Supporting Information for

Effects of copper addition on copper resistance, antibiotic resistance genes and *intl1*during swine manure composting

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S1. Detailed conditions employed for qPCR

The qPCR reaction mixture comprised 1 μL of DNA template, 0.25 μL of each 0.2 μM primer (ShengGong, China), 10 μL of SuperReal PreMix Plus (TianGen, China), and 8.5 μL of RNase-free water. The qPCR conditions comprised an initial hold for 15 min at 95°C, followed by 40 cycles for 10 s at 95°C, 20 s at the annealing temperatures shown in Table S1, and then 32 s at 72°C. The presence of inhibitory compounds in the extracted DNA was tested by qPCR using serially diluted samples. Melting curve analysis was used to detect nonspecific amplification.

S2. Co-occurrence between CRGs/ARGs subtypes and microbial taxa

Figure 5 contains 35 nodes (28 microbial taxa, five CRGs, two ARGs subtypes, and *intl1*) and 130 edges. Some topological properties that are used widely in network analysis were calculated to describe the complex patterns of the interrelationships among ARG subtypes and microbial taxa. The average weighted degree was 2.528. The average network distance between all pairs of nodes, i.e., the average path length, was 1.49 edges with a network diameter of four edges. The average clustering coefficient was 0.352 and the modularity index was 0.504.

Table S1a. PCR primers

Target genes	Primer Sequences	Amplicon size(bp)	Annealing temp(℃)	(GenBank accession no.)	Reference
erm(A)	TCTAAAAAGCATGTAAAAGAA CTTCGATAGTTTATTAATATTAGT	641	52	PRJNA352260	[1]
erm(B)	GATACCGTTTACGAAATTGG GAATCGAGACTTGAGTGTGC	364	58	X52632	[2]
erm(C)	TCA AAA CAT AAT ATA GAT AAA GCT AAT ATT GTT TAA ATC GTC AAT	642	50	M19652	[2]
erm(T)	CAT ATA AAT GAA ATT TTG AG ACG ATT TGT ATT TAG CAA CC	369	51	AF310974	[2]
erm(X)	GAG ATC GGR CCA GGA AGC GTG TGC ACC ATC GCC TGA	488	58	X51472	[2]
tcrB	CATCACGGTAGCTTTAAGGAGATTTTC ATAGAGGACTCCGCCACCATTG	663	55	AY048044.2	[3]
copA	TGCACCTGACVGGSCAYAT GVACTTCRCGGAACATRCC	324	60	M19930 CP000075 AE016853 CP000744 EF587902 CP000304 AE004091 AF455751 CP000076	[4]
cusA	ATGCSACVGGYGTTGGCTGG CCRTTCAGYTCGGCRATRCC	410	60	CU928162.2	[5]
pcoA	GCTGCAGATGGCCAGTATGTAAA CCCTCGAGCGTAACCGGTCC	147	60	DQ517526.1	[6]
cueO	ATGGATATGGAACGGATGAA ATTGCTACAGATGGTGGTT	420	50	HM565232.1	[7]
intI1	CTGGATTTCGATCACGGCACG ACATGCGTGTAAATCATCGTCG	473	60	DQ393784.1	[8]
16s rRNA	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	193	55		[9]

Table S1b. The detailed information of qPCR for each target gene.

Target gene	**R^2**	**amplification efficiencies**	**Detection limits (copies/μL)**
copA	0.998	91.9%	22
cusA	0.992	93.3%	28
tcrB	0.999	92.0%	47
pcoA	0.991	106.9%	57
ermA	0.992	92.1%	25
ermB	0.997	91.4%	92
intI1	0.995	99.2%	35
16s rRNA	0.983	92.9%	250

Table S2. Changes in physical–chemical parameters for three treatments

	compost time (days)	pH	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	exchangeable Cu (mg kg^{-1})
CK	0	7.85	118.20	1046.00	3.10
	2	8.72	221.69	2187.96	3.64
	7	9.14	188.57	2386.95	3.24
	14	8.38	115.31	1440.44	2.32
	21	8.21	97.80	123.34	1.51
	35	8.51	219.66	201.00	1.49
Cu200	0	7.68	150.31	1159.09	9.70
	2	8.80	234.35	2161.43	9.59
	7	8.88	160.95	1946.85	10.69
	14	8.15	88.75	925.64	4.87
	21	8.25	75.61	275.54	2.85
	35	8.32	81.58	280.00	2.76
Cu2000	0	7.92	158.02	1065.70	112.84
	2	8.61	219.97	3380.73	129.57
	7	9.15	222.33	3302.46	68.67
	14	8.44	125.05	2141.44	49.77
	21	7.99	68.28	49.98	40.89
	35	7.90	51.82	62.00	40.08

