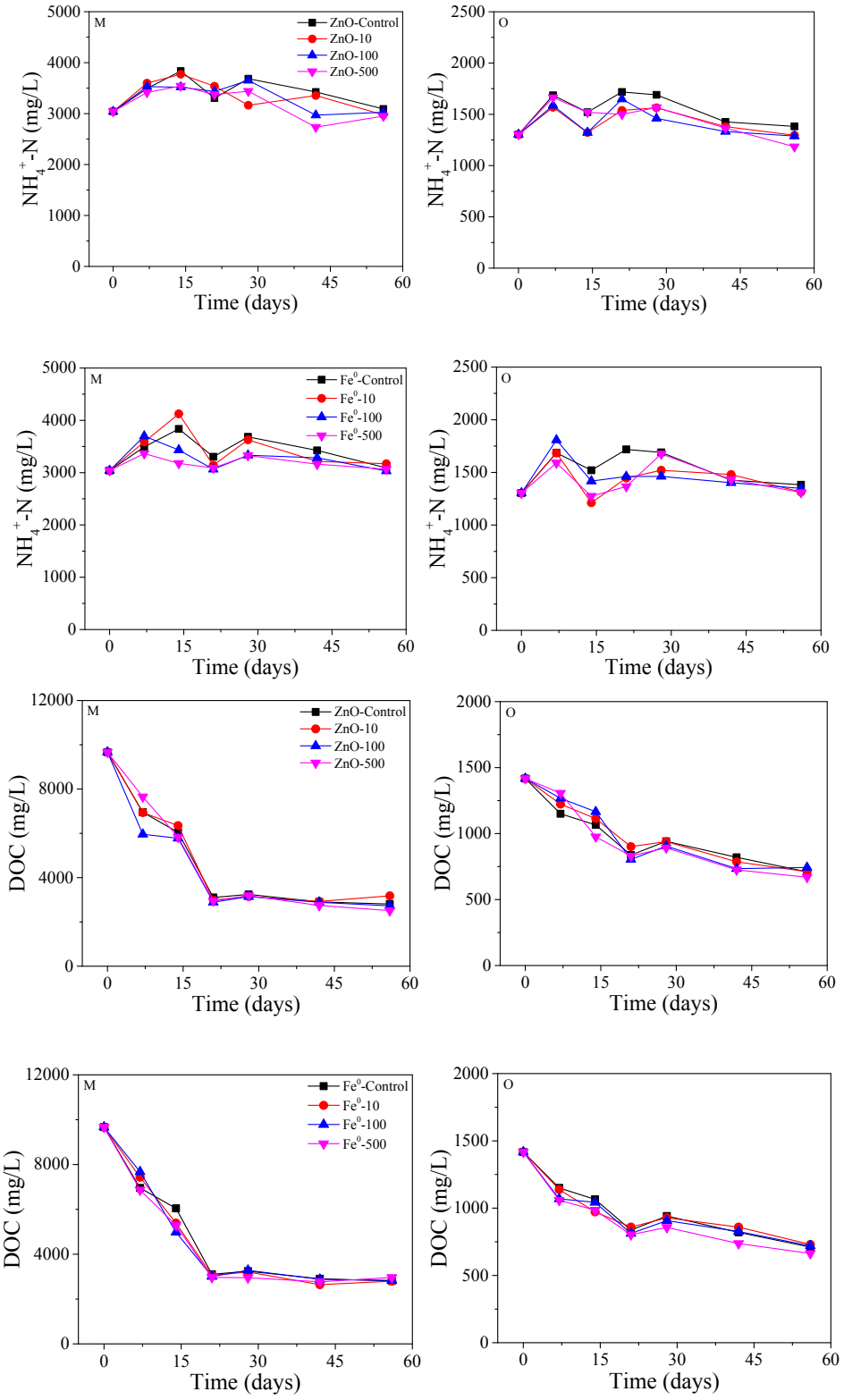


Figure S5 Changes of physicochemical properties (including pH, EC, NH_4^+-N, and DOC) in middle-aged (M) and old-aged (O) leachate with ZnO and Fe^0 NPs. The values represent average amounts of triplicate tests.