

*Supplementary Information*

**Transmission Routes of Microbiome and Resistome from Manure to Soil and Lettuce**

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## RESULTS

### Alpha diversity of microbiome

Alpha diversity indexes for microbiome varied across sample types, with significantly lower (ANOVA and Turkey's HSD,  $p$  values  $< 0.05$ ) average number of genera and Shannon index in root (7 – 15 and 1.29 – 1.44, respectively) and endosphere (2 – 4 and 0.63 – 0.81) than those in other sample types, such as surface soil (40 – 58 and 2.48 – 3.11, Table S3). ANOVA tests show that the manure treatment factor had no significant impacts on the alpha diversity indexes of the microbiomes (Table S4). In comparison, ANOVA tests show that sample type had significant impacts on the alpha diversity indexes of the microbiomes (Table S3 and S4).

### Alpha diversity of resistome

A total of 359 ARG types were detected, with the average number ranging 21 – 145 for each sample type (Table S6). Two-way ANOVA indicates significant differences in the number of ARG types across different sample types ( $p$  values  $< 0.05$ ) and treatment scenarios (Table S7,  $p$  values  $< 0.05$ ). Manure treatment had significant effects on the Shannon index and the number of ARG types in surface soil and episphere (Table S6). For example, manure application (T5 and T10) caused the detection of sulfonamide (e.g., *sul1* and *sul2*) and tetracycline resistance genes (e.g., *tet(A)* and *tet(X)*) in surface soil and episphere, while these ARGs were not detected in the original soil (Figure S5).

Compared to control, T10 had a significantly higher (t-test,  $p$  values  $< 0.05$ ) number of shared ARGs between surface soil and episphere, and among endosphere, rhizosphere and root (Figure S6); T5 had a significant higher (t-test,  $p < 0.05$ ) number of shared ARGs between surface soil and episphere (Figure S6A). These results imply the pivotal role of manure

application in modulating resistome composition (especially in surface soil, episphere and endosphere samples) and transmission from manured soil to lettuce.

**Table S1.** Overall sampling scheme.

<b>Sample type</b>	<b>Treatment</b>	<b>Sampling time</b>	<b>Sampling location</b>	<b># of replicates per combination</b>
Soil		Week 0		3 replicates
Manure		Week 0		3 replicates
Surface soil	C, T5, T10	Week 1 (W1)		3 pooled replicates
	C, T5, T10	Week 3 (W3)		3 pooled replicates
	C, T5, T10	Week 6 (W6)		3 pooled replicates
Rhizosphere soil	C, T5, T10	Week 6	Top (T)	3 pooled replicates
	C, T5, T10	Week 6	Middle (M)	3 pooled replicates
	C, T5, T10	Week 6	Bottom (B)	3 pooled replicates
Root	C, T5, T10	Week 6		3 pooled replicates
Episphere	C, T5, T10	Week 6		3 pooled replicates
Endosphere	C, T5, T10	Week 6		3 pooled replicates

**Table S2.** Total reads and trimmed reads for shotgun metagenomic sequencing.

Sample type	Treatment	Sampling Time	Sampling location	Round					
				1		2		3	
				Total reads ×2	Trimmed reads×2	Total reads ×2	Trimmed reads×2	Total reads ×2	Trimmed reads×2
Soil		W0		16935474	16523066	22255771	21075470	18712521	18398870
Manure		W0		14562266	14250739	23675425	23028860	24931240	24435344
Surface soil	C	W1		25808954	25694705	21274749	20577005	19494638	19091880
		W3		18461872	17760637	32363833	30742031	20961219	20494428
		W6		18383498	17872941	21551647	20652821	28538760	27690754
	T5	W1		28298479	27479742	21315840	20652821	15761754	15470069
		W3		17079039	16799841	17646825	17274068	18905149	18509825
		W6		19722875	19228899	25048543	24150337	29086141	28244532
	T10	W1		15923384	15617345	22035976	21357693	21382195	20929791
		W3		30335850	29542821	24248108	23587006	28785181	28072923
		W6		20825475	20239646	37529771	35733904	20380093	19969219
Rhizosphere soil	C	W6	T	17093537	16779841	23951738	22789792	19848363	19412445
			M	24321049	23490165	31939959	30448574	19657877	19205181
			B	20001952	19503312	25891618	24497597	21013741	20388738
	T5	T	20255196	19746318	20167674	19741777	29064444	28209725	
		M	19735775	19218269	31785831	30270390	40032608	38728078	
		B	24079488	23380364	19939361	19133978	15720659	15451562	
	T10	T	22361166	21605414	37139566	35836685	25376247	24779814	
		M	24739753	23891261	22665021	21779661	23032153	22359571	
		B	25440600	24405457	20244211	19502000	21774836	21257240	
Root	C	W6	T5	18974274	18041989	18869812	18058139	27977283	26564217
			T10	12217629	11865603	27431727	25866078	21087159	20167839
			T10	15122307	14568416	30173319	28436839	21859894	20907222
Episphere	C	W6	T5	17652359	16993401	21471307	21025417	17027900	16733055
			T10	12204532	11857844	24303575	23699086	16679820	16368401
			T10	19425250	18517271	31000122	30099358	42313842	40830779
Endosphere	C	W6	T5	25593188	24777294	17178884	16669714	22205485	21422488
			T10	12400938	12127887	16155839	15481786	14067593	13744787
			T10	19522061	18986575	15068954	14686058	13326235	13002836