Table S3a. Absolute abundance of copper resistance genes and antibiotic resistance*.

		copA	*pcoA*	*cusA*	*tcrB*	*ermB*	*ermA*	*Int1*	16srRNA
0d	CK	2.6E+08	5.3E+07	8.7E+07	2.4E+07	6.3E+06	3.2E+07	2.5E+08	4.3E+10
		(8.6E+06)	(1.8E+06)	(6.8E+06)	(7.0E+06)	(2.4E+06)	(1.6E+06)	(3.1E+07)	(1.4E+09)
	Cu200	1.8E+08	3.2E+07	1.4E+08	3.0E+07	1.7E+07	5.0E+07	4.1E+08	4.2E+10
		(1.3E+07)	(2.6E+06)	(6.6E+06)	(2.0E+06)	(7.3E+06)	(3.3E+06)	(5.7E+07)	(2.4E+09)
	Cu2000	1.4E+08	4.6E+07	1.5E+08	3.0E+07	7.8E+06	4.3E+07	5.4E+08	3.2E+10
		(6.7E+06)	(3.5E+06)	(7.9E+06)	(4.2E+06)	(7.7E+05)	(5.6E+06)	(9.6E+07)	(5.0E+08)
2d	CK	1.8E+09	5.2E+07	4.0E+08	6.3E+07	1.2E+07	2.7E+07	1.9E+09	5.7E+10
		(9.7E+07)	(4.8E+06)	(2.7E+07)	(1.4E+07)	(7.5E+06)	(1.6E+06)	(5.6E+08)	(3.0E+09)
	Cu200	6.0E+08	1.1E+07	2.6E+08	3.7E+07	3.6E+07	2.4E+07	8.5E+08	2.4E+10
		(1.1E+08)	(6.4E+05)	(3.9E+07)	(8.1E+06)	(1.8E+07)	(2.9E+06)	(3.0E+07)	(2.0E+09)
	Cu2000	5.2E+09	2.3E+08	1.1E+09	3.1E+07	3.3E+07	9.1E+07	4.5E+09	9.6E+10
		(3.5E+08)	(2.8E+07)	(7.3E+07)	(1.7E+07)	(6.7E+06)	(2.9E+06)	(7.3E+08)	(4.0E+09)
7d	CK	9.9E+08	1.2E+08	6.8E+08	4.2E+06	3.1E+07	2.4E+07	1.9E+09	1.9E+11
		(3.1E+08)	(5.1E+07)	(7.9E+07)	(1.7E+06)	(7.5E+06)	(1.5E+06)	(8.1E+07)	(7.5E+09)
	Cu200	1.4E+09	1.9E+07	6.7E+08	8.1E+06	1.3E+08	1.2E+07	9.2E+08	1.0E+11
		(1.1E+08)	(7.9E+05)	(9.1E+07)	(3.3E+06)	(1.8E+07)	(1.4E+06)	(1.4E+08)	(7.6E+09)
	Cu2000	1.5E+09	7.7E+06	4.1E+08	6.8E+06	3.3E+07	2.0E+07	1.2E+09	9.6E+10
		(8.1E+08)	(8.2E+04)	(4.9E+07)	(2.9E+06)	(6.7E+06)	(1.6E+06)	(4.5E+07)	(6.5E+08)
14d	CK	1.3E+09	2.6E+06	4.7E+08	1.1E+06	2.4E+06	4.8E+06	1.7E+08	4.9E+10
		(7.4E+07)	(1.9E+06)	(5.5E+07)	(1.6E+06)	(1.1E+06)	(1.6E+06)	(3.8E+07)	(1.6E+08)
	Cu200	2.3E+09	5.8E+06	4.8E+08	9.0E+05	3.1E+06	6.7E+06	2.7E+08	6.3E+10
		(3.2E+08)	(2.7E+06)	(4.1E+07)	(1.7E+05)	(3.1E+05)	(8.4E+05	(1.9E+07)	(5.1E+09)
	Cu2000	8.5E+08	9.8E+06	1.3E+08	3.4E+06	4.4E+06	7.7E+06	3.0E+08	1.5E+10
		(9.9E+07)	(2.1E+06)	(1.7E+07)	(1.6E+06)	(1.1E+06)	(9.2E+05)	(1.1E+07)	(4.8E+08)
21d	CK	9.2E+08	5.8E+06	8.8E+08	2.0E+06	2.4E+06	1.8E+06	2.5E+08	5.8E+10
		(3.7E+08)	(8.8E+05)	(9.9E+07)	(1.2E+06)	(7.4E+05)	(1.1E+05)	(3.8E+07)	(4.3E+09)
	Cu200	3.7E+09	5.6E+05	1.0E+09	8.2E+05	2.5E+06	1.8E+06	1.6E+08	5.9E+10
		(9.2E+08)	(3.0E+05)	(5.3E+07)	(5.1E+05)	(4.0E+05)	(6.7E+04)	(1.9E+07)	(2.1E+09)
	Cu2000	1.7E+09	2.8E+06	8.8E+08	3.9E+06	3.0E+06	9.7E+06	4.0E+08	6.1E+10
		(9.9E+07)	(9.1E+05)	(7.1E+06)	(1.3E+06)	(2.5E+05)	(1.4E+06)	(1.1E+07)	(1.1E+09)
35d	CK	1.9E+09	1.6E+07	9.5E+08	9.7E+05	2.2E+06	1.7E+06	2.1E+08	6.1E+10
		(1.6E+08)	(1.2E+07)	(6.9E+07)	(3.7E+05)	(3.5E+05)	(2.0E+05)	(1.6E+07)	(2.8E+09)
	Cu200	3.4E+09	1.6E+06	5.1E+08	3.7E+05	1.3E+06	1.2E+06	1.5E+08	3.2E+10
		(5.5E+08)	(6.2E+05)	(2.2E+07)	(5.3E+04)	(3.6E+05)	(1.7E+05)	(1.5E+07)	(1.1E+09)
	Cu2000	4.2E+09	1.7E+04	8.5E+08	2.9E+06	8.4E+05	3.0E+06	2.2E+08	3.9E+10
		(1.4E+09)	(4.6E+03)	(9.0E+07)	(1.5E+06)	(8.3E+03)	(2.6E+05)	(1.2E+07)	(7.1E+08)

