

Organic amendment treatments for resistome risk reduction in soil-crop systems

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Supplementary Table S1. Physicochemical properties of the organic amendments studied in the preliminary experiment.

Property	Compost	Manure	Slurry
Dry matter (%)	32.61	22.42	6.56
Electrical conductivity (mS cm ⁻¹)	1.03	1.08	1.00
pH	8.69	8.91	8.67
Organic matter (% DW)	66.23	86.74	78.27
Organic C (%)	12.56	11.31	2.99
N (%)	1.00	0.58	0.32
C/N	12.56	20.56	29.85
K ₂ O (% DW)	2.10	3.10	4.44
P ₂ O ₅ (% DW)	0.59	0.70	1.16
Cd (mg kg ⁻¹ DW)	0.15	0.06	0.14
Co (mg kg ⁻¹ DW)	1.68	1.40	3.25
Cr (mg kg ⁻¹ DW)	7.06	6.74	7.08
Cu (mg kg ⁻¹ DW)	55.31	50.71	116.94
Ni (mg kg ⁻¹ DW)	10.63	4.61	10.78
Pb (mg kg ⁻¹ DW)	2.72	1.44	2.76
Zn (mg kg ⁻¹ DW)	203.07	193.97	441.4

Supplementary Table S2. Primers and PCR conditions for droplet digital PCR (ddPCR) analysis.

Gene	Primers	Amplicon size (bp)	Cycling conditions	Reference
16S rRNA	F: CCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	194	95 °C for 5 min 40 cycles of 95 °C for 30 s and 60 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(1)
tetA	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	95 °C for 5 min 40 cycles of 95 °C for 30 s and 62.5 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(2)
tetX	F: AGCCTTACCAATGGGTGTAAA R: TTCTTACCTTGGACATCCCG	278	95 °C for 5 min 40 cycles of 95 °C for 30 s and 57.5 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(3)
sul1	F: CCGTTGGCCTTCCTGTAAAG R: TTGCCGATCGCGTGAAGT	67	95 °C for 5 min 40 cycles of 95 °C for 30 s and 60 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(4)
sul2	F: CGGCTGCGCTTCGATT R: CGCGCGCAGAAAGGATT	60	95 °C for 5 min 40 cycles of 95 °C for 30 s and 60 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(5)
intl1	F: GCCTTGATGTTACCCGAGAG R: GATCGGTCGAATGCGTGT	196	95 °C for 5 min 40 cycles of 95 °C for 30 s and 61 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(6)
tnpA-04	F: CCGATCACGGAAGCTCAAG R: GGCTCGCATGACTTCGAATC	101	95 °C for 5 min 40 cycles of 95 °C for 30 s and 61 °C for 1 min, with a ramping rate of 2.5 °C s ⁻¹ 4 °C for 5 min 90 °C for 5 min	(7)

Supplementary Table S3. Primer sets used in high-throughput qPCR (HT-qPCR) analysis.

