

Supporting Information for

Long-term Effect of Different Fertilization and Cropping Systems on the Soil Antibiotic Resistome

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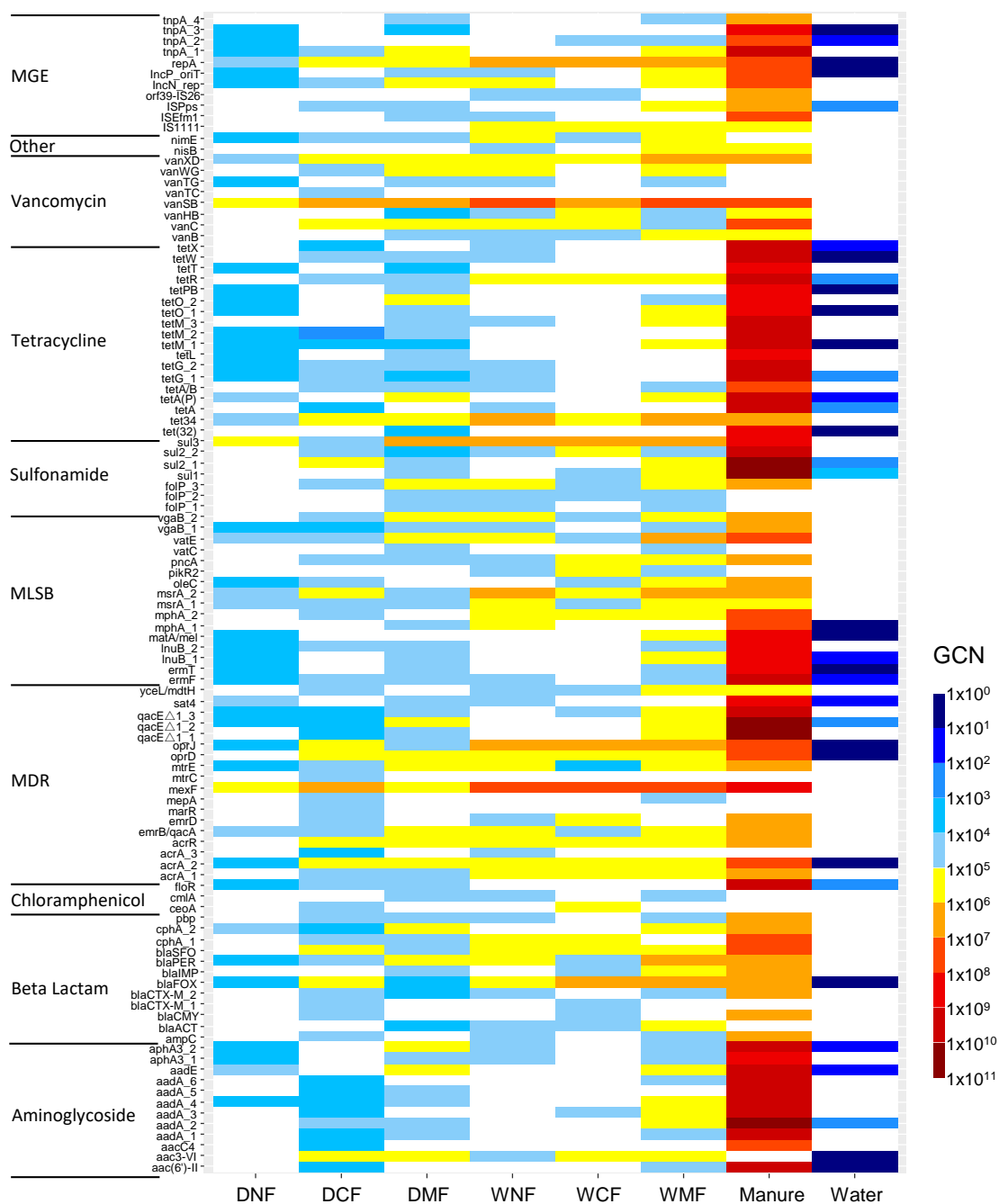


Figure S1 Heatmap showing absolute gene copy number of antibiotic resistance genes and mobile genetic elements in the manure and soil with different fertilizer and different cropping system. The resistance genes detected in composted manure or water, but not in the soil were not shown. DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with chemical fertilizer; WMF, paddy soil with composted manure.