*mean ± (S.E.)

Table S3b. The dynamic changes of log transformed gene copies during swine manure composting

Target genes	Control						Cu200						Cu2000					
	D0	D2	D7	D14	D21	D35	D0	D2	D7	D14	D21	D35	D0	D2	D7	D14	D21	D35
copA	8.4	9.3	9.0	9.1	9.0	9.3	8.3	8.8	9.1	9.4	9.6	9.5	8.1	9.7	9.2	8.9	9.2	9.6
pcoA	7.7	7.7	8.1	6.4	6.8	7.2	7.5	7.0	7.3	6.8	5.7	6.2	7.7	8.4	6.9	7.0	6.4	4.2
cusA	7.9	8.6	8.8	8.7	8.9	9.0	8.1	8.4	8.8	8.7	9.0	8.7	8.2	9.0	8.6	8.1	8.9	8.9
tcrB	7.4	7.8	6.6	6.1	6.3	6.0	7.5	7.6	6.9	6.0	5.9	5.6	7.5	7.5	6.8	6.5	6.6	6.5
ermA	7.5	7.4	7.4	6.7	6.3	6.2	7.7	7.4	7.1	6.8	6.2	6.1	7.6	8.0	7.3	6.9	7.0	6.5
ermB	6.8	7.1	7.5	6.4	6.4	6.3	7.2	7.6	8.1	6.5	6.4	6.1	6.9	7.5	7.5	6.6	6.5	5.9
intI1	8.4	9.3	9.3	8.2	8.4	8.3	8.6	8.9	9.0	8.4	8.2	8.2	8.7	9.7	9.1	8.5	8.6	8.3

Table S4. The Spearman's correlation analysis between relative abundances of CRGs, ARGs, *intl1* and exchangeable Cu during the swine manure composting

	copA	*CusA*	*pcoA*	*tcrB*	*emA*	*emB*	*Intl1*	Cu
copA	1	0.723**	-0.388**	-0.411**	-0.365**	-0.303*	-0.139	-0.031
CusA		1	-0.590**	-0.408**	-0.580**	-0.463**	-0.352**	-0.245
pcoA			1	0.572**	0.724**	0.516**	0.670**	0.326*
tcrB				1	0.747**	0.492**	0.639**	0.399**
emA					1	0.645**	0.787**	0.662**
emB						1	0.721**	0.535**
Intl1							1	0.716**
Cu								1

* Correlation significant at $p < 0.05$.