Gene	Forward Primer	Reverse Primer	Target antibiotics (major)
16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCTCAGCTCGTG	16S rRNA
Bacteroidetes	GGARCATGTGGTTTAATTCGATGAT	AGCTGACGACAACCATGCAG	Taxonomic
Firmicutes	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC	Taxonomic
aac(3)-iid_ia	CGATGGTGC GGGTTGGTC	TCGGCGTAGTGCAATGCG	Aminoglycoside
aac(6)-ig	GCGATGTTAGAAGCCTCAATTCG	CACACTTCGGCCTGTGCGAA	Aminoglycoside
aac(6)-ir	GCTATAACGATCAGCAGCAAGC	CGCGATGCATGGCATGAC	Aminoglycoside
aac(6)-is_iu_ix	AAGCTTACTCTGGCCTGATCATG	TGCCTGAACGTCGATATTCAGG	Aminoglycoside
aac(6')-Iy	GCCTCAATCCGCCACGATTA	ACGCGCTCTGTTTCTCTCAA	Aminoglycoside
aac3-IVa	CCAACACGACGCTGCATC	GCTGTGCGCCACAATGTCTG	Aminoglycoside
aac6-aph2	CCAAGAGCAATAAGGGCATACCAA	GCCACACTATCATAACCACTACCG	Aminoglycoside
aadA1	TGTACGGCTCCGCAGTG	CACGGAATGATGTCTGCTGTG	Aminoglycoside
aadA16	ACGGTGGCCTGAAGCC	GAATTGCAGTTCCTCGTCTGG	Aminoglycoside
aadA5	ATCACGATCTTGGCATTTTGCT	CTGCGGATGGGCCTAGAAG	Aminoglycoside
aadA6	CCATCGAGCGTCATCTGGAA	CCCGTCTGGCCGGATAAC	Aminoglycoside
aadA7	CACTCCGCGCCTTGGA	TGTGGCGGGCTCGAAG	Aminoglycoside
aadB	CCTGCTTGGTGGGACAGAC	CGGCACGCAAGACCTCAA	Aminoglycoside
aph3-III	CAGAAGGCAATGTCATACCACTTG	GACAGCCGCTTAGCCGAA	Aminoglycoside
aph4-Ib	GGGAACACCGTGCTCACC	GTTGGTCCCGTGCAAGTC	Aminoglycoside
aph6-Ia	CGCTGGGAGCTGAAGAGG	AGCATCGTGCTGCTCTCC	Aminoglycoside
apmA	GGCGCACATGCATTCATCA	CTATACTCCAGTCCCACCATTTGA	Aminoglycoside
armA	TCTTCGACGAATGAAAGAGTCG	GCTAATGGATTGAAGCCACAACC	Aminoglycoside
spcN	GCTATGTGCTGGTGGACTGG	GGAACCACTCGACGAACCTCG	Aminoglycoside
strB	GCTCGGTCTGTGAGAACAATCT	CAATTTCCGTCGCTGGTAGT	Aminoglycoside
bla1	GCAAGTTGAAGCGAAAGAAAAGA	TACCAGTATCAATCGCATATACACCTAA	β -lactam
blaACT	AAGCCGCTCAAGCTGGA	GCCATATCCTGCACGTTGG	β -lactam
blaBEL-nonmobile	ATGTCCATGGCAGACTGTG	CCTGTCTGTGACCCGTTACC	β -lactam
blaMIR	CGGTCTGCCGTTACAGGTG	AAAGACCCGCGTCGTCATG	β -lactam
blaOXY_1	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTGAGAATT	β -lactam
blaOXY_2	AAAGGTGACCGCATTTCGC	CCAGCGTCAGCTTGCG	β -lactam
blaSFO	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT	β -lactam
cphA	GCGAGCTGCACAAGCTGAT	CGGCCAGTCGCTCTTC	β -lactam
intI1_1	CGAACGAGTGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA	Integrans
intI1_2	CGAAGTCGAGGCATTCTGTGTC	GCCTTCCAGAAAACCGAGGA	Integrans
intI3	CAGGTGCTGGGCATGGA	CCTGGGCAGCATCACCA	Integrans
acrA	GGTCTATCACCTACGCGCTATC	GCGCGCACGAACATAACC	MDR
cefa_qacelta	TAGTTGGCGAAGTAATCGCAAC	TGCGATGCCATAACCGATTATG	MDR
czcA	GCCTTGTTTCATCGGCGAAC	GGCAATGTGCTCTCGTTC	MDR
emrD	CTCAGCAGTATGGTGTAAGCATT	ACCAGGCGCCGAAGAAC	MDR