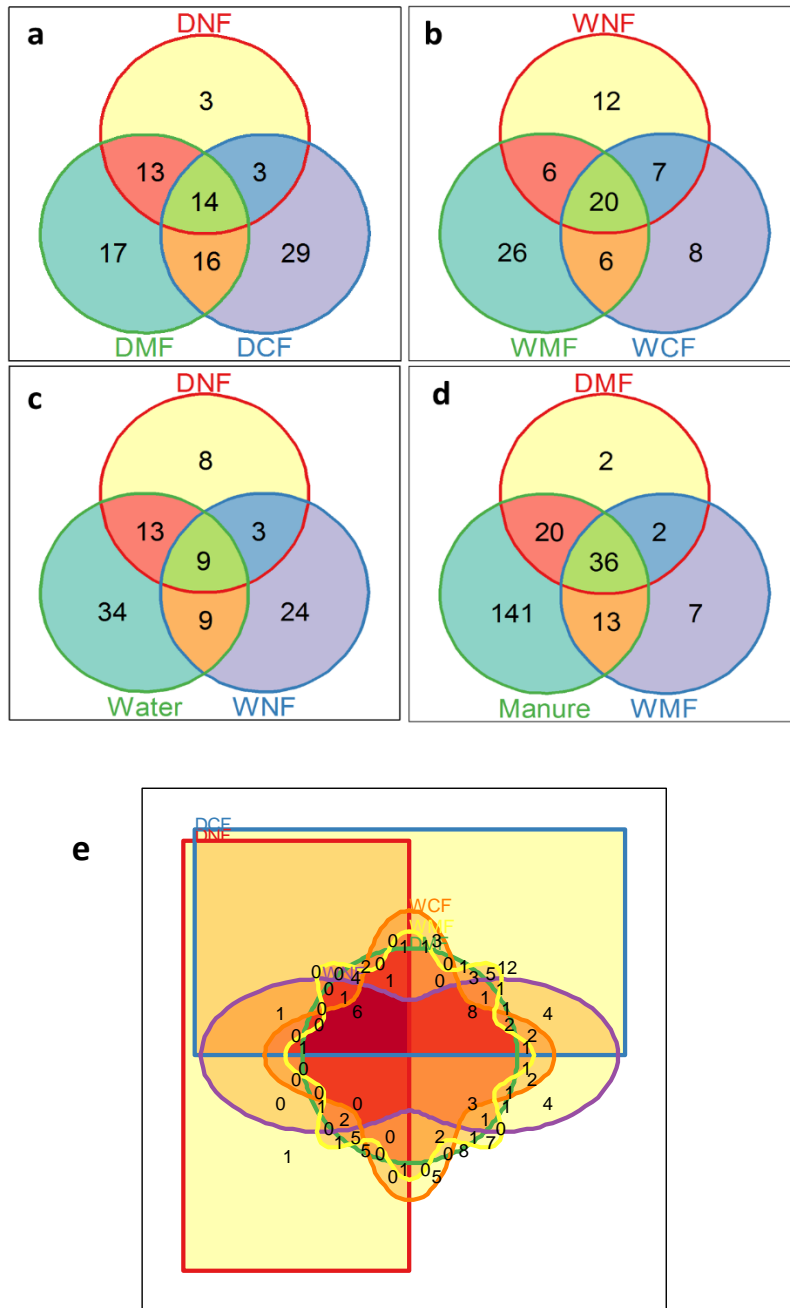


Figure S2 Number of antibiotic resistance genes and mobile elements genes shared in the dryland soil with different fertilization (a), paddy field soil with different fertilization (b), irrigation water and unfertilized soil (c), composted manure and composted manure applied soil (d) and all the soil (e). DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with chemical fertilizer; WMF, paddy soil with composted manure.

