Supplemental Information

Composting reduces the risks of resistome in beef cattle manure at the transcriptional level

Yuepeng Sun, [1,2,+] Zachery R. Staley, [2,+] Bryan Woodbury, [3] Jean-Jack Riethoven,[5] and Xu Li [2,4,]*

[1]School of Ecology and Environment, Inner Mongolia University, Hohhot 010021, China

[2]Department of Civil and Environmental Engineering, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

[3]USDA-ARS U.S. Meat Animal Research Center, Clay Center, NE 68933, USA

[4]Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

[5]Center for Biotechnology, University of Nebraska-Lincoln, Lincoln, Nebraska, 68588, USA

[+] These authors contributed equally to this work

Correspondence:

*Xu Li
Department of Civil and Environmental Engineering
University of Nebraska - Lincoln
900 N. 16th St, W150D Nebraska Hall
Lincoln, NE 68588-0531
Email: xuli@unl.edu

Number of Pages: 18
Number of Figures: 8
Number of Tables: 7

RESULTS

Microbial Community in Both Piles

More specifically, stockpiling pile reduced the abundance of Actinobacteria at the center and surface position from 12.0% to 6.2% and 30.6% to 0.32%, respectively, and Firmicutes at the center and surface position from 16.0% to 10.0% and 30.1% to 1.8%, respectively. Correspondingly, the composting pile reduced the abundance of Actinobacteria at the center and surface position from 11.7% to 6.0% and 20.7% to 11.1%, respectively, and Firmicutes at the center and surface position from 23.5% to 11.0% and 30.7% to 2.1%, respectively.

Table S1. Mean daily temperatures (°C) for all thermocouples in each layer (layers 1-4 from bottom to top) for the composting pile and stockpile for all days where one layer in at least one pile had temperatures above 55°C during the summer-fall round. Temperatures exceeding 55°C are bolded.

	Stockpiling				Composting			
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
8/8/2018	45.0	45.0	53.1	49.0	**55.5**	46.7	52.5	51.1
8/9/2018	49.7	47.8	**61.8**	**55.5**	**62.9**	50.6	**60.8**	**62.0**
8/10/2018	46.5	48.6	**62.2**	**59.4**	**63.1**	51.7	**62.6**	**67.9**
8/11/2018	45.7	49.8	**62.8**	**63.0**	**63.8**	53.2	**64.2**	**70.3**
8/12/2018	46.0	51.1	**63.2**	**64.3**	**64.4**	**55.0**	**65.5**	**71.0**
8/13/2018	45.9	52.1	**62.7**	**65.1**	**64.8**	**56.2**	**65.8**	**70.9**
8/14/2018	45.0	52.8	**61.4**	**65.4**	**64.5**	**56.9**	**65.4**	**70.8**
8/15/2018	45.8	53.6	**60.8**	**64.1**	**64.3**	**57.7**	**65.8**	**69.3**
8/16/2018	47.3	54.1	**61.6**	**63.1**	**64.2**	**58.0**	**65.5**	**69.4**
8/17/2018	47.2	54.1	**61.3**	**63.0**	**64.2**	**58.4**	**65.1**	**69.2**
8/18/2018	47.4	54.5	**60.3**	**63.3**	**63.7**	**62.8**	**64.2**	**68.9**
8/19/2018	45.8	54.7	**58.6**	**64.0**	**62.3**	**61.8**	**63.0**	**67.7**
8/20/2018	45.9	**55.9**	**57.8**	**62.6**	**63.9**	**62.9**	**64.1**	**68.9**
8/21/2018	47.1	**55.6**	**57.7**	**60.9**	**65.3**	**63.6**	**64.5**	**67.1**
8/22/2018	48.2	**55.0**	**57.3**	**58.8**	**64.8**	**62.9**	**63.6**	**65.5**
8/23/2018	47.1	54.1	**56.3**	**58.0**	**62.8**	**61.6**	**62.4**	**67.4**
8/24/2018	47.4	53.7	**55.6**	**59.1**	**62.2**	**61.2**	**61.9**	**66.7**
8/25/2018	48.2	53.3	**56.0**	**58.9**	**62.6**	**61.2**	**62.1**	**66.2**
8/26/2018	47.5	53.0	**56.0**	**59.0**	**61.6**	**60.7**	**61.6**	**68.3**
8/27/2018	46.9	53.0	**55.5**	**61.4**	**61.1**	**60.2**	**61.0**	**68.0**
8/28/2018	46.4	53.1	**55.5**	**61.2**	**61.7**	**60.4**	**61.2**	**67.4**
8/29/2018	45.5	53.4	54.3	**60.6**	**60.9**	**59.1**	**60.3**	**66.3**
8/30/2018	45.9	53.4	54.7	**60.5**	**60.9**	**59.9**	**60.8**	**67.5**
8/31/2018	46.3	53.6	54.8	**61.6**	**60.6**	**59.6**	**60.6**	**66.4**
9/1/2018	46.9	54.7	**55.3**	**63.9**	**60.7**	**59.2**	**60.1**	**65.7**
9/2/2018	45.7	54.6	54.0	**63.5**	**59.8**	**58.0**	**59.1**	**64.2**
9/3/2018	45.7	54.3	53.3	**60.7**	**59.8**	**59.2**	**60.4**	**65.4**
9/4/2018	44.3	53.9	50.0	**57.7**	**57.9**	**59.3**	**56.1**	**58.6**
9/5/2018	44.3	52.7	50.1	53.6	**57.9**	**57.9**	**57.4**	**58.1**
9/6/2018	43.7	51.5	50.1	51.7	**58.2**	**57.2**	**58.9**	**61.5**
9/7/2018	43.3	50.6	49.7	51.1	**58.0**	**56.9**	**59.1**	**63.7**
9/8/2018	42.8	50.0	49.2	51.1	**57.3**	**56.9**	**59.0**	**63.4**
9/9/2018	42.8	49.6	48.5	51.0	**57.1**	**56.9**	**58.5**	**60.1**
9/10/2018	42.4	49.1	48.3	50.9	**56.8**	**57.0**	**57.8**	**58.7**