**Table S3.** The alpha diversity, Shannon Index and the number of genera, of the microbiome in various sample types.

Sample type	Treatment	Time/Location	Shannon index	Number of Genera
Soil		Week 0	$2.75 \pm 0.33$	$38 \pm 8$
Manure		Week 0	$2.51 \pm 0.54$	$50 \pm 6$
Surface soil	C	Week 1	$2.84 \pm 0.08$	$40 \pm 5$
		Week 3	$2.75 \pm 0.37$	$51 \pm 5$
		Week 6	$3.06 \pm 0.40$	$56 \pm 19$
	T5	Week 1	$2.55 \pm 0.06$	$50 \pm 7$
		Week 3	$2.38 \pm 0.29$	$40 \pm 10$
		Week 6	$3.11 \pm 0.29$	$55 \pm 8$
	T10	Week 1	$2.48 \pm 0.15$	$47 \pm 1$
		Week 3	$2.80 \pm 0.23$	$58 \pm 9$
		Week 6	$2.81 \pm 0.17$	$48 \pm 2$
Rhizosphere soil	C	Bottom	$2.97 \pm 0.21$	$47 \pm 15$
		Middle	$3.04 \pm 0.11$	$47 \pm 2$
		Top	$2.75 \pm 0.52$	$43 \pm 6$
	T5	Bottom	$3.19 \pm 0.13$	$53 \pm 13$
		Middle	$3.14 \pm 0.20$	$50 \pm 11$
		Top	$2.64 \pm 0.75$	$53 \pm 12$
	T10	Bottom	$2.83 \pm 0.45$	$49 \pm 12$
		Middle	$2.97 \pm 0.06$	$47 \pm 14$
		Top	$3.12 \pm 0.16$	$55 \pm 5$
Root	C	Week 6	$1.41 \pm 0.64$	$15 \pm 4$
	T5	Week 6	$1.29 \pm 0.26$	$9 \pm 4$
	T10	Week 6	$1.44 \pm 0.59$	$7 \pm 4$
Episphere	C	Week 6	$1.82 \pm 0.26$	$44 \pm 4$
	T5	Week 6	$2.29 \pm 0.81$	$49 \pm 6$
	T10	Week 6	$2.34 \pm 0.74$	$50 \pm 9$
Endosphere	C	Week 6	$0.63 \pm 0.16$	$2 \pm 1$
	T5	Week 6	$0.68 \pm 0.48$	$2 \pm 1$
	T10	Week 6	$0.81 \pm 0.34$	$4 \pm 2$

**Table S4.** The  $p$  and F values of the two-way ANOVA analyses, on manure treatment and sample type, for the alpha diversity indexes of the microbiome at the genus level.

<b>Factors</b>	<b>Number of Genera</b>		<b>Shannon index</b>	
	<b><math>p</math> value</b>	<b>F value</b>	<b><math>p</math> value</b>	<b>F value</b>
Manure Treatment	0.701	0.357	0.677	0.407
Sample Type	<2e-16	68.515	<2e-16	52.615
Treatment×Type	0.929	0.377	0.918	0.667

**Table S5.** The  $p$ -values from the ANOVA analyses on ARG abundance among sample types, treatments, sampling times, sampling locations. Significance is indicated by asterisks, with  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  indicated by \*, \*\* and \*\*\*, respectively. For surface soil samples, manure treatment was defined as main treatment while time as a repeated-measure.