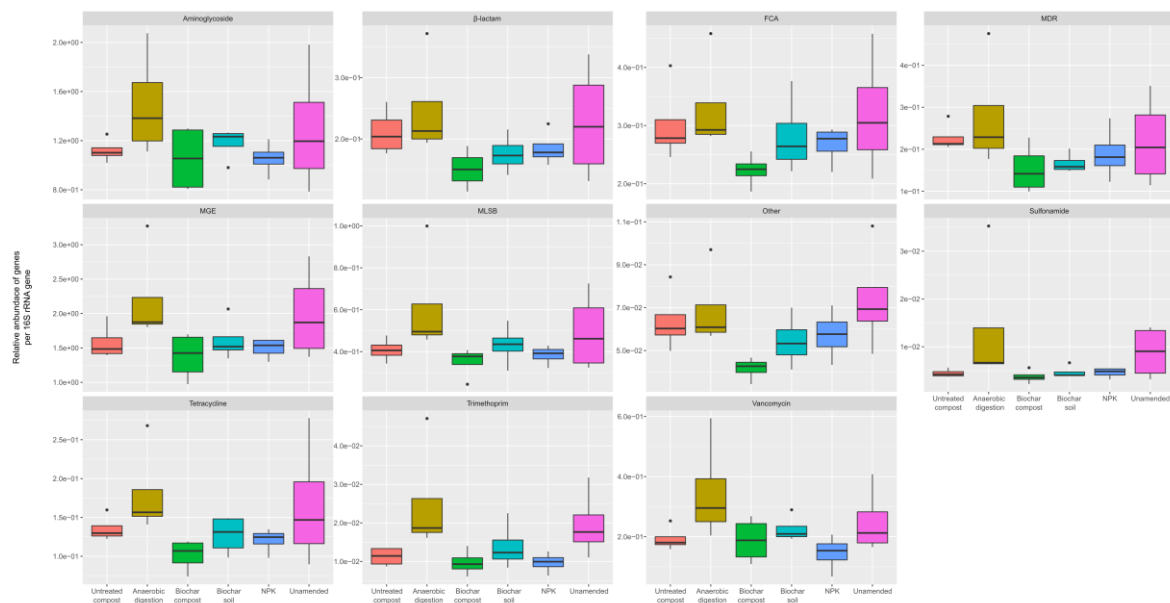
mepA	ATCGGTCGCTCTTCGTTAC	ATAAATAGGATCGAGCTGCTGGAT	MDR
oprD	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA	MDR
pbrT	GATGCGCACTGGGCTTG	TCGGAATATGCGGAAATGCG	MDR
sugE	CTTAGTTATTGCTGGTCTGCTGGA	GCATCGGGTTAGCGGACTC	MDR
ttgA	ACGCCAATGCCAAACGATT	GTCACGGCGCAGCTTGA	MDR
IncN_rep	AGTTCACCACCTACTCGCTCCG	CAAGTTCTTCTGTTGGGATTCCG	MGE
IS1111	GTCTTAAGGTGGGCTGCGTG	CCCCGAATCTCATTGATCAGC	MGE
IS1133	GCAGCGTCGGGTTGGA	ACGCGTTCGAACAACTGTAATG	MGE
IS1247_1	CGGCCGTCAC TGACCAA	TCGGCAGGTTGGTGACG	MGE
IS1247_2	TGGATCGACCGGTTCCAT	GCTGACCGAGCTGTCCATGT	MGE
IS6100	CGCACCGGCTTGATCAGTA	CTGCCACGCTCAATACCGA	MGE
IS630	CCGCCACCACTGTGATGG	TTGGCGCTGACTGGATGC	MGE
ISEcp1	CATGCTCTGCGGTCACTTC	GACGCACCTTCTTGATGACC	MGE
ISPPs	CACACTGCAAAAACGCATCCT	TGCTTTTGGCGTCACAGTTCTC	MGE
orf37-IS26	GCCGGGTTGTGCAAATAGAC	TGGCAATCTGTCGCTGCTG	MGE
Tn5403	AAGCGAATGGCGCGAAC	CGCGCAGGGTAAACTGC	MGE
tnpA_2	CCGATCACGGAAGCTCAAG	GGCTCGCATGACTTCGAATC	MGE
tnpA_3	GGGCGGGTCGATTGAAA	GTGGGCGGGATCTGCTT	MGE
trbC	CGGYATWCCGSCSACRCTGCG	GCCACCTGYSBGCAGTCMCC	MGE
ereA	GATAATTCTGCTGGCGCACA	GCAGGCGTGGTCACAAC	MLSB
erm35	CCTTCAGTCAGAACCGGCAA	GCTGATTTGACAGTTGGTGGTG	MLSB
erm36	GGCGGACCGACTTGCAAT	TCTGCGTTGACGACGGTTAC	MLSB
ermE	GTCACGCAGCTGGAGTTCTG	CGGTGAAGCACAGCTCGAC	MLSB
ermO	GAGTACGCCCCGAAACG	GCGTTCGATCCGGAGGA	MLSB
ermX	TGATGACGGCTCAGTGG	GTGCACCAGCGCCTGA	MLSB
lnuB	GGATCGTTTACCAAAGGAGAAGG	AGCATAGCCTTCGTATCAGGAA	MLSB
lnuC	GGGTGTAGATGCTCTTCTTGGA	CTTTACCCGAAAGAGTTTCTACCG	MLSB
mefA_1	TAATTATCGCAGCAGCTGGTTC	GTTCCCAAACGGAGTATAAGAGTG	MLSB
mefA_2	CCGTAGCATTGGAACAGCTTTT	AAACGGAGTATAAGAGTGCTGCAA	MLSB
mphA	TCAGCGGGATGATCGACTG	GAGGGCGTAGAGGGCGTA	MLSB
oleC	CCCGGAGTCGATGTTCTGA	GCCGAAGACGTACACGAACAG	MLSB
pncA	GCAATCGAGGCGGTGTTT	TTGCCGCAGCCAATTCA	MLSB
vat(A)	ATGAACGGAGCGAATCATCGG	CCATACCGATCCAAACGTCATTTT	MLSB
bacA	ATCCGCGGCACCCTGA	CCTGCTTGATGGACTTGATGAAGA	Other
fabK	CAGGAGCAGGAAATCCAAGC	CCAGCTTCCATTCTTCTGC	Other
mcr1	CACATCGACGGCGTATTCTG	CAACGAGCATACCGACATCG	Other
merA	GTGCCGTCCAAGATCATG	GGTGAAGTCCAGTAGGGTGA	Other
ttgB	TCGCCCTGGATGTACACCTT	ACCATTGCCGACATCAACAAC	Other
catQ	AGGTGCACTTACAGTATGACTGC	AACGTGGGAAGTTCTCGTCATAC	Phenicol
cmlV	GCCCTCATCACCGTCTTCG	GGACGTTGGCGATGGAGAG	Phenicol
optrA	GGTGGATGAAGTCCGTACGG	AGGTTAGACCTCCAAGAGCCA	Phenicol
qepA	GGGCATCGCGCTGTTT	GCGCATCGGTGAAGCC	Quinolone
qnrB	GCGACGTTCACTGGTTCAGA	GCTGCTCGCCAGTCGAA	Quinolone

