Supplementary Information

Supplementary materials and methods

Qualitative PCR and quantitative real-time PCR

According to Liang et al. (2017), all qualitative PCR tests were performed in a 50 μL reaction system with an ABI 2720 Thermocycler (ABI-2720, Applied Biosystems, USA). The specific components of the reaction system are shown in Table S3. The specific primers designed are presented in Table S4. The specificity tested was for each of them. The thermocycler was programmed to denature at 94℃ for 4 min, followed by 35 cycles of 30 s at 94℃, 30 s at different annealing temperatures and 30 s at 72℃, with a final extension step for 7 min at 72℃. The annealing temperature varied depending on the target gene: 55℃ for *tet*W, *tet*M, *tet*O, *tet*Q, *tet*H, *gyr*A, and *qnr*A and 60℃ for *sul*1, *sul*2, *erm*F, and 16S rRNA. To ensure reproducibility, triplicate PCRs were performed for each sample. Sterile water was used as the negative control in every run.

PCR products were excised and purified using a TIANGEN Universal DNA Purification Kit (Tiangen, China) according to the manufacturer's instructions. The purified PCR products were ligated into a pEASY-T3 Cloning Kit (TransGen) and then transformed into Trans1-T1 Phage Resistant Chemically Competent Cells (TransGen), as described in the manufacturer's manual. Plasmids carrying the target genes were extracted using a TIAN Pure Midi Plasmid Kit (Tiangen) and used as standards for real-time fluorescent quantitative PCR. The CFX96 real-time PCR System (CFX96, Bio-Rad, USA) was used for quantitative real-time PCR analysis of target ARGs and 16S rRNA. The real-time PCR primers for ARGs and the 16S rRNA gene were the same as those used in the qualitative PCR (Table S4). Each reaction was performed in a 20 μL system, including 10 μL SYBR®Premix Ex TaqTM (TaKaRa, Japan).

GeoChip hybridization to detect ARGs

The DNA extracted from the samples was labeled fluorescently, purified, and resuspended in 50 μL of hybridization solution containing 45% formamide, 5 × SSC, 0.1% SDS, 0.1 mg/mL salmon sperm DNA, and 2 μL of common oligonucleotide reference standard (0.1 pmol/μL) (Liang et al., 2010). The labeled DNA was hybridized with GeoChip 5.0 on a MAUI® Hybridization System (BioMicro Systems, UT, USA). A ScanArray 5000 Microarray Analysis System (PerkinElmer, Wellesley, USA) was used to scan microarrays at 95% laser power and 68% photomultiplier tube gain. The scanned images were quantified using ImaGene® version 6.0 (BioDiscovery, Inc., Los Angeles, USA) to extract the digital signal at each spot and obtain the original data. The spots with signal-to-noise ratios lower than 2.0 were removed (Zhou et al., 2015). And those data were processed in the Microarray Data Manager system on the Institute for Environmental Genomics website.

Supplementary Tables

Table S1 Locations, climate zone, mean annual temperature, mean annual precipitation, and soil type of the sampling regions

Sites	Latitude	Longitude	Climate zone	MAT (℃)	MAP (mm)	Soil type[a)]	
						Soil subgroup	Soil parent suborder
TZ	32.40°N	119.58°E	Subtropical monsoon climate	14.7	1037.7	Typic Fe-accumuli-Stagnic Anthrosols	Udic Cambosols
MG	32.32°N	118.11°E	Subtropical to warm temperate climate	15.0	934.1	Albic Fe-laechi-Stagnic Anthrosols	Udic Cambosols
DY	32.00°N	119.27°E	Subtropical monsoon climate	15.8	1050.0	Albic Fe-laechi-Stagnic Anthrosols	Udic Cambosols
CZ	31.60°N	119.48°E	Subtropical monsoon climate	15.4	1066.0	Typic Fe-accumuli-Stagnic Anthrosols	Udic Cambosols
LA	32.01°N	116.41°E	Subtropical humid monsoon climate	15.1	1250.0	Typic Hapli-Stagnic Anthrosols	Udic Argosols
SH	31.37°N	121.27°E	Subtropical monsoon climate	17.6	1173.4	Typic Fe-accumuli-Stagnic Anthrosols	Alluvic Primosols
JX	30.79°N	120.73°E	Subtropical monsoon climate	15.9	1168.6	Typic Fe-accumuli-Stagnic Anthrosols	Alluvic Primosols
YW	29.20°N	119.55°E	Subtropical monsoon climate	17.0	1350.0	Typic Hapli-Stagnic Anthrosols	Udic Ferralosols
SG	28.14°N	115.00°E	Subtropical monsoon climate	17.6	1718.4	Typic Hapli-Stagnic Anthrosols	Udic Ferralosols
ZS	28.01°N	115.54°E	Subtropical monsoon climate	17.7	1710.7	Typic Hapli-Stagnic Anthrosols	Udic Ferralosols
FZ	26.00°N	119.27°E	Subtropical maritime monsoon climate	22.5	1500.0	Typic Hapli-Stagnic Anthrosols	Alluvic Primosols