ARG family	Surface Soil		Rhizosphere soil		Root	Episphere	Endosphere	Sample Type
	Treatment	Time	Treatment	Location	Treatment	Treatment	Treatment	
aminoglycoside	<b>0.009 *</b>	0.2360	0.1240	<b>0.0198*</b>	0.6870	0.7760	0.5730	<b>1.96e-07 ***</b>
bacitracin	0.2790	0.3790	0.5840	0.6580	0.3330	0.5260	<b>0.0438 *</b>	<b>0.0001 ***</b>
carbomycin	0.1660	0.5170	0.0844	0.0819		0.4220		0.1690
chloramphenicol	<b>0.006 *</b>	0.3700	0.2402	<b>0.0033 **</b>	0.5990	0.4520	0.5950	<b>2.56e-07 ***</b>
fosfomycin	0.1840	0.6240	0.6140	0.3420	0.7150	0.3950		0.0957
fosmidomycin	<b>0.029 *</b>	0.0949	0.2171	<b>0.00789 **</b>	0.4400	0.9570	<b>0.0246 *</b>	<b>3.93e-14 ***</b>
kasugamycin	0.8710	0.2110	0.9660	0.6590	0.6290	0.4470		<b>1.05e-06 ***</b>
MLS	<b>0.015 *</b>	0.2760	0.1470	0.9840	0.2720	0.3130	0.6100	<b>&lt; 2e-16 ***</b>
multidrug	0.4180	0.3360	0.9030	0.3850	0.3500	0.3330	0.1800	<b>0.0004 ***</b>
polymyxin	0.1560	0.2922	0.7564	0.0557		0.3260	0.4420	0.4560
puromycin	0.0788	0.4150	0.3600	0.6780	0.2760	0.4560		<b>0.0327 *</b>
quinolone	0.3500	0.5310	0.2020	0.2280	0.4990	0.3940	0.4420	<b>5.21e-07 ***</b>
rifamycin	<b>0.0296 *</b>	0.8310	0.7590	<b>0.0126 *</b>	0.4450	0.7870	<b>0.0490 *</b>	<b>1.65e-09 ***</b>
sulfonamide	<b>0.0003 **</b>	0.5360	<b>0.0200 *</b>	<b>0.0185 *</b>	0.5350	0.5290	0.5760	<b>3.98e-09 ***</b>
tetracycline	<b>0.0143 *</b>	<b>0.0290*</b>	0.5050	0.9510	0.7060	0.6280	0.5530	<b>1.23e-05 ***</b>
vancomycin	0.0643	0.1700	0.4370	0.1380	0.9220	0.6370	0.8860	<b>0.0038 **</b>
trimethoprim	0.1160	0.8040	0.4020	0.5910		0.4020		<b>1.09e-05 ***</b>
tetracenomycin_C	0.4410	0.3070	0.7400	0.9540	0.4570	0.7300	0.4220	<b>0.0168 *</b>
total	0.4900	0.2420	0.7730	0.8510	0.3200	0.5850	0.2110	<b>7.84e-10 ***</b>

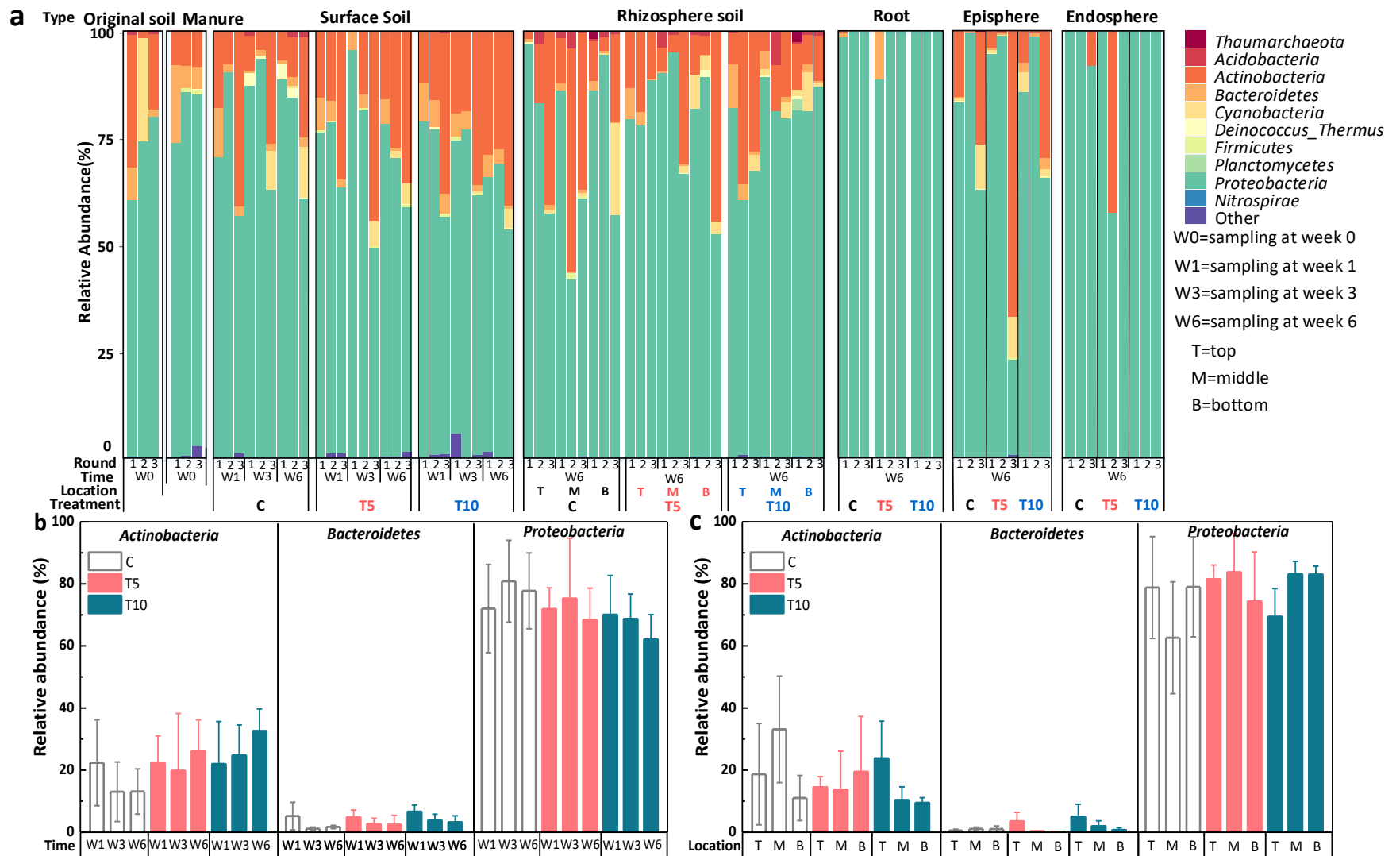


**Table S6.** The alpha diversity, Shannon Index and the number of ARGs, of the resistome in various sample types. Items with different letters represent significant differences at the  $p < 0.05$  level according to Tukey's HSD tests.