qnrB4	TCACCACCCGCACCTG	GGATATCTAAATCGCCCAGTTCC	Quinolone
sul3	TCCGTTTCAGCGAATTGGTGCAG	TTCGTTTCACGCCTTACACCAGC	Sulfonamide
tetD	AATTGCACTGCCTGCATTGC	GACAGATTGCCAGCAGCAGA	Tetracycline
tetG	TCGCGTTCCTGCTTGCC	CCGCGAGCGACAAACCA	Tetracycline
tetL	ATGGTTGTAGTTGCGCGCTATAT	ATCGCTGGACCGACTCCTT	Tetracycline
tetM	GGAGCGATTACAGAATTAGGAAGC	TCCATATGTCCTGGCGTGTG	Tetracycline
tetPA	GGAAACCTTAGTTCAGTGACTTGG	CCCATTTAACCACGCACTGAA	Tetracycline
tetPB	TGGGCGACAGTAGGCTTAGAA	TGACCCTACTGAAACATTAGAAAATATACCT	Tetracycline
tetR	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC	Tetracycline
tetW	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC	Tetracycline
dfrA27	GCCGCTCAGGATCGGTA	GTCGAGATATGTAGCGTGTG	Trimethoprim
vanA	GGGCTGTGAGGTCGGTTG	TTCAGTACAATGCGGCCGTTA	Vancomycin
vanB	TTGTCGGCGAAGTGGATCA	AGCCTTTTCCGGCTCGTT	Vancomycin
vanHB	GAGGTTTCCGAGGCGACAA	CTCTCGGCGGCAGTCGTAT	Vancomycin
vanTC	ACAGTTGCCGCTGGTGAAG	CGTGGCTGGTCGATCAAAA	Vancomycin