** Correlation significant at $p < 0.01$.

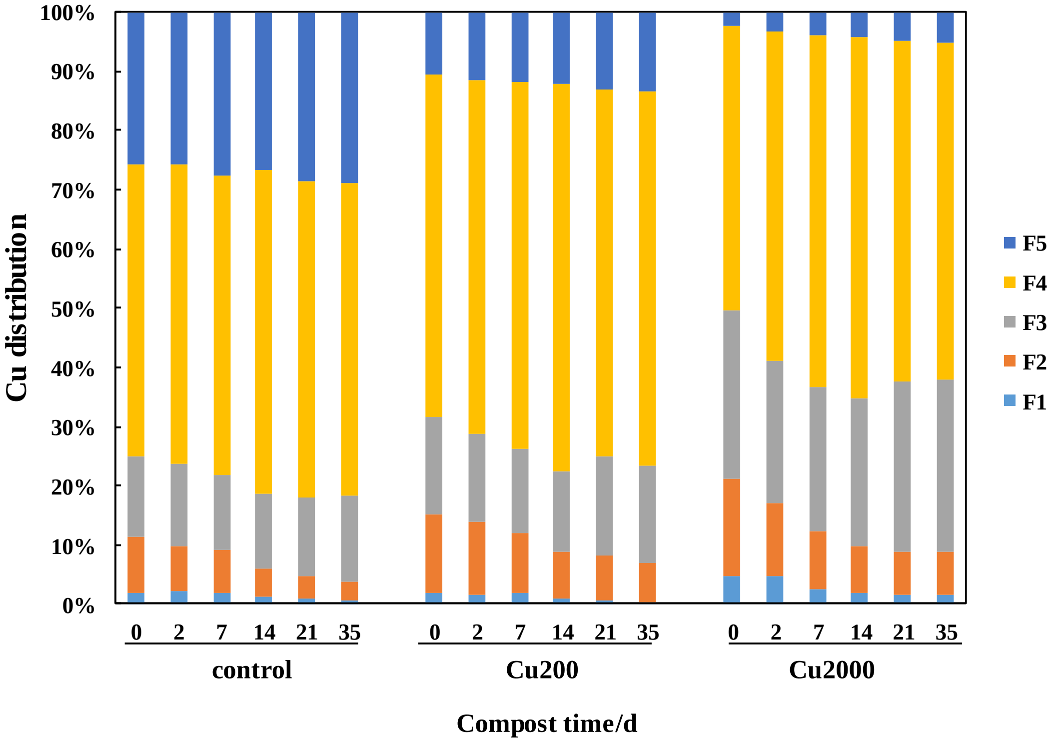

Figure S1. Changes in Cu speciation during manure composting of control (A), Cu200 (B) and Cu2000(C), respectively.