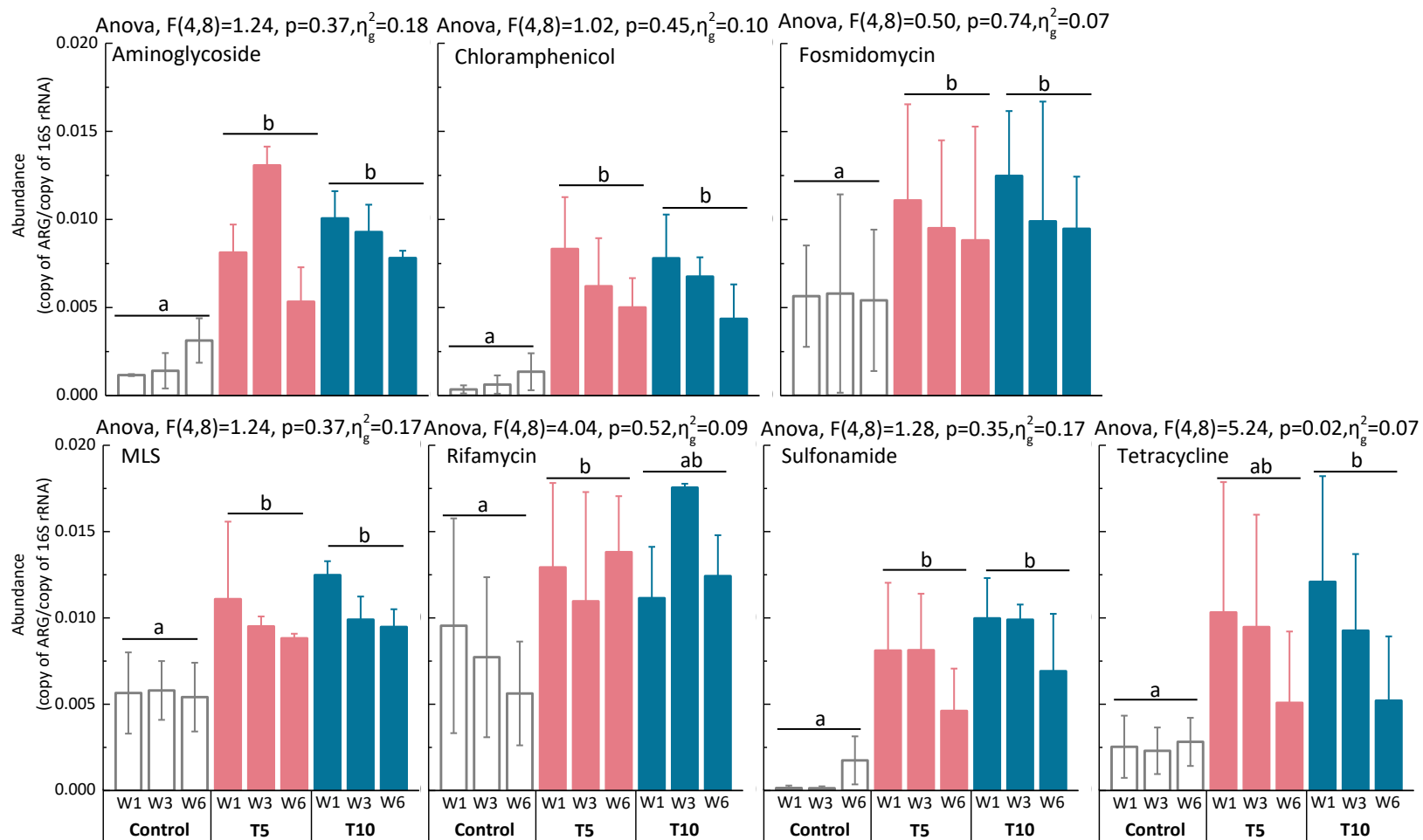
Sample type	Treatment	Time/Location	Shannon index	Number of ARGs	
Soil		Week 0	$2.72 \pm 0.33$	$75 \pm 8$	
Manure		Week 0	$3.79 \pm 0.09$	$145 \pm 9$	
Surface soil	C	Week 1	$2.92 \pm 0.27$	$88 \pm 3$	
		Week 3	$3.19 \pm 0.17$ <b>a</b>	$103 \pm 5$ <b>a</b>	
		Week 6	$3.24 \pm 0.10$	$105 \pm 19$	
	T5	Week 1	$3.76 \pm 0.13$	$137 \pm 21$	
		Week 3	$3.39 \pm 0.16$ <b>b</b>	$102 \pm 9$ <b>b</b>	
		Week 6	$3.47 \pm 0.09$	$111 \pm 2$	
	T10	Week 1	$3.74 \pm 0.07$	$127 \pm 9$	
		Week 3	$3.56 \pm 0.10$ <b>c</b>	$130 \pm 17$ <b>b</b>	
	Rhizosphere soil	C	Bottom	$3.09 \pm 0.16$	$83 \pm 4$
			Middle	$3.04 \pm 0.21$	$90 \pm 8$
Top			$3.20 \pm 0.09$	$89 \pm 17$	
T5		Bottom	$3.02 \pm 0.12$	$85 \pm 6$	
		Middle	$3.10 \pm 0.12$	$93 \pm 10$	
		Top	$3.34 \pm 0.05$	$105 \pm 15$	
T10		Bottom	$3.24 \pm 0.13$	$93 \pm 17$	
		Middle	$3.12 \pm 0.07$	$89 \pm 11$	
Root		C	Top	$3.35 \pm 0.08$	$107 \pm 5$
		T5	Week 6	$3.14 \pm 0.23$	$45 \pm 15$
	T10	Week 6	$2.97 \pm 0.11$	$35 \pm 11$	
Episphere	T10	Week 6	$3.17 \pm 0.48$	$39 \pm 16$	
	C	Week 6	$3.10 \pm 0.31$	$93 \pm 15$ <b>a</b>	
	T5	Week 6	$3.72 \pm 0.28$	$123 \pm 11$ <b>ab</b>	
Endosphere	T10	Week 6	$3.62 \pm 0.07$	$134 \pm 4$ <b>b</b>	
	C	Week 6	$2.77 \pm 0.10$	$21 \pm 2$	
	T5	Week 6	$2.84 \pm 0.18$	$23 \pm 4$	
	T10	Week 6	$2.83 \pm 0.11$	$24 \pm 4$	