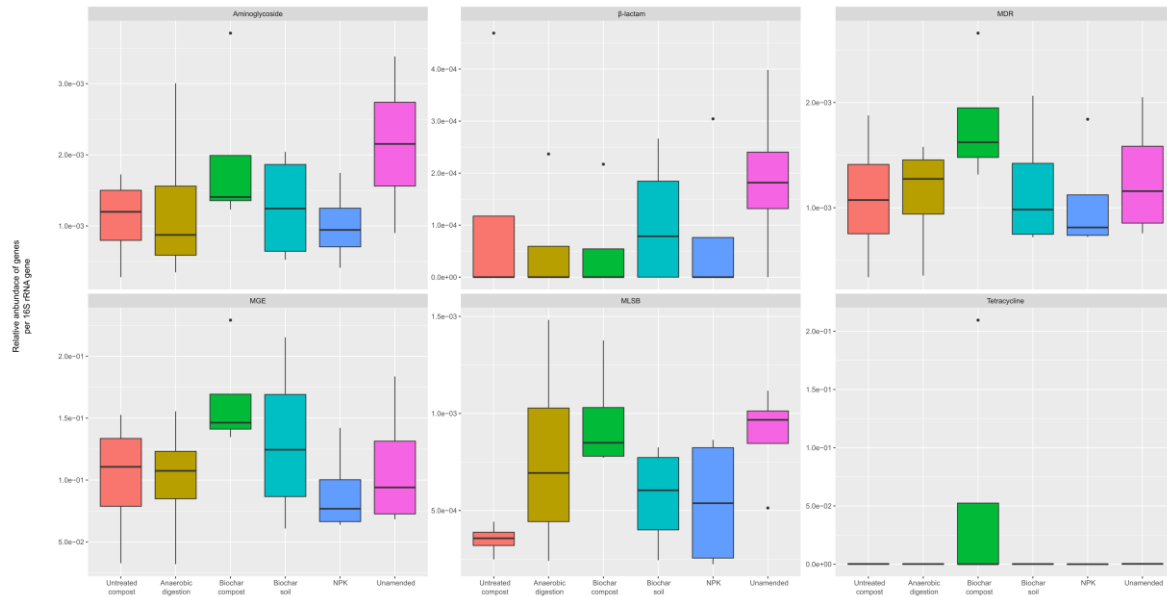
Supplementary Table S4. Comparison of the relative abundances of antibiotic resistance genes and mobile genetic element genes in soil (S) and lettuce (L) samples for each treatment, based on Tukey's post-hoc test. ns: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Gene	Untreated compost	Anaerobic digestion	Biochar compost	Biochar soil	NPK	Unamended
aac(6)-Ig	S > L ***	S > L **	S > L **	S > L ***	S > L ***	S > L *
aac3-IVa	S > L ***	S > L **	S > L *	S > L ***	S > L **	S > L **
aadA5	S > L *	ns	ns	ns	ns	S > L *
blaBEL-nonmobile	ns	ns	ns	ns	ns	ns
cmlV	S > L ***	S > L **	S > L **	S > L ***	S > L *	S > L ***
czcA	S > L ***	S > L **	S > L **	S > L ***	S > L ***	S > L **
erm35	S > L **	S > L *	S > L *	S > L **	S > L **	S > L **
ermX	S > L ***	S > L **	S > L ***	S > L ***	S > L ***	S > L **
fabK	S > L ***	S > L ***	S > L ***	S > L ***	S > L **	S > L *
intI1_1	S > L ***	S > L **	S > L ***	S > L ***	S > L ***	S > L **
intI1_2	ns	ns	ns	S > L *	ns	ns
IS6100	S > L ***	S > L **	S > L **	S > L ***	S > L ***	S > L *
ISEcp1	S > L ***	S > L *	S > L **	S > L **	S > L **	S > L **
mefA	S > L ***	S > L *	S > L **	S > L ***	S > L **	S > L *
mepA	S > L ***	S > L **	S > L ***	S > L **	S > L ***	S > L **
optrA	S > L ***	S > L *	S > L ***	S > L ***	S > L ***	S > L ***
orf37-IS26	S > L ***	S > L **	S > L ***	ns	S > L ***	S > L **
spcN	S > L ***	S > L ***	S > L **	S > L ***	S > L ***	S > L ***
tetPA	ns	ns	ns	ns	ns	ns
ttgA	S > L ***	S > L *	S > L ***	S > L ***	S > L ***	S > L **

