

Rothamsted Repository Download

A - Papers appearing in refereed journals

Cui, E., Fan, X., Hu, C., Neal, A. L., Cui, B., Liu, C. and Gao, F. 2022. Individual applications of N, P, K fertilizer on the reduction of antibiotic resistance genes in reclaimed water irrigated soil: N has the best outcome. *Ecotoxicology and Environmental Safety*. 231, p. 113185. <https://doi.org/10.1016/j.ecoenv.2022.113185>

The publisher's version can be accessed at:

- <https://doi.org/10.1016/j.ecoenv.2022.113185>

The output can be accessed at:

<https://repository.rothamsted.ac.uk/item/98772/individual-applications-of-n-p-k-fertilizer-on-the-reduction-of-antibiotic-resistance-genes-in-reclaimed-water-irrigated-soil-n-has-the-best-outcome>.

© 12 January 2022, Please contact library@rothamsted.ac.uk for copyright queries.

Supplementary information for
Individual applications of N, P, K fertilizer on the reduction of
antibiotic resistance genes in reclaimed water irrigated soil: N
has the best outcome

Erping Cui ^a, Xiangyang Fan ^a, Chao Hu ^a, Andrew L. Neal ^b, Bingjian Cui ^a,
Chuncheng Liu ^a, Feng Gao ^{a,*}

*a Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences,
Xinxiang 453002, China*

*b Department of Sustainable Agricultural Sciences, Rothamsted Research, North
Wyke, Devon EX22 2SB, UK*

* Corresponding author.

E-mail address: gaofengcaas@sina.com (Feng Gao)

Tel: +86 373 3393402

Figure captions:

Fig. S1: Numbers of antibiotic resistance genes (ARGs) detected in different treatments (A and C) and proportions of different types of ARGs in soil samples (B and D).

Fig. S2: Percentage of antibiotic resistance genes (ARGs) in different classes, based on normalized abundance values.

Fig. S3: Effect of **individual N, P, or K** fertilization on the fold-changes of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) abundance in rhizosphere and bulk soil.

Fig. S4. Composition and diversity of bacterial communities in rhizosphere and bulk soils under **individual N, P, or K** fertilization.

Fig. S5. (A and C) Spearman's **rank** correlation between bacterial family (>1% in any sample) and antibiotic resistance genes (ARGs) in rhizosphere and bulk soil **under N fertilization**. (B and D) Bacterial families (>1% in any sample) in rhizosphere and bulk soil showing significant differences in abundance **between N fertilized and unfertilized soils**.

Table captions:

Table S1: Properties of groundwater and reclaimed water.

Table S2: Effects of **individual N, P, or K** fertilization on tomato yield and soil physicochemical properties.

Table S3: Shannon-Wiener and Simpson **diversity indices** based on abundance of antibiotic resistance genes (ARGs) detected **in unfertilized or individual N, P, or K fertilization**.

Table S4: Distribution of antibiotic resistance gene (ARG) types and **putative** ARG hosts at the phylum level **under individual N, P, or K fertilization**, as determined by network analysis.