**Table S7.** The  $p$  and F values of the two-way ANOVA analyses, on manure treatment and sample type, for the alpha diversity indexes of the resistome at the ARG level.

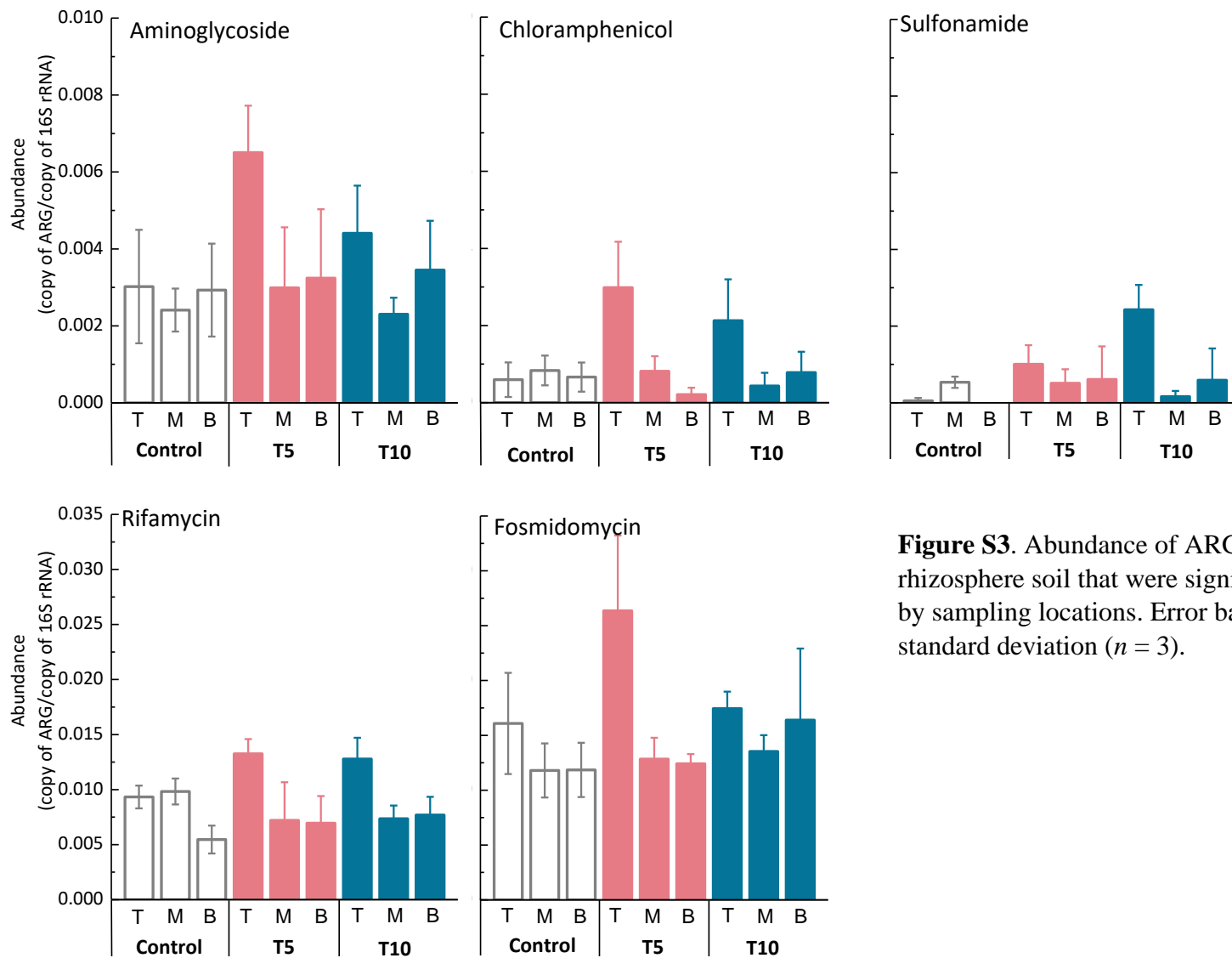
<b>Factors</b>	<b>Number of ARGs</b>		<b>Shannon index</b>	
	<b><math>p</math> value</b>	<b>F value</b>	<b><math>p</math> value</b>	<b>F value</b>
<b>Treatment</b>	0.00443	5.855	8.81e-05	10.680
<b>Sample Type</b>	< 2e-16	67.714	1.04e-11	16.317
<b>Treatment*Type</b>	0.03115	2.368	0.0156	2.695