9/11/2018	42.2	48.6	48.3	50.5	**57.0**	**57.1**	**57.7**	**58.7**
9/12/2018	41.6	48.2	47.9	50.5	**56.5**	**57.5**	**57.1**	**61.4**
9/13/2018	41.7	48.1	48.3	51.7	**56.6**	**57.8**	**56.9**	**63.4**
9/14/2018	42.3	48.3	48.8	52.2	**57.1**	**58.5**	**57.5**	**61.8**
9/15/2018	42.8	48.4	49.3	52.7	**57.4**	**58.7**	**57.7**	**60.0**
9/16/2018	42.6	48.3	49.6	53.5	**57.2**	**58.7**	**57.4**	**59.2**
9/17/2018	42.6	48.3	49.9	53.8	**57.0**	**58.9**	**57.2**	**58.5**
9/18/2018	42.9	48.3	50.6	53.7	**57.0**	**58.7**	**57.5**	**59.3**
9/19/2018	42.5	48.2	50.8	54.4	**56.4**	**58.4**	**57.5**	**60.6**
9/20/2018	41.7	48.6	50.8	**55.4**	**56.9**	**59.6**	**56.4**	**56.9**
9/21/2018	-	-	-	-	**57.4**	**60.2**	**56.8**	**55.8**
9/22/2018	-	-	-	-	**56.9**	**59.6**	**56.0**	**56.4**
9/23/2018	-	-	-	-	**56.7**	**59.4**	**55.3**	**55.9**
9/24/2018	-	-	-	-	**56.6**	**59.3**	**55.5**	**56.1**

- Denotes temperature not recorded due to a thermocouple error.

Table S2. The GC%, N50, total size, size of the largest DNA contig, and number of DNA contigs in samples. The light shades are added to differentiate the sampling locations (Center vs. Surface).

Treatment	Depth	Day	GC%	N50	Total Size (bp)	Largest DNA Contig (bp)	# of DNA Contigs
Compost	Center	1	55.62	1208	241100633	101656	211506
Compost	Center	5	54.47	1288	191762636	130220	159647
Compost	Center	19	49.89	1426	234781769	168248	183598
Compost	Center	33	57.72	1414	224336362	212979	178116
Compost	Center	47	55.14	1129	283728692	249797	260324
Compost	Surface	1	53.75	1978	176686740	148258	119497
Compost	Surface	5	54.58	1647	155079049	86715	113497
Compost	Surface	19	61.67	1150	281495770	97311	256195
Compost	Surface	33	60.93	1322	291358901	470598	240485
Compost	Surface	47	62.66	1029	210331119	108386	205790
Stockpile	Center	1	55.39	1229	280174973	90806	245359
Stockpile	Center	5	54.14	1466	181017520	174199	141588
Stockpile	Center	19	51.9	1224	253717506	129302	219778
Stockpile	Center	33	50.48	1504	250323373	324555	191159
Stockpile	Center	47	57.32	1190	319337703	115399	283745
Stockpile	Surface	1	56.16	1283	228829668	92976	192126
Stockpile	Surface	5	58.08	1643	236657956	192576	174242
Stockpile	Surface	19	59.24	1285	290292903	94860	245994
Stockpile	Surface	33	58.77	1044	274903405	131009	268367
Stockpile	Surface	47	58.76	1090	203137258	361672	189986

Table S3. The GC%, N50, total size, size of the largest transcript contig, and the number of transcript contigs in samples. The light shades are added to differentiate the sampling locations (Center vs. Surface).

Treatment	Depth	Day	GC%	N50	Total Size (bp)	Largest Transcript Contig (bp)	# of Transcript Contigs
Compost	Center	1	52.57	1083	29366635	12493	28701
Compost	Center	5	58.87	1428	106639503	74354	86001
Compost	Center	19	63.12	971	78839057	40012	82081
Compost	Center	33	54.15	829	19683932	9251	23403
Compost	Center	47	56.02	832	16713082	8331	19832
Compost	Surface	1	59.95	1128	48317909	19353	45691
Compost	Surface	5	68.34	1240	27791205	28188	24825
Compost	Surface	19	63.51	1180	129737670	96886	117294
Compost	Surface	33	58.63	995	25025129	19811	25086
Compost	Surface	47	62.1	965	42849588	25337	44427
Stockpile	Center	1	62.38	1033	55745813	26494	55198
Stockpile	Center	5	56.35	1121	131671510	44976	123404
Stockpile	Center	19	61.12	1041	59679315	87632	57895
Stockpile	Center	33	66.13	1130	171462195	39117	161317
Stockpile	Center	47	65.06	1210	298022193	88588	268233
Stockpile	Surface	1	61.46	1077	43246575	13692	42592
Stockpile	Surface	5	63.51	1217	49260530	35673	43428
Stockpile	Surface	19	60.78	965	38219819	89441	38772
Stockpile	Surface	33	67.2	1052	212636456	105085	207594
Stockpile	Surface	47	59.82	952	22584762	11324	23825

Table S4. The treatment averages of the gene abundance of ARG families for storage treatment and pile depth, as well as the p values for the ANOVA tests on the effects of treatment and depth.