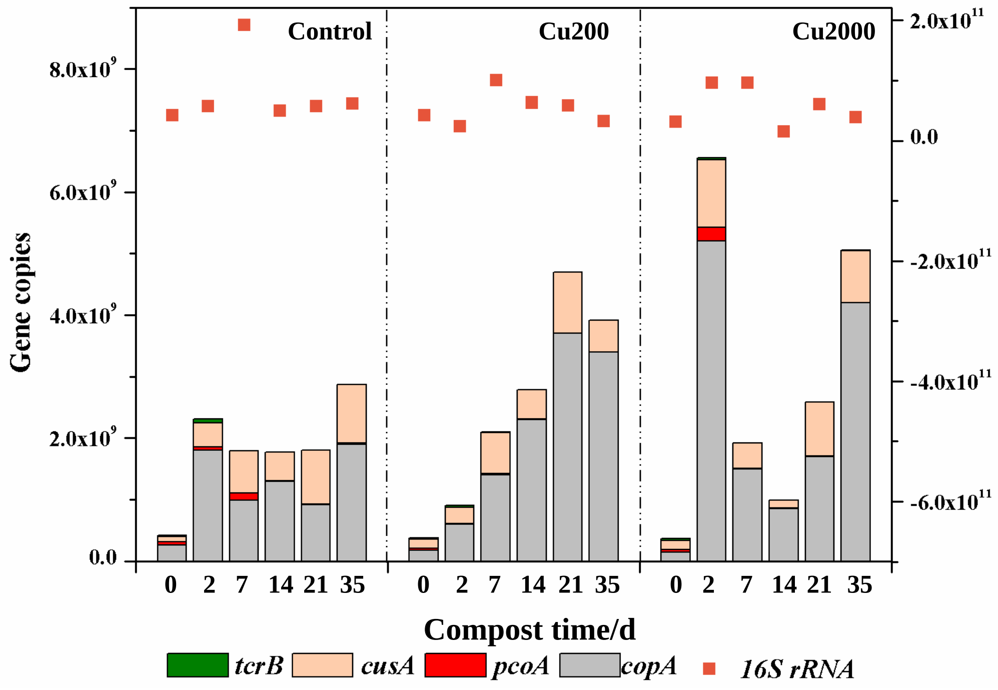

Figure S2. Changes of absolute abundance CRGs and 16S rRNA gene during manure composting of control (A), Cu200 (B) and Cu2000(C), respectively.

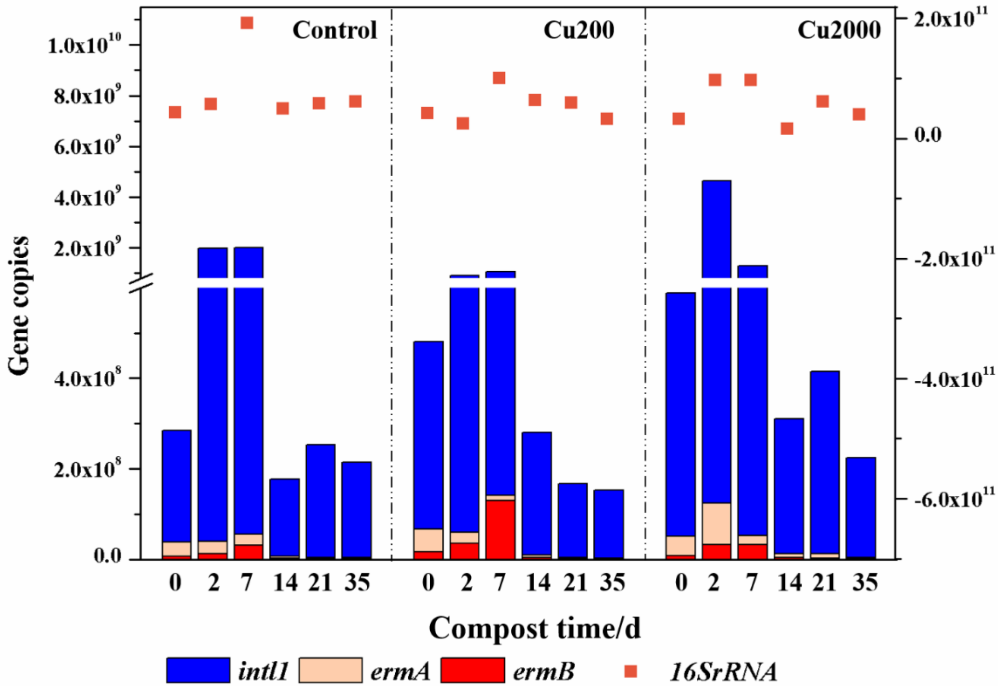

Figure S3. Changes of absolute abundance ARGs and *intI1* gene during manure composting of control (A), Cu200 (B) and Cu2000 (C), respectively.