Notes: TZ: Taizhou; MG: Mingguang; DY: Danyang; CZ: Changzhou; LA: Lu'an; SH: Shanghai; JX: Jiaxing; YW: Yiwu; SG: Shanggao; ZS: Zhangshu; FZ: Fuzhou. MAT: mean annual temperature; MAP: mean annual precipitation. a) Soil type was classified according to Chinese Soil Taxonomy

Table S2 The instrument detection limit (LOD) of four antibiotics in soils, quantitative limit (LOQ), method detection limit (MQL), and the standard recoveries

Antibiotics	LOD (mg/L)	LOQ (mg/L)	MQL in soils (μg/kg)	MQL in manure (mg/kg)	Recovery (%)
Tetracyclines	0.029–0.086	0.090–0.260	0.750–1.700	0.030–0.180	63.200–89.300
Sulfonamides	0.008–0.069	0.023–0.208	0.200–0.800	0.030–0.090	58.700–78.800
Quinolones	0.002–0.046	0.007–0.138	0.450–1.900	0.060–0.150	68.100–83.900
Macrolides	0.017–0.031	0.052–0.095	1.300–2.700	0.010–0.050	81.200–97.600

Table S3 Reaction system for quantitative real time polymerase chain reaction (qPCR)

Component	Dosage (μ L)
2×PCR Buffer	5
Mg^{2+} (25 mM)	3
dNTP (each 10 mM)	1
forwarding primers (10–20 pmol)	2
reverse primers (10–20 pmol)	2
Taq DNA Polymerase (5 U/μL)	1
template	1
ddH_2O	35
total system	50

Table S4 Primers used in this study for quantitative real time polymerase chain reaction (qPCR)

Genes	Sequences (5'→3')	Amplicon size (bp)	Annealing temperature (℃)	Reference
*tet*W	FW_GAGAGCCTGCTATATGCCAGC RV_GGGCGTATCCACAATGTTAAC	168	55	Aminov et al., 2001
*tet*M	FW_ACAGAAAGCTTATTATATAAC RV_TGGCGTGTCTATGATGTTCAC	171	55	Aminov et al., 2001
*tet*O	FW_ACGGARAGTTTATTGTATACC RV_TGGCGTATCTATAATGTTGAC	171	55	Aminov et al., 2001
*tet*Q	FW_AGAATCTGCTGTTTGCCAGTG RV_CGGAGTGTCAATGATATTGCA	169	55	Aminov et al., 2001
*tet*H	FW_CAACCCATTACGGTGTGCTA RV_AAGTGTGGTTGAGAATGCCA	164	55	Tamminen et al., 2011
*sul*1	FW_CGCACCGGAAACATCGCTGCAC RV_TGAAGTTCCGCCGCAAGGCTCG	163	60	Pei et al., 2007
*sul*2	FW_TCCGGTGGAGGCCGGTATCTGG RV_CGGGAATGCCATCTGCCTTGAG	191	60	Pei et al., 2007
*gyr*A	FW_ACGTACTAGGCAATGACTGG RV_AGAAGTCGCCGTCGATAGAAC	192	55	Everett et al., 1996
*qnr*A	FW_AGGATTTCTCACGCCAGGATT RV_CGCTTTCAATGAAACTGCA	124	55	Cummings et al., 2011
*erm*F	FW_CGACACAGCTTTGGTTGAAC RV_GGACCTACCTCATAGACAAG	309	60	Chen et al., 2007
16S rDNA	515FW_GTGCCAGCMGCCGCGG 806RV_GGACTACHVGGGTWTCTA-AT	292	60	Caporaso et al., 2011

Notes: FW: forward; RV: reverse

Table S5 Paddy soil physicochemical properties in 11 rice planting regions with inorganic-fertilization and manure-fertilization