ARG Family	Treatment (gene copy of ARG per 16S)		Depth (gene copy of ARG per 16S)		p values	
	Stockpiling[A,B]	Composting[A,B]	Center[A,B]	Surface[A,B]	Treatment	Depth
aminoglycoside	0.021	0.019	0.028 a	0.015 b	0.360	0.042*
bacitracin	0.011	0.014	0.013	0.011	0.263	0.333
beta-lactam	0.001	0.001	0.000 a	0.002 b	0.938	0.011 *
bleomycin	0.000	0.000	0.000	0.000	0.699	0.289
carbomycin	0.000	0.000	0.000	0.000	0.894	0.241
chloramphenicol	0.007	0.005	0.005	0.006	0.351	0.317
fosfomycin	0.007	0.007	0.004	0.009	0.732	0.253
fosmidomycin	0.007	0.005	0.002 a	0.009 b	0.695	0.009 **
kasugamycin	0.000	0.000	0.000	0.000	0.178	0.684
MLS	0.023	0.015	0.025	0.019	0.096	0.475
multidrug	0.046	0.037	0.035	0.049	0.381	0.181
polymyxin	0.000	0.000	0.000	0.000	0.168	0.168
puromycin	0.000	0.000	0.000	0.000	0.503	0.462
quinolone	0.002	0.002	0.001 a	0.002 b	0.307	0.022 *
rifamycin	0.005	0.003	0.003	0.005	0.123	0.093
sulfonamide	0.009	0.006	0.006	0.010	0.182	0.125
tetracenomycin_C	0.000	0.000	0.000	0.001	0.633	0.150
tetracycline	0.008 a	0.005 b	0.009	0.006	0.003 **	0.054
trimethoprim	0.001	0.000	0.000 a	0.001 b	0.387	0.013 *
vancomycin	0.023	0.024	0.015 a	0.028 b	0.171	0.005 **
total	0.174	0.148	0.149	0.177	0.140	0.108

[A]Values are treatment/depth averages, which were calculated based on all data for one particular treatment or depth.

[B]Values followed by different letter are statistically significantly different at the 0.05 (*) or the 0.01 (**) levels based on Tukey's post hoc test.

Table S5. The treatment averages of the transcript abundance of ARG families for storage treatment and pile depth, as well as the p values for the ANOVA tests on the effects of treatment and depth.

ARG Family	Treatment (transcript copy of ARG per 16S)		Depth (transcript copy of ARG per 16S)		p values	
	Stockpiling[A, B]	Composting[A, B]	Center[A, B]	Surface[A, B]	Treatment	Depth
aminoglycoside	0.00093	3.77E-05	0.001051	0.000185	0.164	0.341
bacitracin	0.000933	9.83E-05	0.000623	0.000154	0.113	0.164
beta-lactam	0.000102	7.26E-06	3.89E-05	5.06E-05	0.070	0.875
bleomycin	0	2.28E-07	8.95E-06	2.28E-07	0.347	0.347
carbomycin	1.68E-05	0	0.000205	3.39E-06	0.257	0.491
chloramphenicol	0.000419	1.83E-05	0.00029	0.000134	0.150	0.528
fosfomycin	0.000149	5.57E-05	0.000425	2.5E-05	0.415	0.188
fosmidomycin	0.000747	1.69E-05	0.000308	0.000302	0.161	0.750
kasugamycin	2.59E-07	6.87E-07	0.000588	6.67E-08	0.572	0.293
MLS	0.001054	5.75E-05	0.003163	0.000193	0.098	0.219
multidrug	0.005146	0.000161	0.002614	0.001388	0.162	0.464
polymyxin	2.55E-06	1.39E-07	5.73E-06	3.43E-08	0.365	0.327
puromycin	5.95E-06	0	3.74E-05	0	0.210	0.210
quinolone	7.51E-05	6.51E-06	0.000229	2.79E-05	0.135	0.557
rifamycin	0.000395	1.49E-05	0.000198	0.000113	0.151	0.472
sulfonamide	0.001392	6.4E-05	0.000805	0.000263	0.089	0.223
tetracenomycin_C	3.4E-05	4.2E-07	0.000163	2.02E-05	0.204	0.819
tetracycline	0.000271 a	3.2E-05 b	0.000174	4.94E-05	0.048*	0.086
trimethoprim	1.02E-05	1.29E-06	0.000294	3.88E-06	0.290	0.652
vancomycin	0.002414	0.000208	0.008909	0.000578	0.191	0.375
total	0.014655	0.000801	3.674554	0.003625	0.141	0.372

[A]Values are treatment/depth averages, which were calculated based on all data for one particular treatment or depth.
[B]Values followed by different letter are statistically significantly different at the 0.05 (*) or the 0.01 (**) levels based on Tukey's post hoc test.

Table S6. Numbers of low-, moderate-, high-risk ARGs on DNA contigs as well as the pathogen hosts identified from the metagenomic data. The light shades are added to differentiate the sampling locations (Center vs. Surface).

Treatment	Depth	Day	DNA Contigs containing ARGs	Number of DNA Contigs in Risk Level			Pathogenic host of the high-risk DNA contigs
				Low (ARG)	Moderate (ARG & MGE)	High (ARG, MGE & Pathogen)	
Stockpiling	Center	1	0.62%	1510	13	2	*S. enterica, E. coli* O157:H7
Stockpiling	Center	5	0.29%	406	9	1	*S. enterica*
Stockpiling	Center	19	0.29%	629	10	0	
Stockpiling	Center	33	0.29%	545	9	1	*L. pneomophila*
Stockpiling	Center	47	0.29%	819	7	0	
Stockpiling	Surface	1	0.31%	598	5	1	*S. enterica*
Stockpiling	Surface	5	0.29%	496	4	1	*K. pneumoniae*
Stockpiling	Surface	19	0.31%	754	4	2	*E. coli* O25b:H4, *E. coli* O157:H7
Stockpiling	Surface	33	0.68%	1824	10	3	*S. enterica, E. coli* O157:H7
Stockpiling	Surface	47	1.14%	2154	6	2	*S. enterica*
Composting	Center	1	0.29%	609	8	1	*S. enterica*
Composting	Center	5	0.30%	476	4	1	*S. enterica*
Composting	Center	19	0.29%	530	3	1	*S. enterica*
Composting	Center	33	0.31%	552	5	1	*S. enterica*
Composting	Center	47	0.31%	804	3	1	*E. coli* O157:H7
Composting	Surface	1	0.26%	304	7	2	*S. enterica, K. pneumonia*
Composting	Surface	5	0.31%	352	0	2	*K. pneumoniae*
Composting	Surface	19	0.31%	793	10	0	
Composting	Surface	33	0.31%	732	10	0	
Composting	Surface	47	0.39%	802	5	1	*S. enterica*