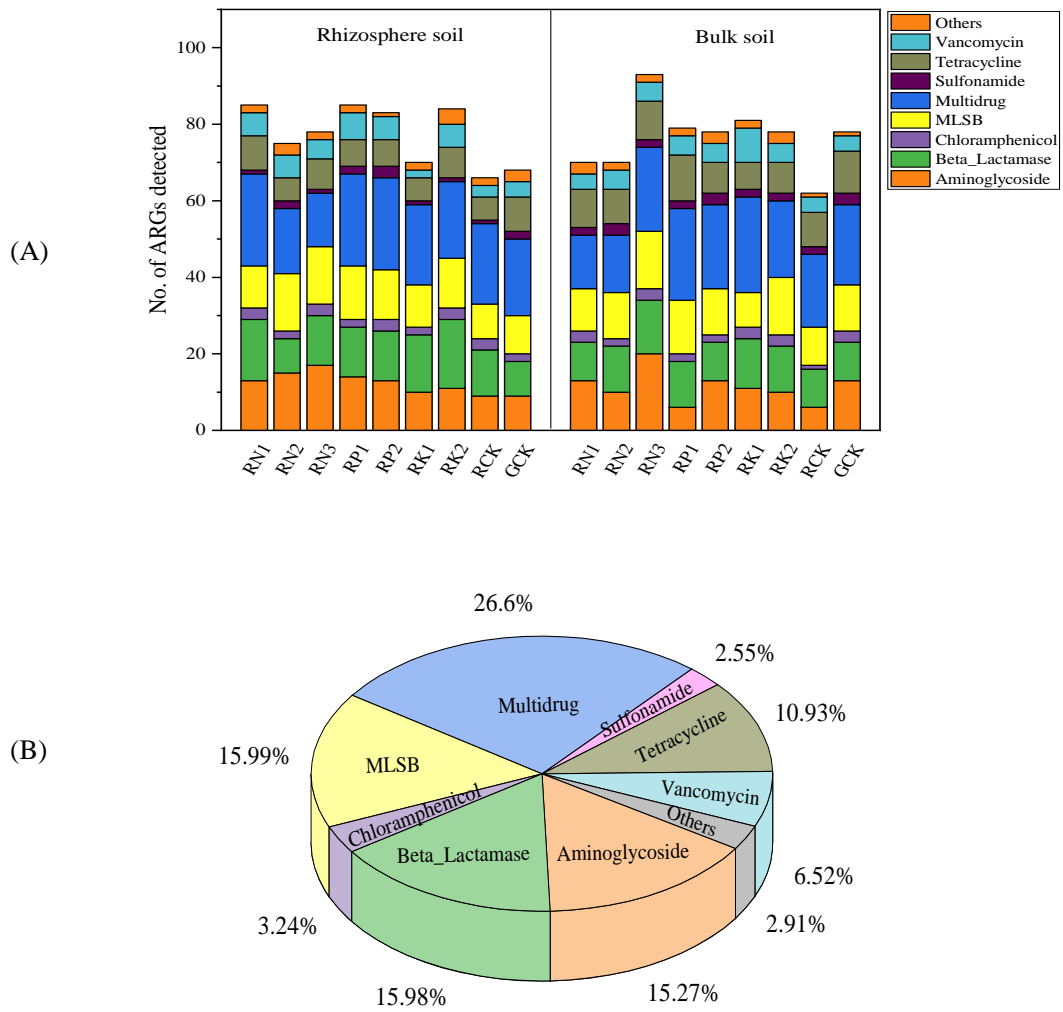


Fig. S1: Numbers of antibiotic resistance genes (ARGs) detected in different treatments (A) and proportions of different types of ARGs in soil samples (B).

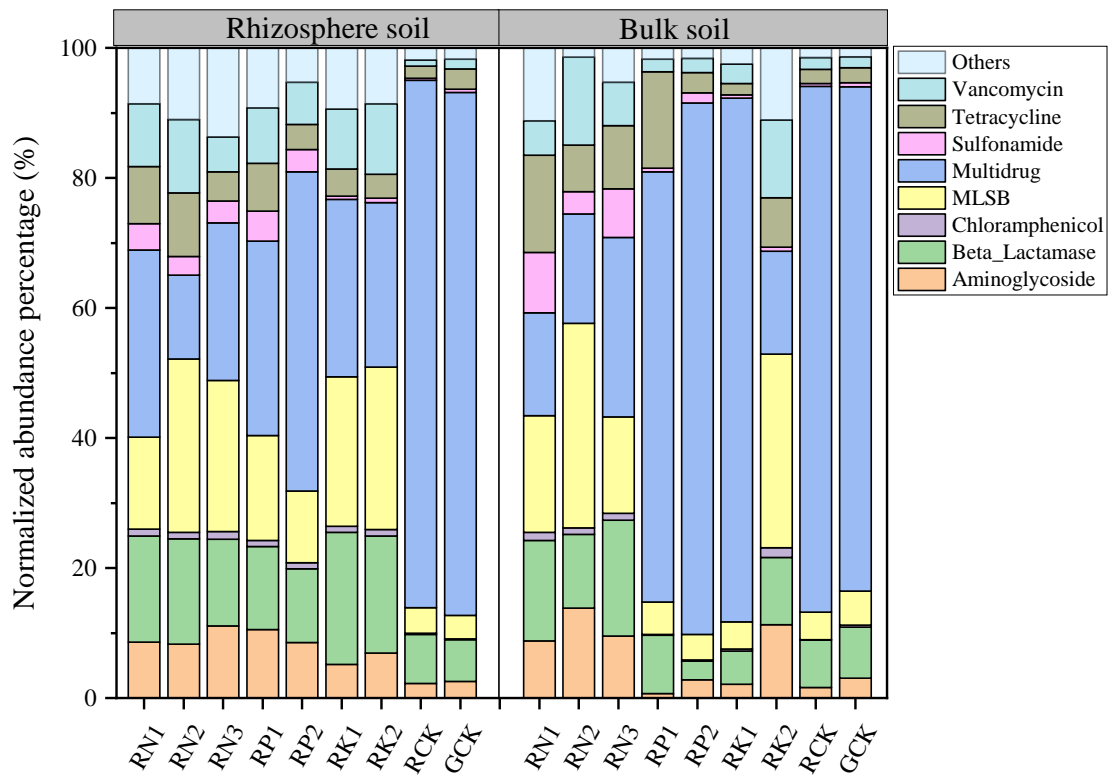


Fig. S2: Percentage of antibiotic resistance genes (ARGs) in different classes, based on normalized abundance values.