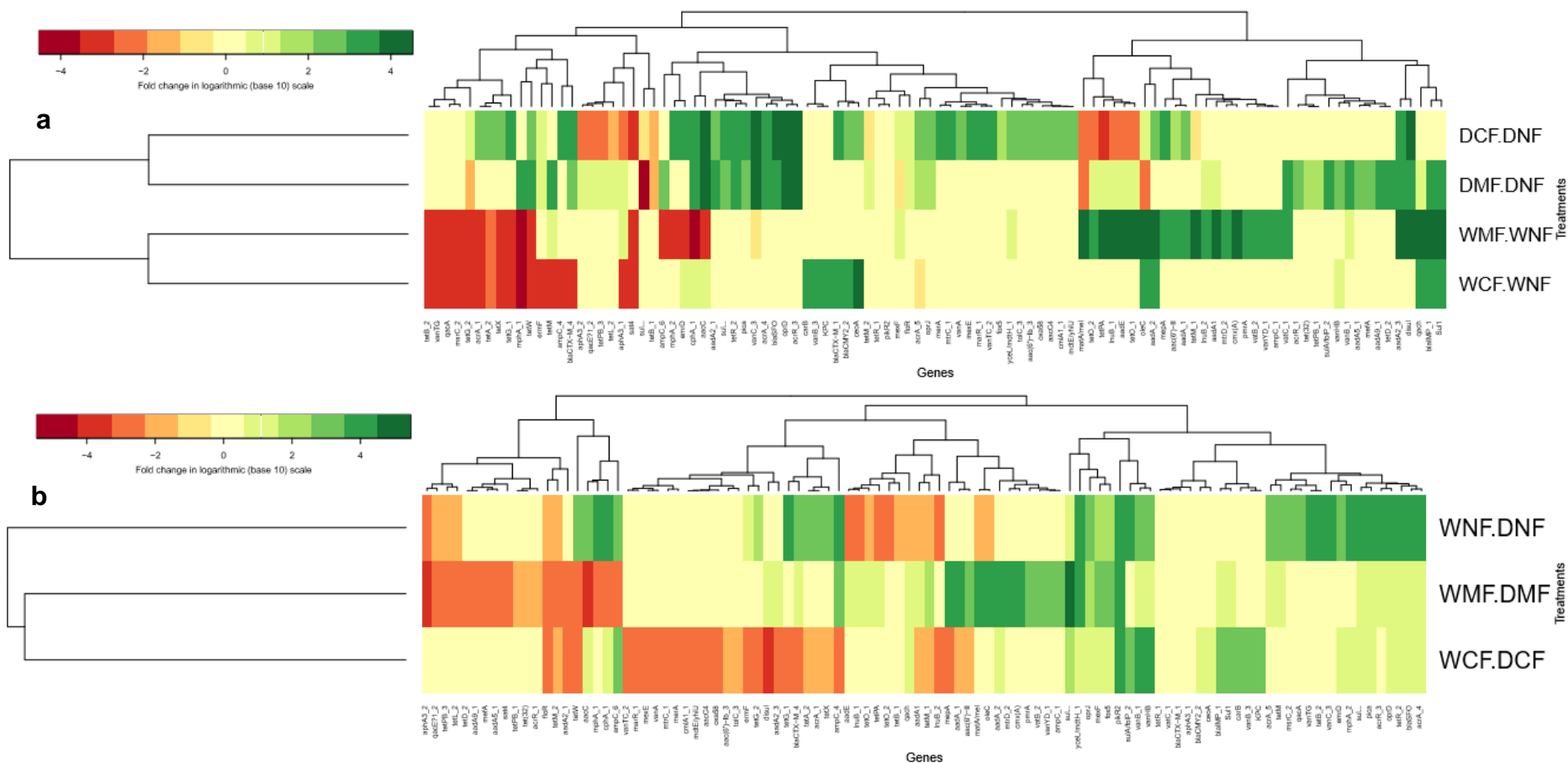


Figure S3 Log-transformed fold change of gene copy numbers of antibiotic resistance genes in the soil with different fertilizer (a) and different cropping system (b). DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with chemical fertilizer; WMF, paddy soil with composted manure.

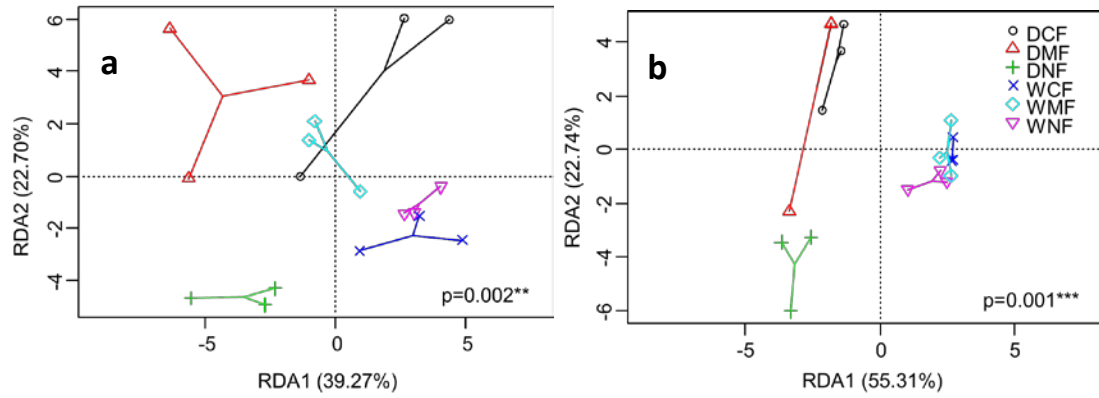
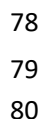