Table S7. Numbers of low-, moderate-, high-risk ARGs on transcript contigs as well as the pathogen hosts identified from the metatranscriptomic data. The light shades are added to differentiate the sampling locations (Center vs. Surface).

| Treatment	Depth	Day	Transcript contigs containing ARGs	Number of Transcript Contigs in Risk Level			Pathogenic host of the high-risk transcript contigs
				Low (ARG)	Moderate (ARG&MGE)	High (ARG, MGE and Pathogen)	
Stockpiling	Center	1	0.51%	277	7	0	
Stockpiling	Center	5	0.44%	526	8	3	*S. enterica, K. pneumoniae*
Stockpiling	Center	19	0.43%	246	1	2	*S. enterica, E. coli* O157:H7
Stockpiling	Center	33	0.38%	612	4	1	*S. enterica*
Stockpiling	Center	47	0.38%	1020	6	2	*S. enterica, E. coli* O157:H7
Stockpiling	Surface	1	0.37%	155	0	2	*K. pneumoniae*
Stockpiling	Surface	5	0.47%	204	0	1	*K. pneumoniae*
Stockpiling	Surface	19	0.48%	183	5	0	
Stockpiling	Surface	33	0.43%	883	6	1	*E. coli* O25b:H4
Stockpiling	Surface	47	0.30%	70	1	0	
Composting	Center	1	0.38%	106	2	0	
Composting	Center	5	0.28%	236	1	0	
Composting	Center	19	0.30%	245	2	1	*K. pneumoniae*
Composting	Center	33	0.35%	79	1	2	*S. enterica*
Composting	Center	47	0.52%	101	2	0	