Site	NH_4^+-N (mg/kg)			TP (g/kg)			AP (mg/kg)			TK (g/kg)		
	Inorganic-amended soils	Manure-amended soils	*p*	Inorganic-amended soils	Manure-amended soils	*p*	Inorganic-amended soils	Manure-amended soils	*p*	Inorganic-amended soils	Manure-amended soils	*p*
TZ	28.1	20.9	<0.001	0.9	0.7	<0.001	0.6	0.4	0.062	14.6	14.6	0.974
MG	56.3	30.3	0.157	1.2	0.4	<0.001	1.5	2.2	0.187	15.3	18.7	0.004
DY	70.0	48.9	0.007	0.9	0.4	<0.001	1.5	0.5	<0.001	14.8	14.7	0.603
CZ	47.8	31.2	0.004	0.4	0.7	<0.001	0.5	0.8	<0.001	12.3	17.1	<0.001
LA	48.5	26.6	0.003	0.4	0.8	<0.001	0.6	3.7	<0.001	14.8	11.8	<0.001
SH	36.3	36.6	<0.001	0.8	2.5	<0.001	0.3	8.0	<0.001	17.6	17.4	0.405
JX	51.5	41.7	0.130	0.7	2.2	<0.001	0.8	11.7	<0.001	17.3	18.0	0.005
YW	60.5	83.3	0.386	0.4	1.9	<0.001	0.4	6.2	<0.001	16.5	20.9	<0.001
SG	20.9	35.4	0.002	0.9	0.6	<0.001	3.0	1.8	0.018	18.9	16.1	<0.001
ZS	71.4	36.6	<0.001	1.0	1.5	0.074	1.4	8.2	0.008	8.0	13.2	<0.001
FZ	15.9	22.8	0.014	1.5	1.6	0.425	3.3	7.9	<0.001	44.5	52.9	<0.001

Notes: TZ: Taizhou; MG: Mingguang; DY: Danyang; CZ: Changzhou; LA: Lu'an; SH: Shanghai; JX: Jiaxing; YW: Yiwu; SG: Shanggao; ZS: Zhangshu; FZ: Fuzhou. TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; AP: available phosphorus; TK: total potassium. The significant differences ($p < 0.05$) between inorganic-amended ($n = 143$) and manure-amended soils ($n = 143$) are colored in red

Supplementary Figures

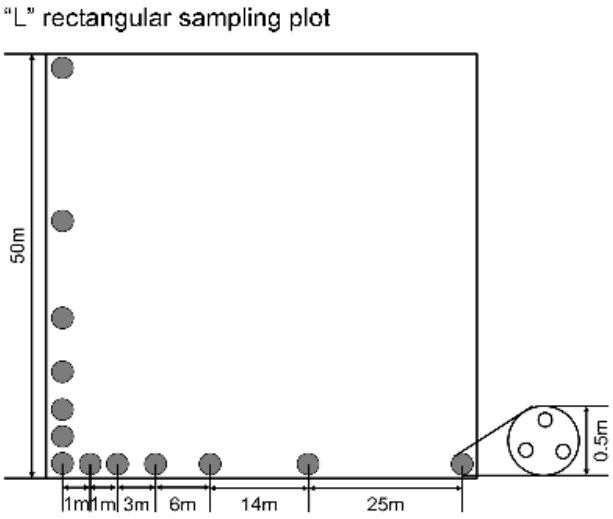

Fig. S1 'L-shaped' sampling layout. A 50 m × 50 m rectangular plot was established at each sampling site. Thirteen subplots were placed apart along the transect, resembling an "L", with distances between the adjacent subplots of 0, 1, 1, 3, 6, 14, and 25 m