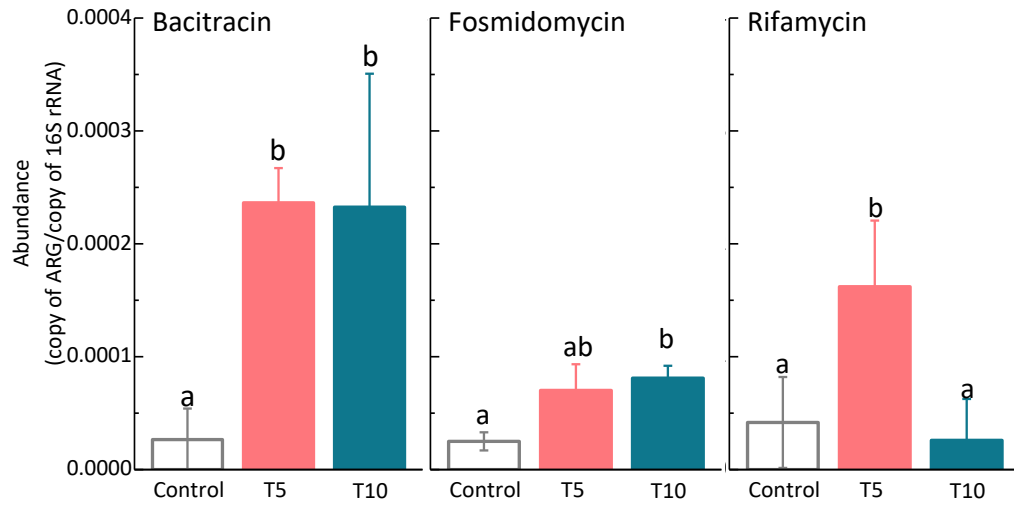
**Figure S1.** Relative abundance of phyla in different sample types (a), dynamic change of major phyla in surface soil at three sampling times (b), and spatial variations of major phyla in rhizosphere soil (c). Error bars indicate standard deviation ( $n = 3$ ).