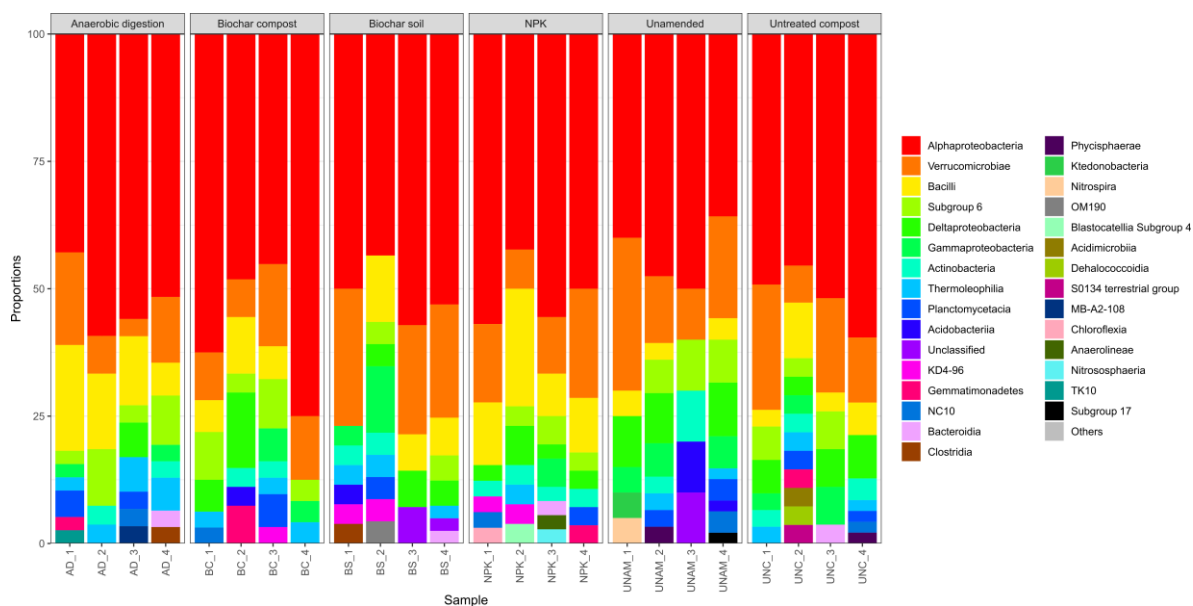
Supplementary Figure S1. Relative abundance (via HT-qPCR analysis) of antibiotic resistance genes and mobile genetic element genes in the studied soils.