Figure S4 Redundancy Analysis (RDA) of log₂ transformed relative abundance of antibiotic resistance genes in the soil (a) and bacteria at genus level (b). P values indicate that ordination is significant between different treatments: DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with chemical fertilizer; WMF, paddy soil with composted manure.



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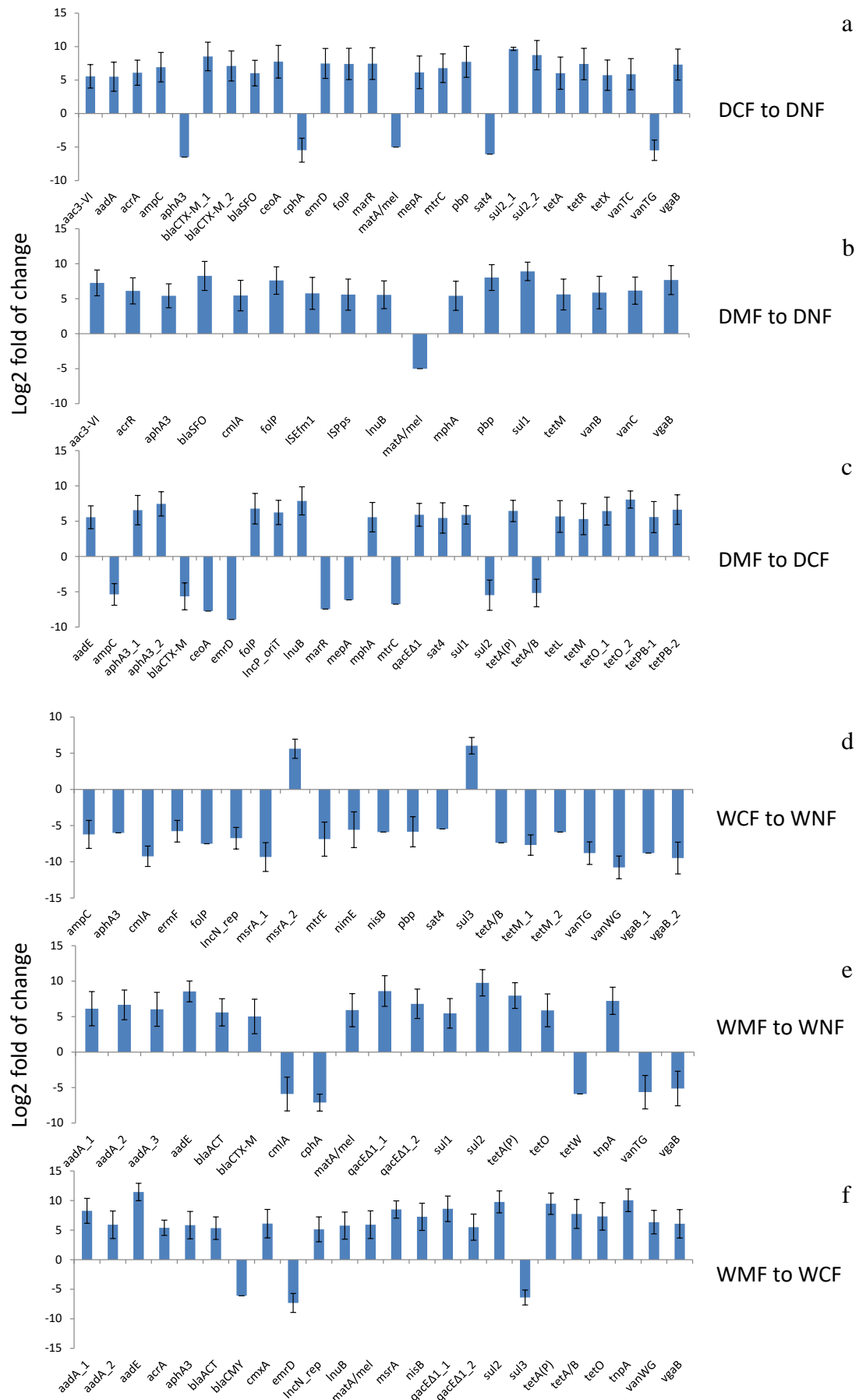
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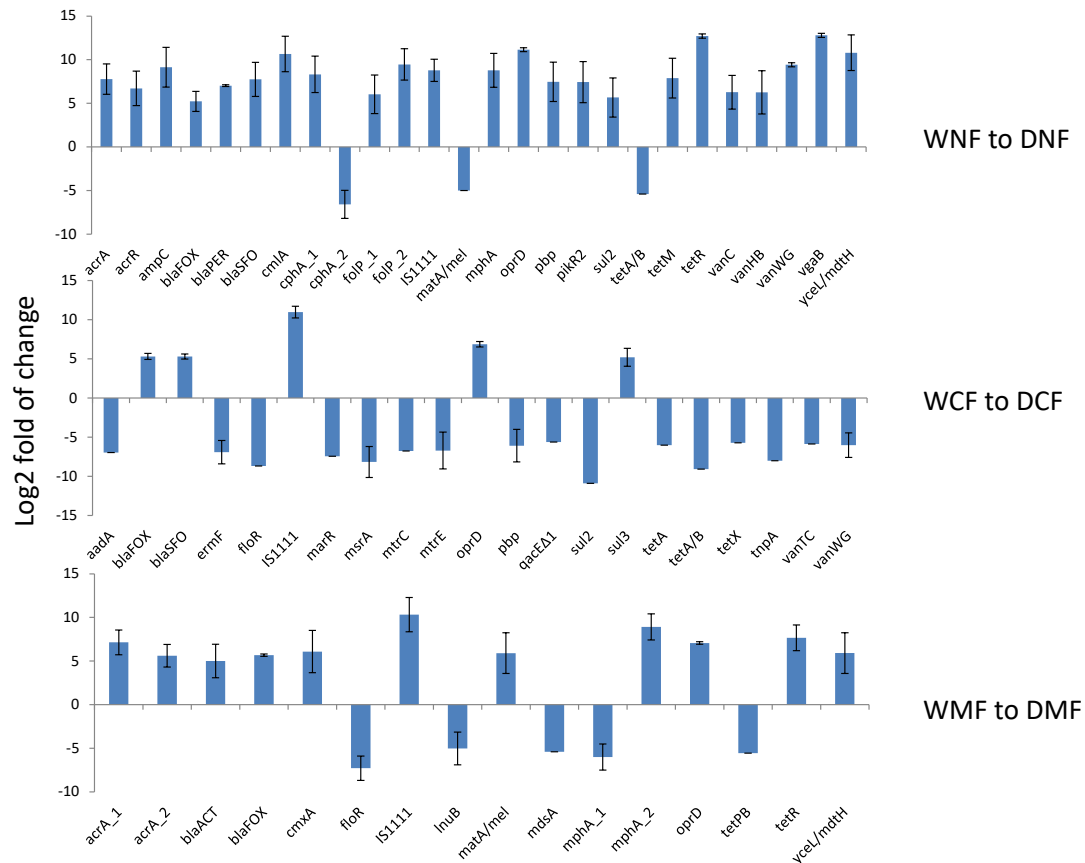
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90 Figure S6 Fold of change of antibiotic resistance genes (ARGs), mobile genetic
 91 elements (MGEs) from different fertilization and cropping system. Fold of change is
 92 log2 transformed. Only genes at least 5-log2fold increase or decrease are shown. DNF,
 93 dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland
 94 soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with
 95 chemical fertilizer; WMF, paddy soil with composted manure.