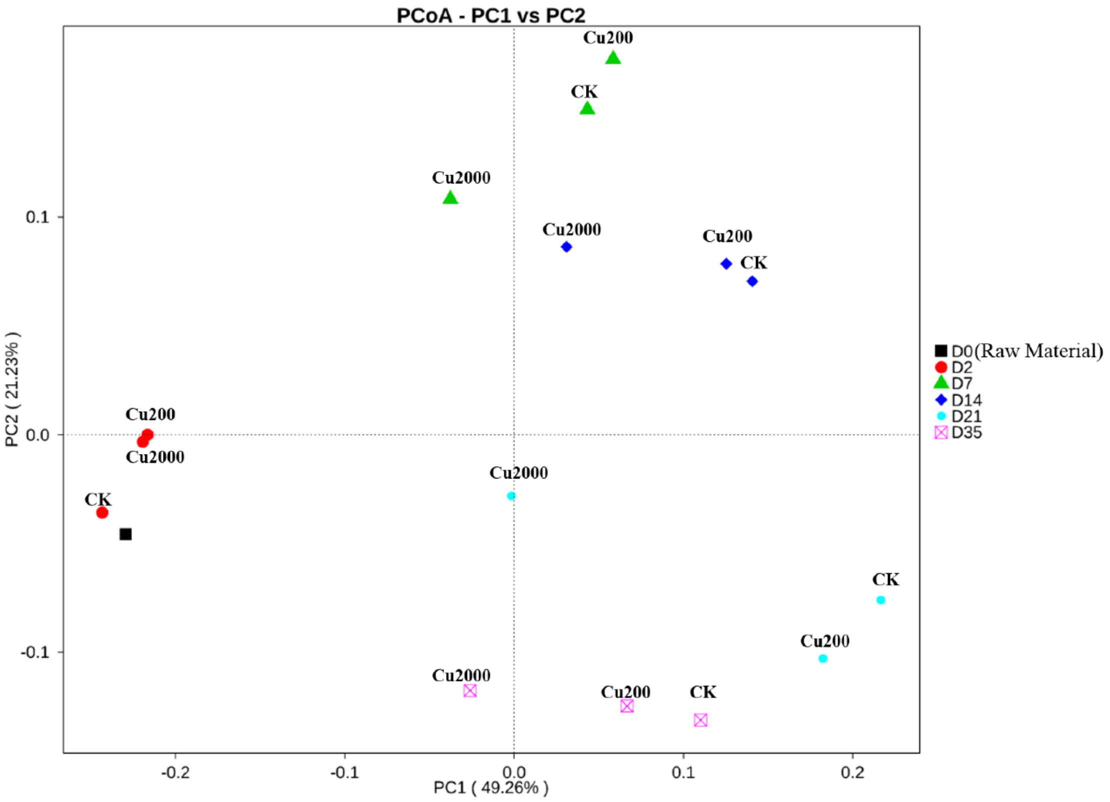

Figure S4. Principal coordinate analysis of the bacterial communities during manure composting of control (A), Cu200 (B) and Cu2000 (C), respectively.

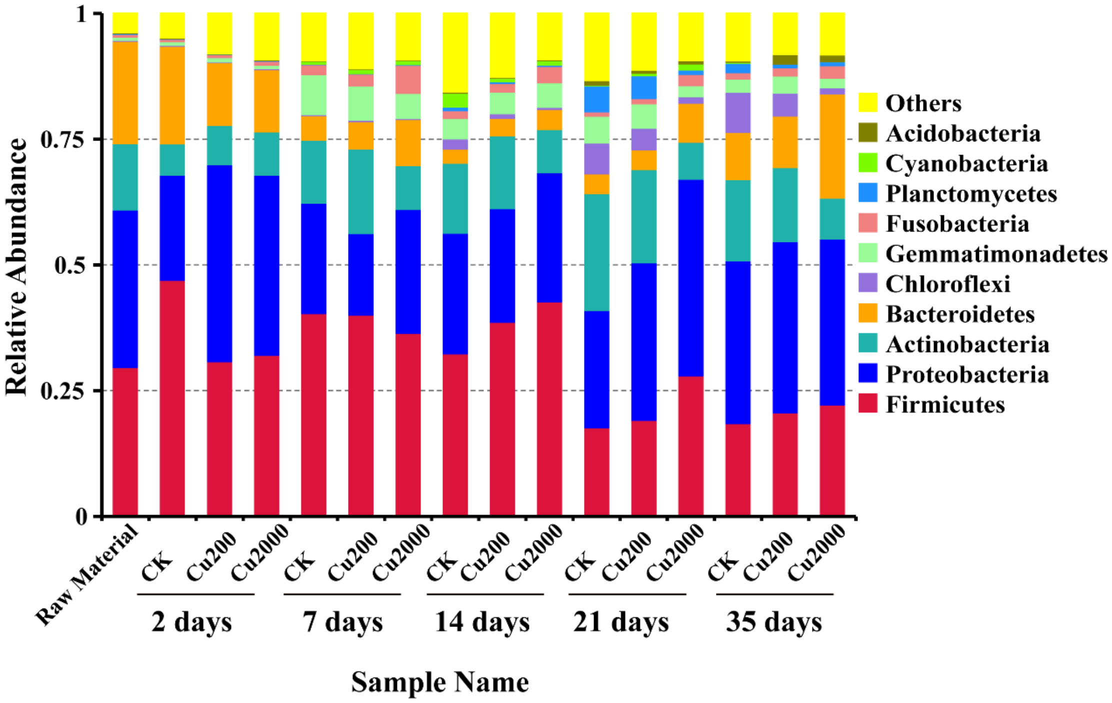

Figure S5. Variations in the main phyla detected during manure composting of control (A), Cu200 (B) and Cu2000 (C), respectively.

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