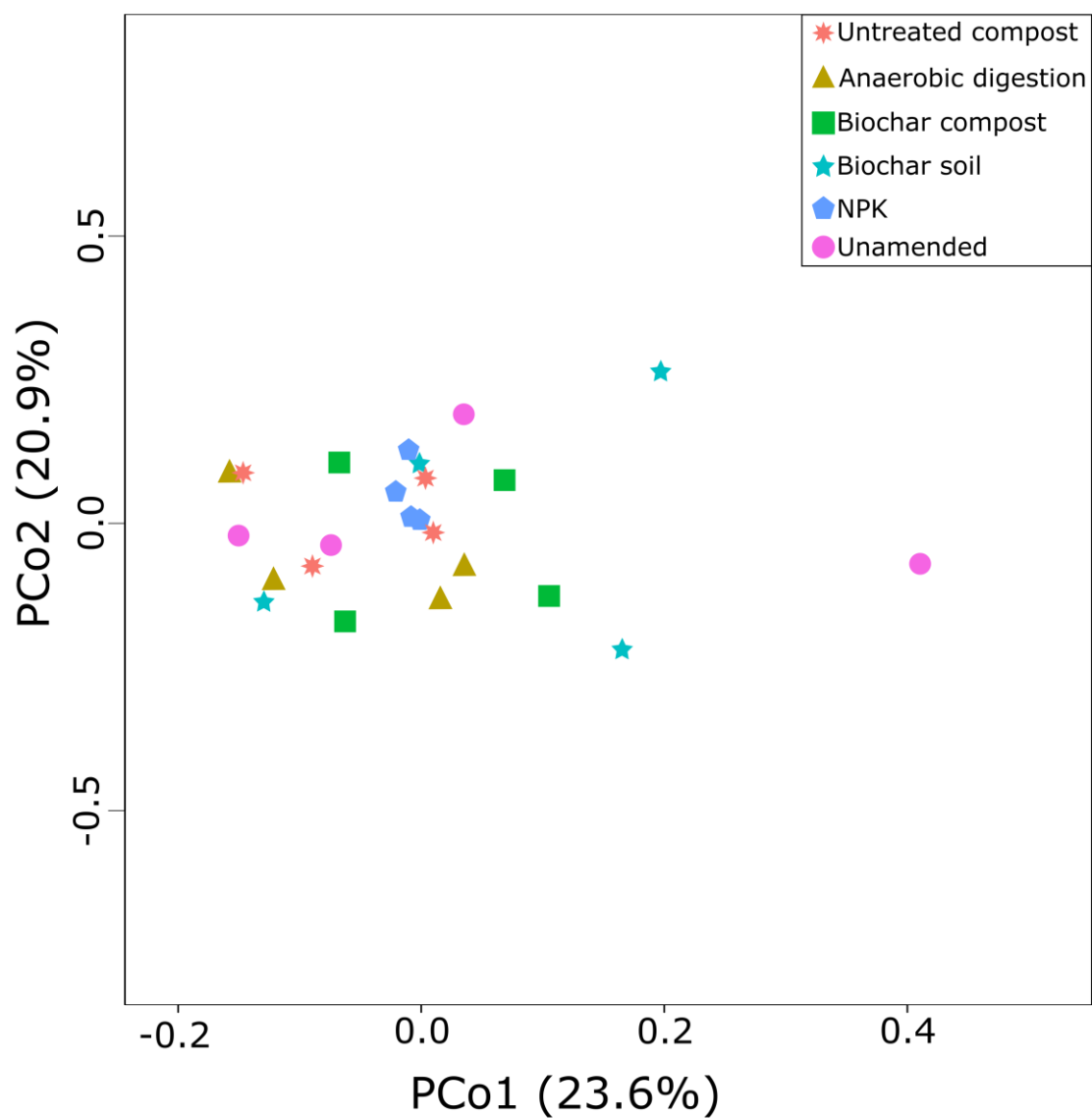
Supplementary Figure S2. Relative abundance (via HT-qPCR analysis) of antibiotic resistance genes and mobile genetic element genes in lettuce samples.



Supplementary Figure S3. The 30 most abundant bacterial classes in the studied soils.



Supplementary Figure S4. Principal coordinates analysis (PCoA) plot of soil microbial community composition based on Bray-Curtis distance metric for bacterial class in the studied soils.



References

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