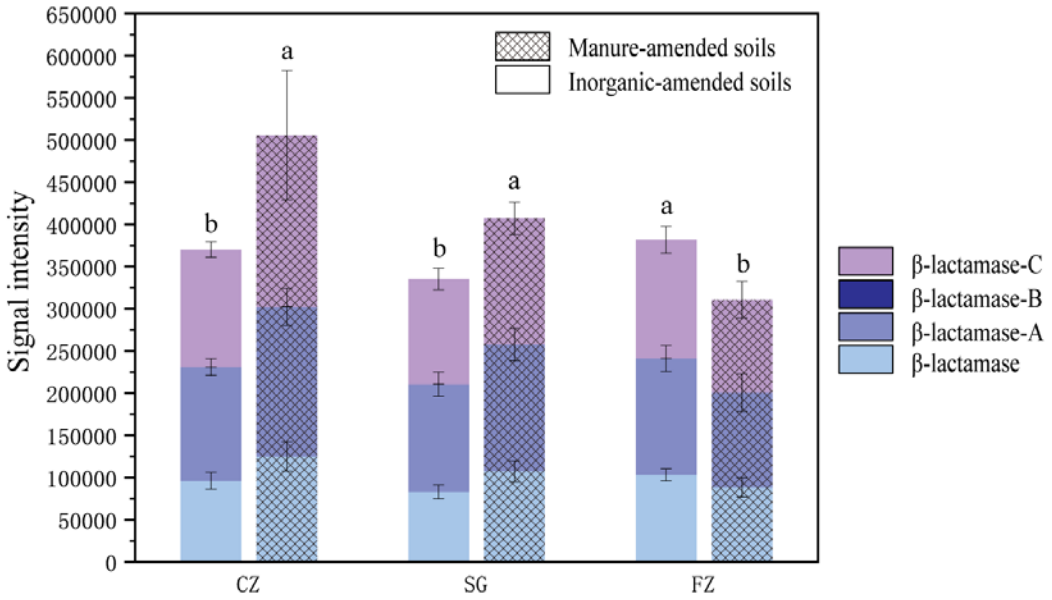

Fig. S2 The absolute abundance of β-lactam resistance genes in inorganic-amended and manure-amended paddy soils in 3 rice planting regions in eastern China. CZ: Changzhou; SG: Shanggao; FZ: Fuzhou. Different letters above the bars indicate the difference in the sum abundance of antibiotic resistance genes between inorganic-amended and manure-amended paddy soils at $p < 0.05$

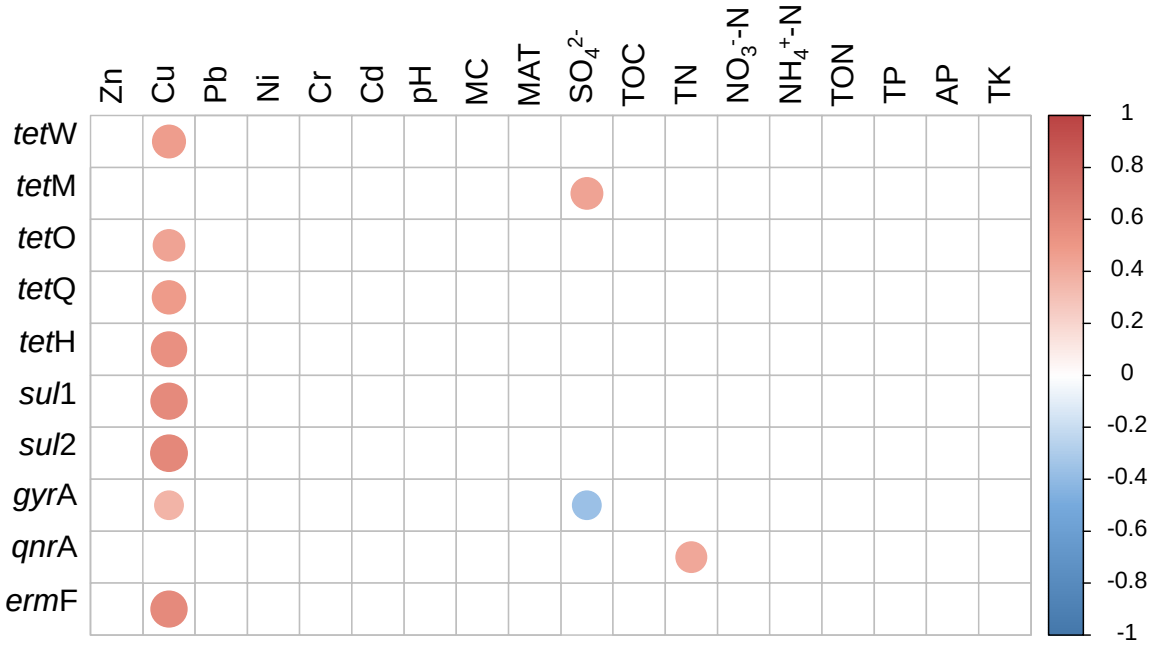

Fig. S3 Pearson correlations between the antibiotic resistance genes and the metals and others environmental variables in inorganic-amended paddy soils. The color points indicate the significant correlation coefficient ($p < 0.05$; bluer indicates stronger negative correlation and redder indicates stronger positive correlation). MC: moisture content; MAT: mean annual temperature; SO_4^{2-}: sulfate; TOC: total organic carbon; TN: total nitrogen; NO_3^-: nitrate nitrogen; NH_4^+: ammonium nitrogen; TON: total organic nitrogen; TP: total phosphorus; AP: available phosphorus; TK: total potassium

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