Composting	Surface	1	0.33%	149	1	1	*K. pneumoniae*
Composting	Surface	5	0.37%	88	2	1	*K. pneumoniae*
Composting	Surface	19	0.45%	520	4	2	*S. enterica, Mycobacterium chelonae*
Composting	Surface	33	0.46%	113	3	0	
Composting	Surface	47	0.62%	271	4	0	
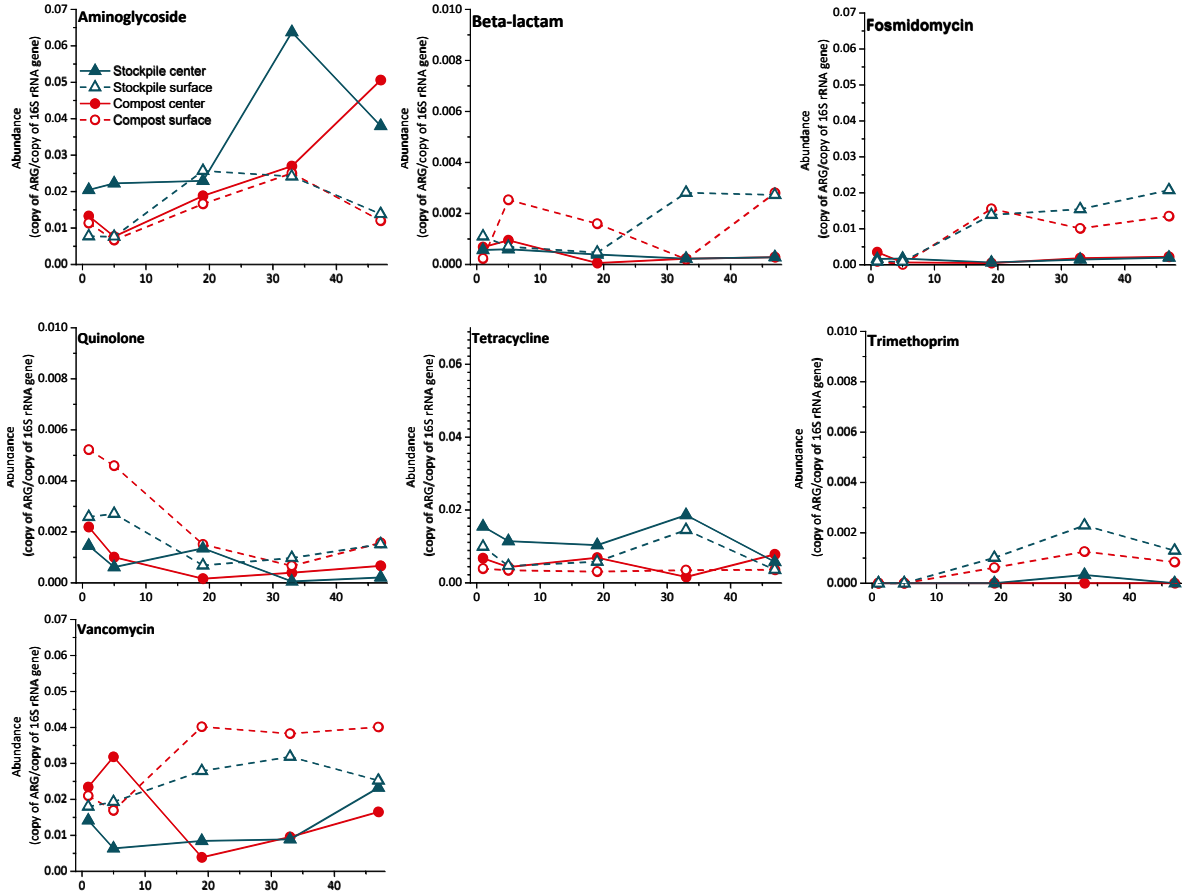
Figure S1. Variation of ARG gene abundance in the center and surface of the composting and stockpiling piles at each sampling day.
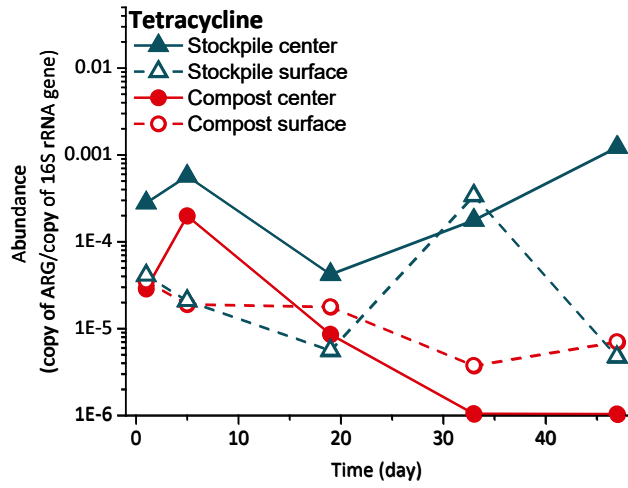
Figure S2. Variation of ARG transcript abundance in the center and surface of the composting and stockpiling piles at each sampling day.
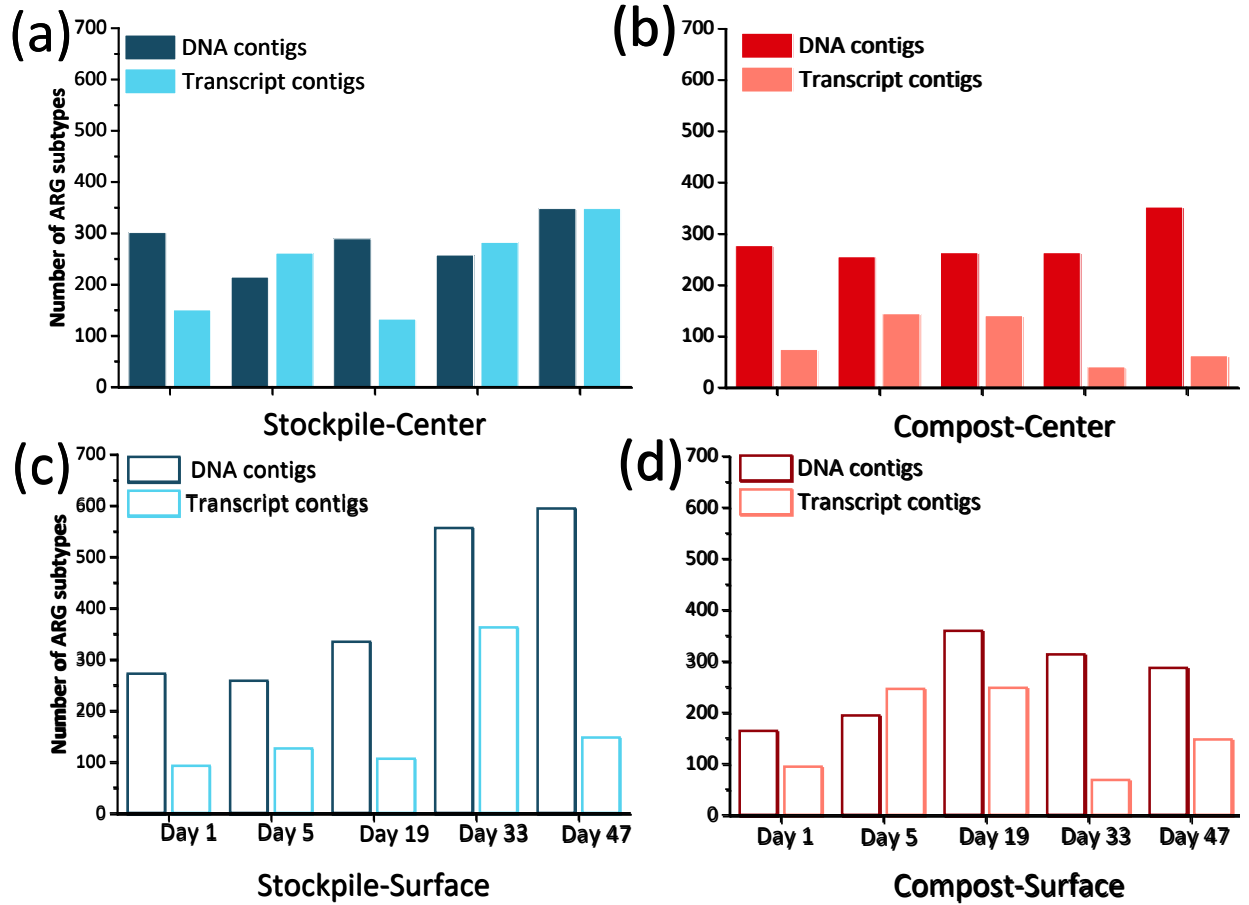