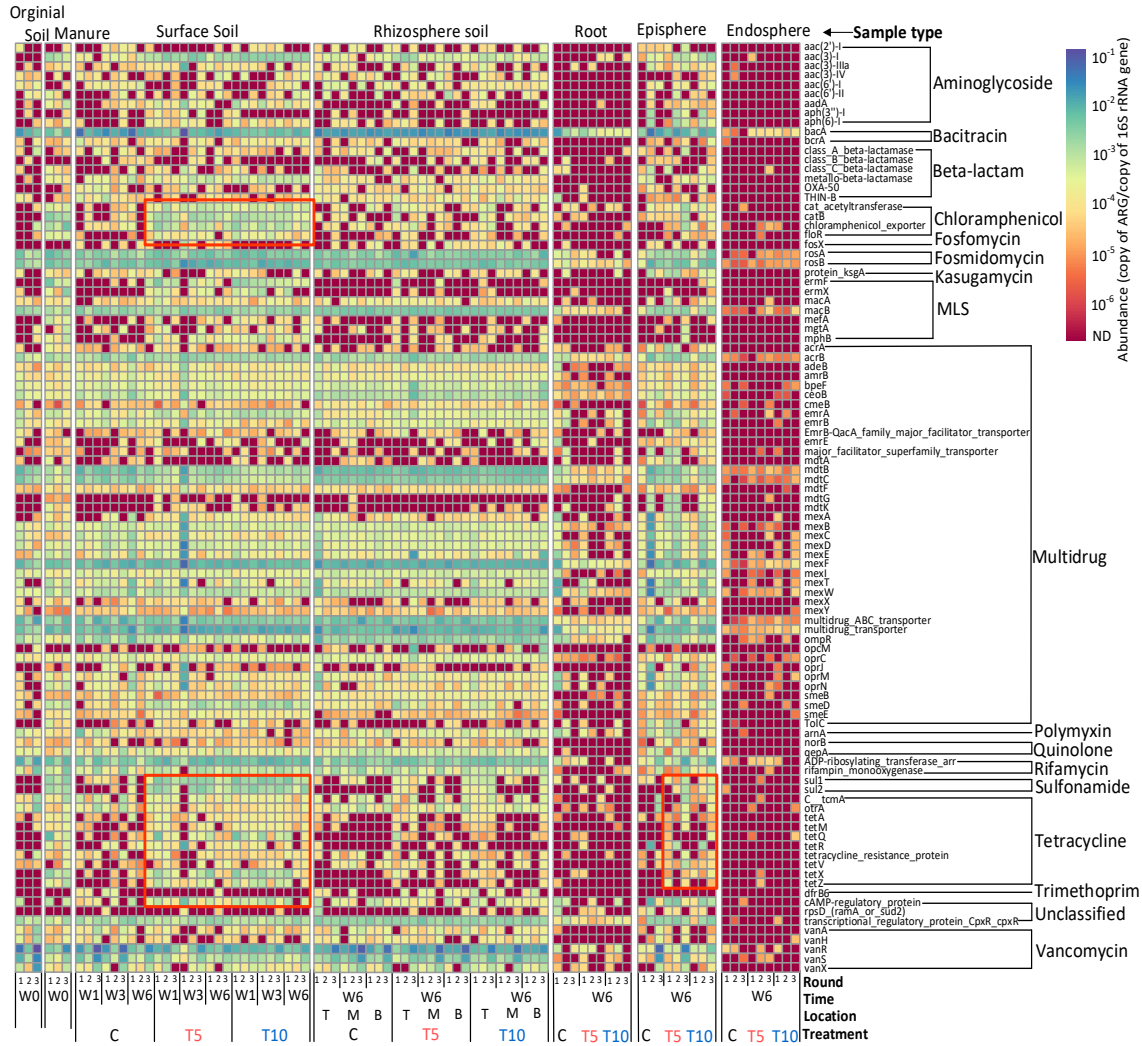
**Figure S2.** Abundance of ARG families in surface soil that were significantly impacted by manure treatment. Different letters represent significant differences according to Tukey's HSD at  $p < 0.05$ . Two-way interactions between treatment and time on the abundance of ARG families are shown. Error bars represent standard deviations ( $n = 3$ ).



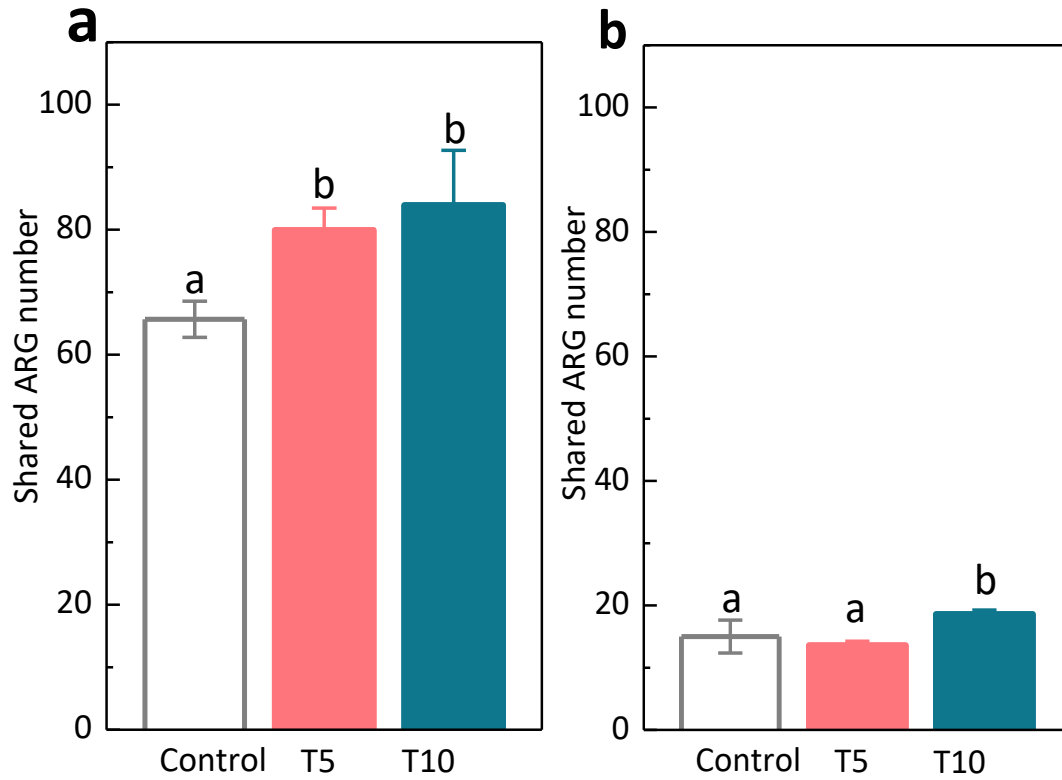
**Figure S3.** Abundance of ARG families in rhizosphere soil that were significantly impacted by sampling locations. Error bars indicate standard deviation ( $n = 3$ ).