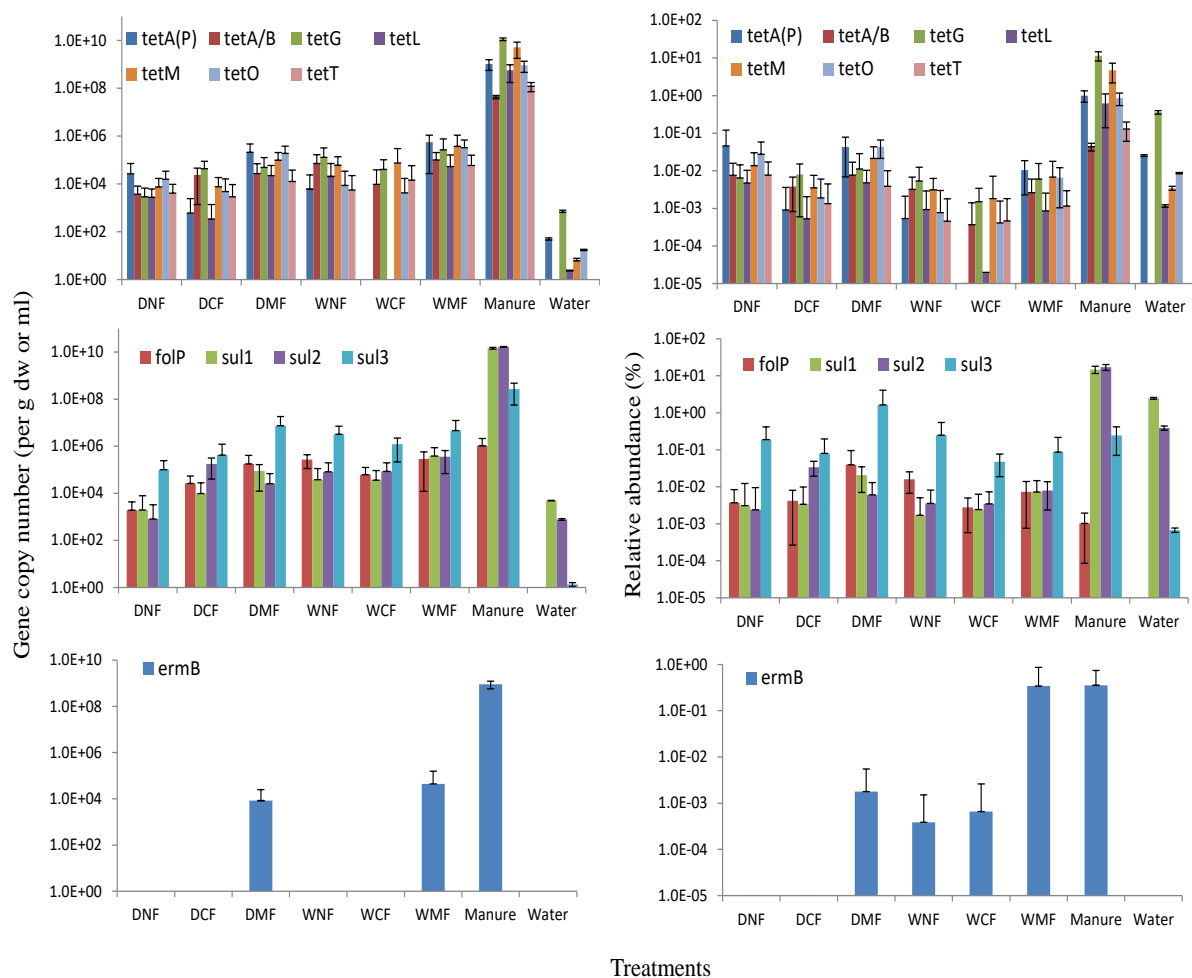


Figure S7 Gene copy numbers and relative abundance of some special antibiotic resistance genes in soil, composted manure and irrigation water. DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy soil with no fertilizer; WCF, paddy soil with chemical fertilizer; WMF, paddy soil with composted manure.