Figure S3. The number of ARG subtypes on DNA contigs and transcript contigs in the surface and center of the stockpiling (**a**, **c**) and composting (**b**, **d**) piles of manure at each sampling event.
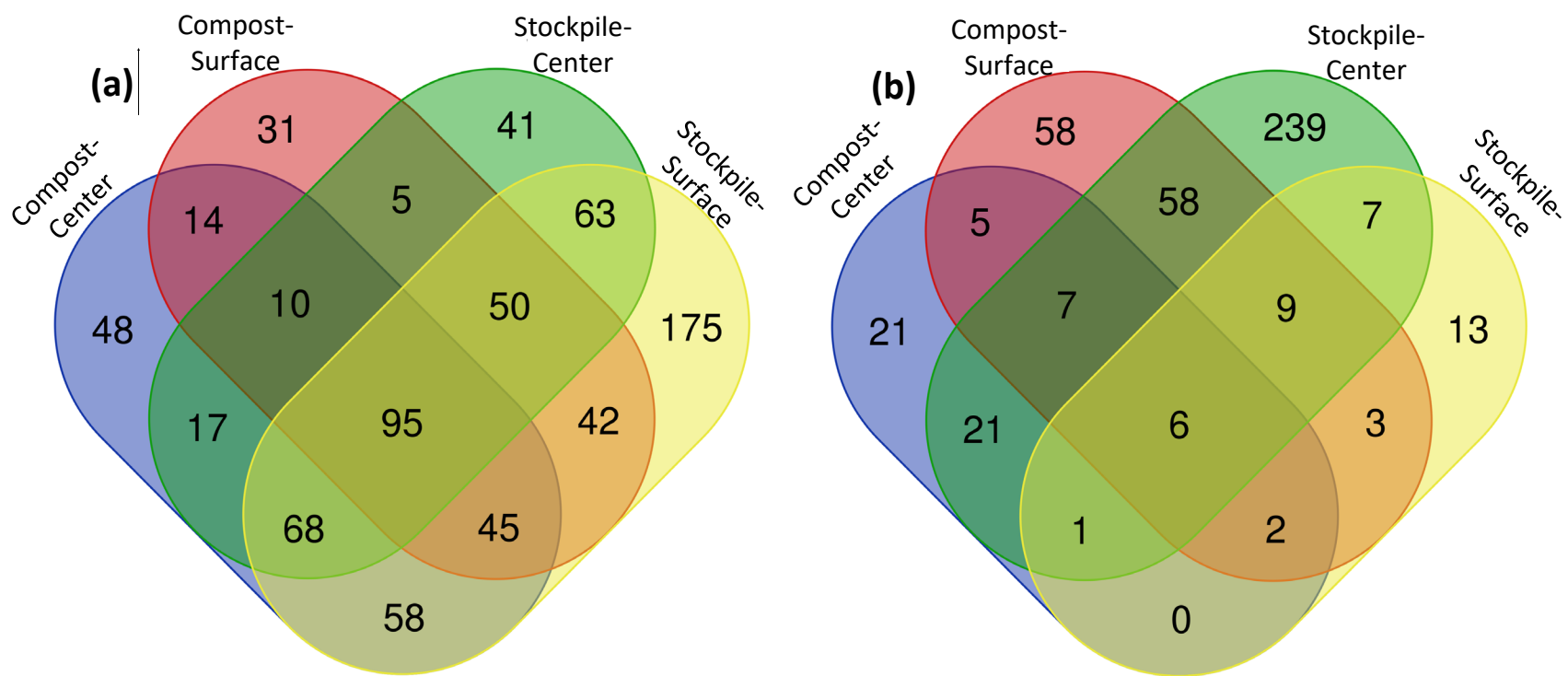
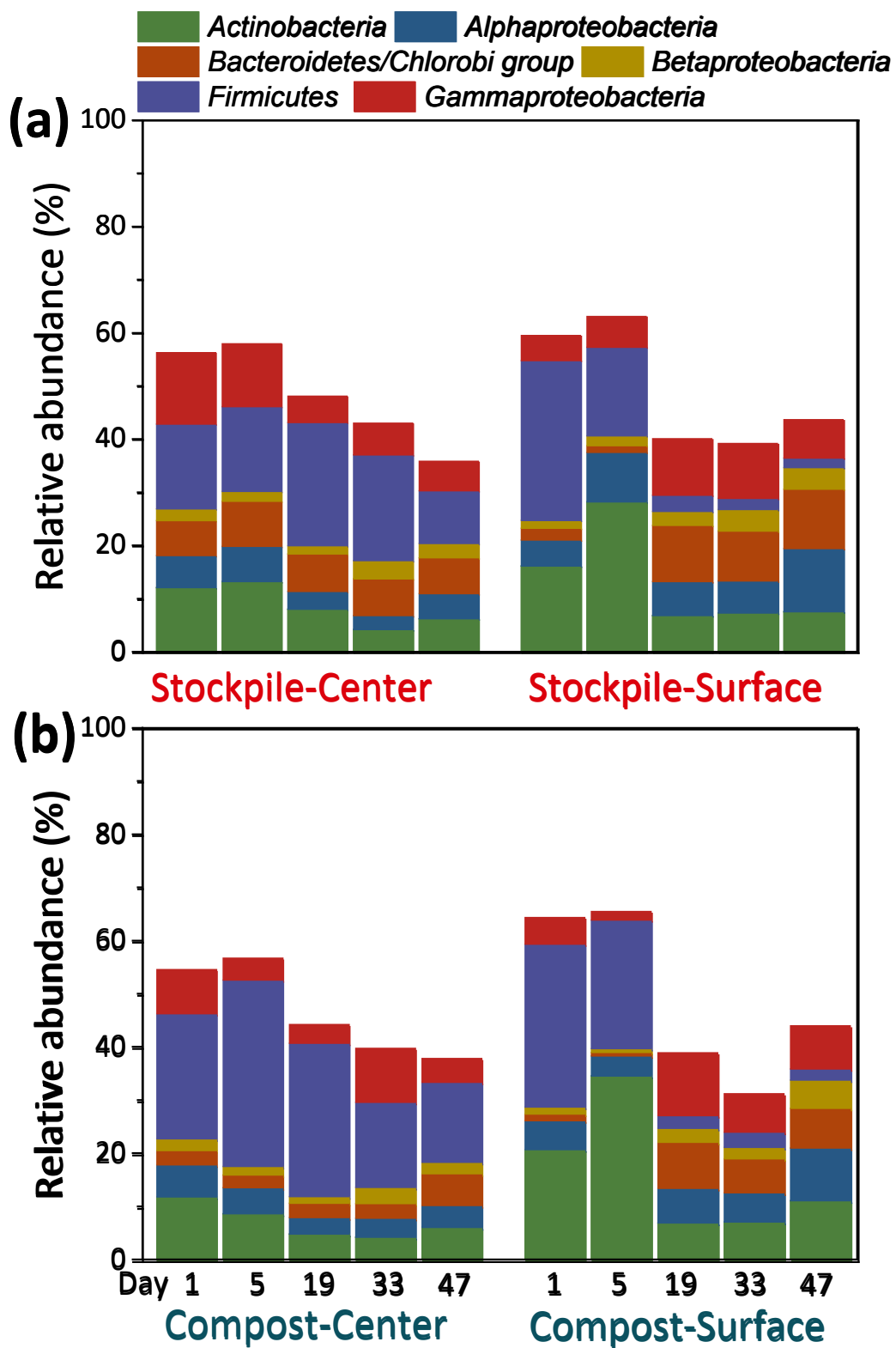**Figure S4.** Venn diagrams showing the numbers of unique ARG subtypes on DNA contigs (a) and transcript contigs (b) of the stockpiling and the composting piles.
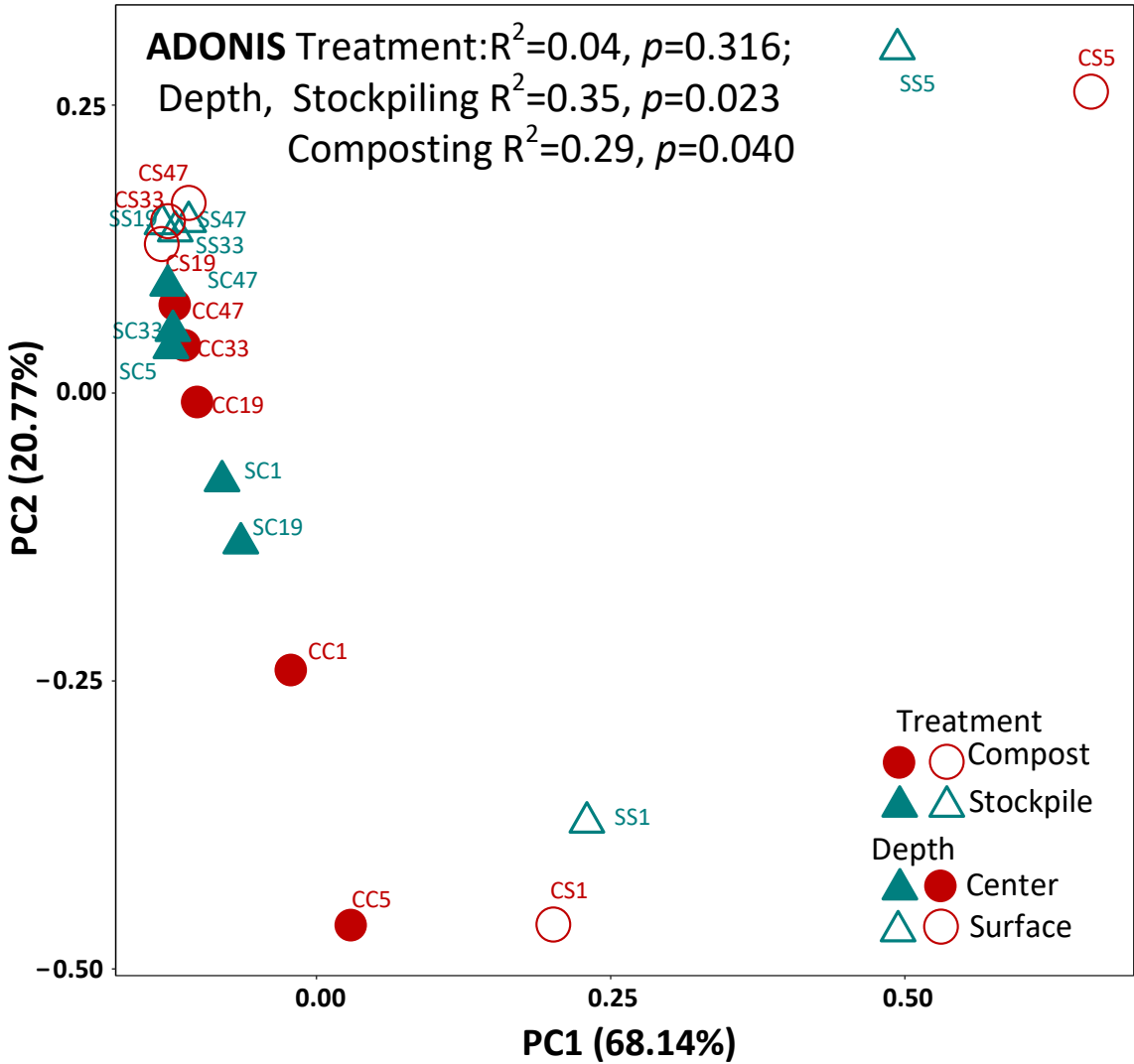
Figure S5. Microbial community at the phylum level in stockpiling (a) and composting (b) piles.