**Figure S4.** Abundance of ARG families in endosphere that were significantly impacted by manure treatment. Column with different letter indicates significant difference according to Tukey's HSD with  $p < 0.05$ . Error bars indicate standard deviation ( $n = 3$ ).

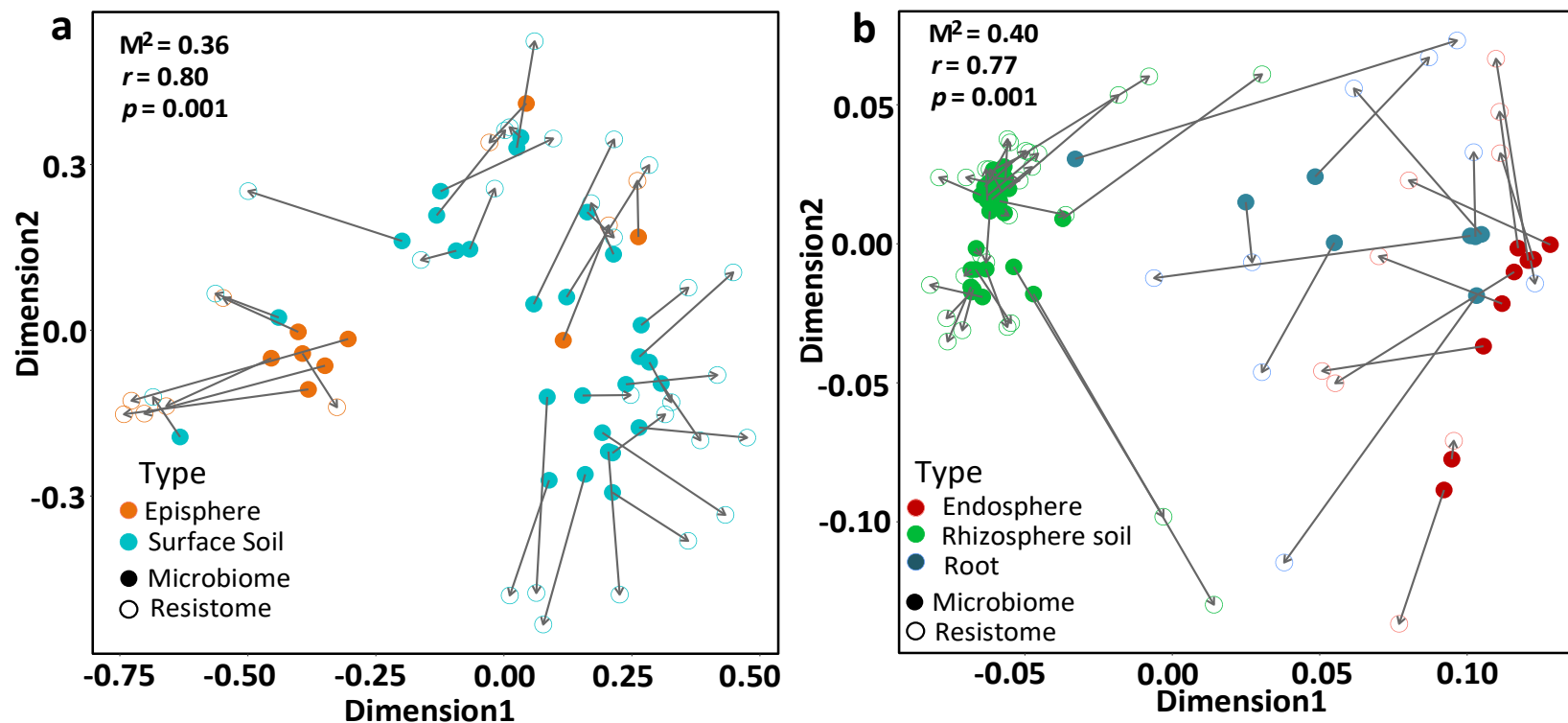


**Figure S5.** Abundance of the top 100 ARGs (i.e., average abundance higher than 10<sup>-5</sup> copy of ARG per copy of the 16S rRNA gene across all samples) among different sample types and treatments. Red frames represent genes detected following manure application.



**Figure S6.** Numbers of ARGs shared between surface soil and episphere (A), and shared among endosphere, rhizosphere soil, and root (B). Column with different letters indicate significant difference ( $t$ -test,  $p < 0.05$ ). Error bars indicate standard deviation ( $n = 3$ ).





**Figure S7.** Procrustes test depicting the significant correlations between resistomes and microbiomes of surface soil and episphere (a), and of rhizosphere soil, root, and endosphere (b). Bray-Curtis distance with 9999 permutations were applied.