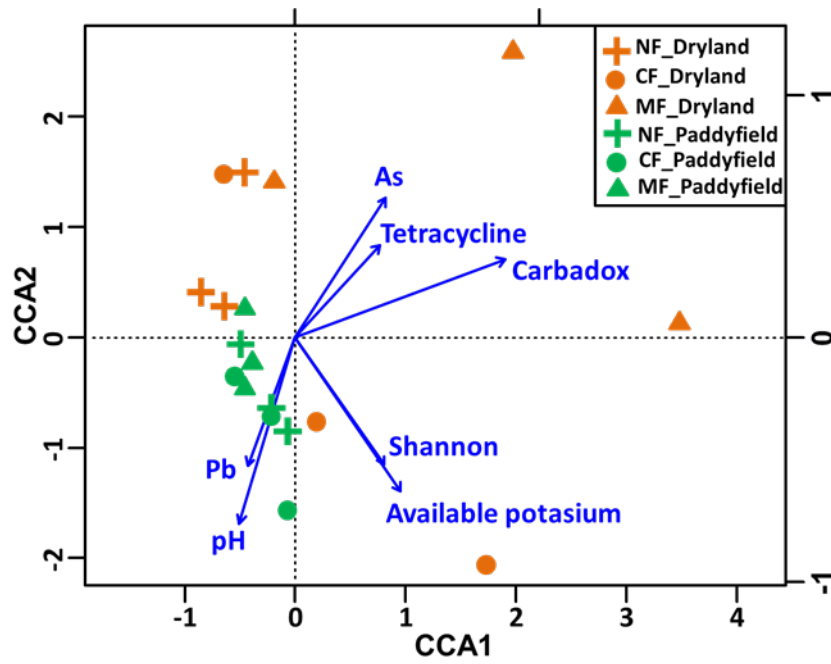


Figure S8 Canonical correspondence analysis (CCA) of soil characters and relative abundance of antibiotic resistance genes and mobile genetic element in the dryland soil and paddy soil with application of chemical fertilizer (CF) or composted manure (MF) and without fertilizer (NF). Symbols are for soils.

107 **Table S1** Physicochemical properties of composted manure (CM) and soil in different treatment

Sample	Moist ure (%)	pH	CEC cmol kg ⁻¹	Pb mg kg ⁻¹	Zn mg kg ⁻¹	Cu mg kg ⁻¹	Cd mg kg ⁻¹	Cr mg kg ⁻¹	Hg μg kg ⁻¹	As mg kg ⁻¹	OM g kg ⁻¹	TN g kg ⁻¹	TP g kg ⁻¹	TK g kg ⁻¹	AN mg kg ⁻¹	AP mg kg ⁻¹	AK mg kg ⁻¹
DNF	11.95	4.80c	14.11b	30.10ab	78.27c	28.90c	0.25b	52.05c	63.45a	15.40a	10.71e	0.73e	0.55f	9.25c	85.75b	15.23f	141.67b
DCF	9.86	5.24b	14.42b	32.23a	84.48c	29.16c	0.21b	63.18b	59.95a	16.89a	10.44e	0.71e	0.53f	15.23a	83.30b	45.34d	186.67a
DMF	11.33	4.84c	14.42b	28.43b	89.86b	33.66b	0.28b	64.86b	64.52a	15.62a	12.53d	0.87d	0.99d	10.1b	84.53b	38.26e	162.50a
WNF	46.94	5.55b	18.87a	36.48a	79.21c	25.26d	0.24b	43.87d	31.12c	7.66b	17.57c	1.10c	0.81e	17.35a	94.33b	72.95c	146.67b
WCF	53.99	5.63b	19.11a	32.52a	83.16c	27.27cd	0.25b	64.38b	34.30bc	8.14b	19.67b	1.48b	1.17c	15.93a	123.73a	119.47b	181.25a
WMF	60.00	5.57b	17.10a	31.57a	105.31a	41.23a	0.58a	72.78a	39.21b	8.22b	21.50a	1.43b	1.45b	15.85a	129.85a	183.28a	176.25a
CM		7.43a										20.97a	22.47a	9.55c			

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109 Note: DNF, dryland soil with no fertilizer; DCF, dryland soil with chemical fertilizer; DMF, dryland soil with composted manure; WNF, paddy

110 field soil with no fertilizer; WCF, paddy field soil with chemical fertilizer; WMF, paddy field soil with composted manure.

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Table S2 Mantel test on the relationship (r) between the relative abundance of the antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) and main soil factors influencing the distribution of ARGs in soil^a.

	Pearson		Spearman	
	r	p	r	p
pH	0.17	0.02*	0.22	0.01*
Pb	0.06	0.32	0.04	0.40
As	0.15	0.02*	0.17	0.02*
Available potassium	0.24	0.14	0.18	0.14
Carbadox	0.60	0.007**	0.45	0.03*
Tetracycline	0.11	0.20	0.15	0.16
Shannon index	0.64	0.001**	0.55	0.001**

^a: Significance level: p<0.05(*), p<0.01(**)