Figure S6. PCA plot of beta diversity on the microbial community at the genus level. The sample ID: the first letter represents the treatment (C for composting and S for stockpiling); the second letter represent the depth (C for center and S for surface); and the number after each sample name represents sampling days.

(a)

(b)

● Firmicutes	● Firmicutes
● Actinobacteria	● Actinobacteria
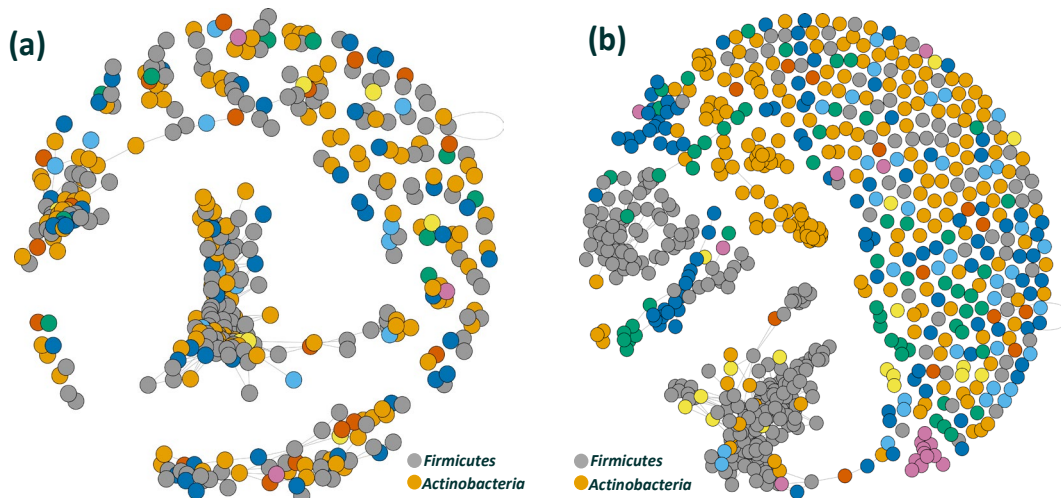
Topological property of networks.

	Stockpiling (n=10)	Composting (n=10)
Number of edges	3910	2516
Number of vertices	460	466
Diameter	13.0	14.0
Average degree	17.000	10.798
Clustering coefficient	0.046	0.578
Centralization betweenness	0.014	0.016
Connectance	0.037	0.023
Average path length	3.047	3.503

Figure S7. Bacterial network analysis showing the correlations between microorganisms at the genus level of the stockpiling (a) and the composting (b) pile. Nodes are colored by phylum. Nodes are connected when Pearson correlation coefficients higher than $|\pm0.9|$ ($p < 0.001$).
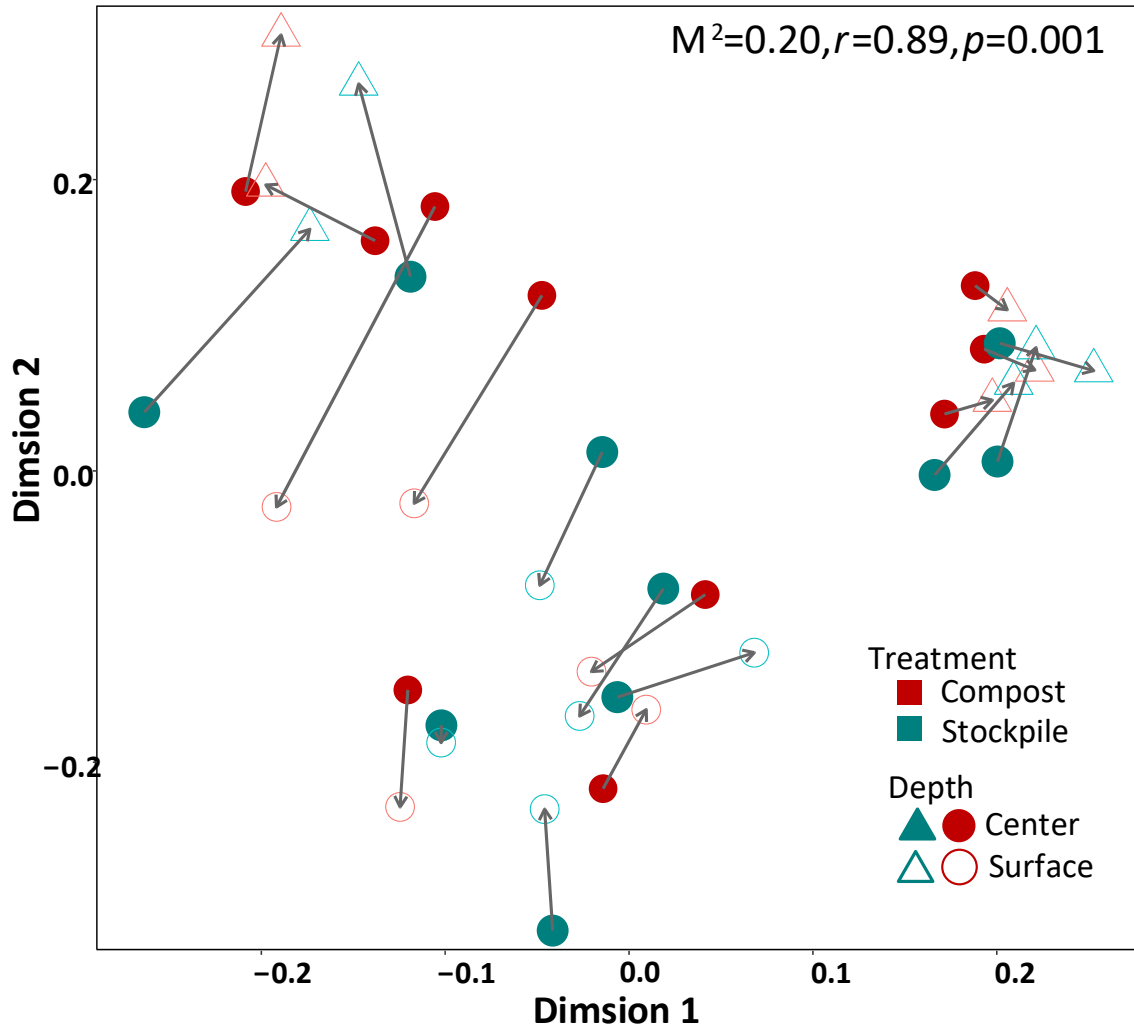
Figure S8. Procrustes test depicting the significant correlation between resistomes and microbiomes in the center and surface of stockpiling and composting piles.