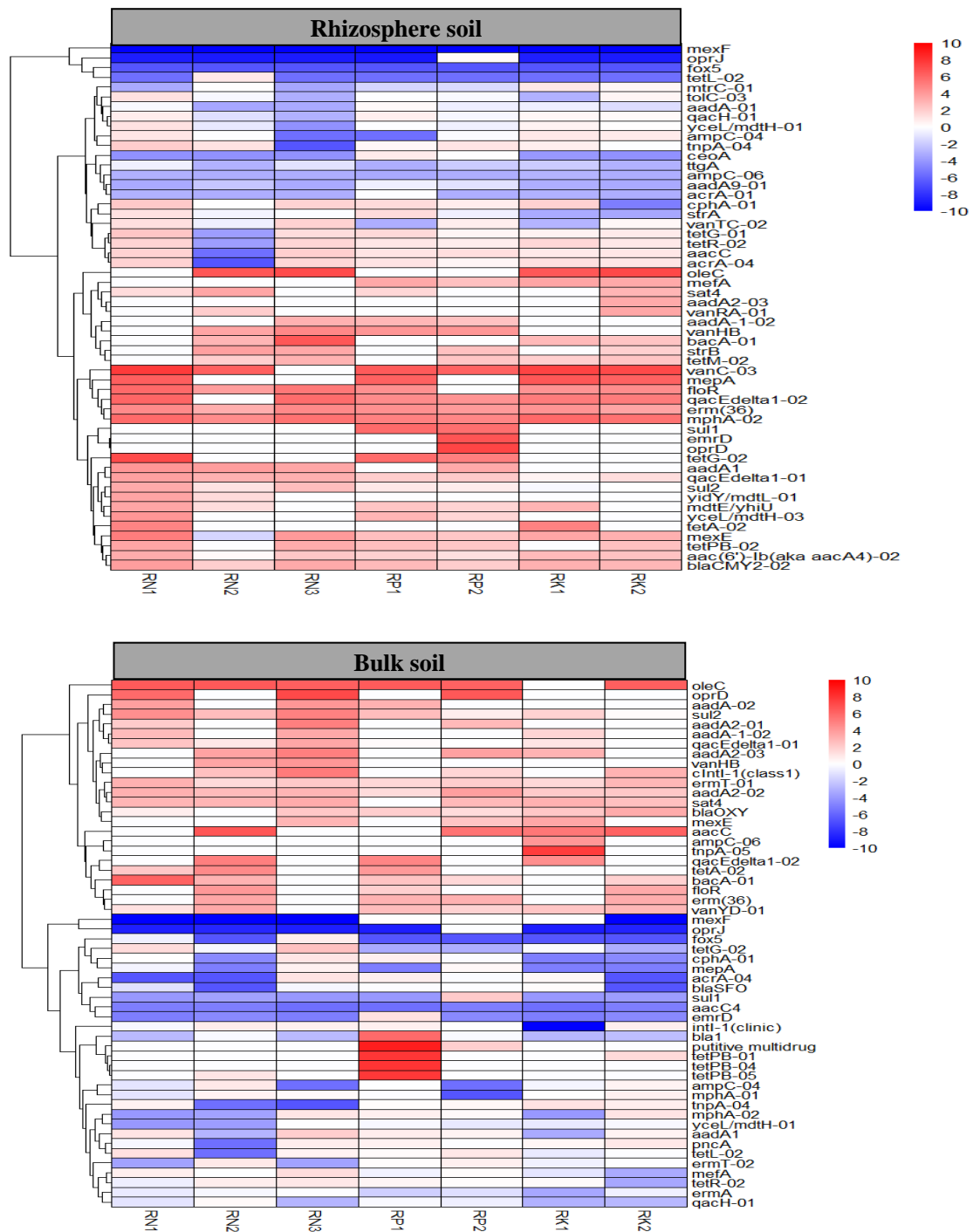


Fig. S3: Effect of individual N, P, or K fertilization on the fold-changes of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) abundance in rhizosphere and bulk soil. The Ct values of undetected ARGs or MGEs are replaced by 31, and the number in brackets stands for fold of change that is log₂ transformed. Only the values >3 and <-3 in any sample are displayed. The value of 0 means no change, and the positive and negative number means an increase or decrease, respectively.

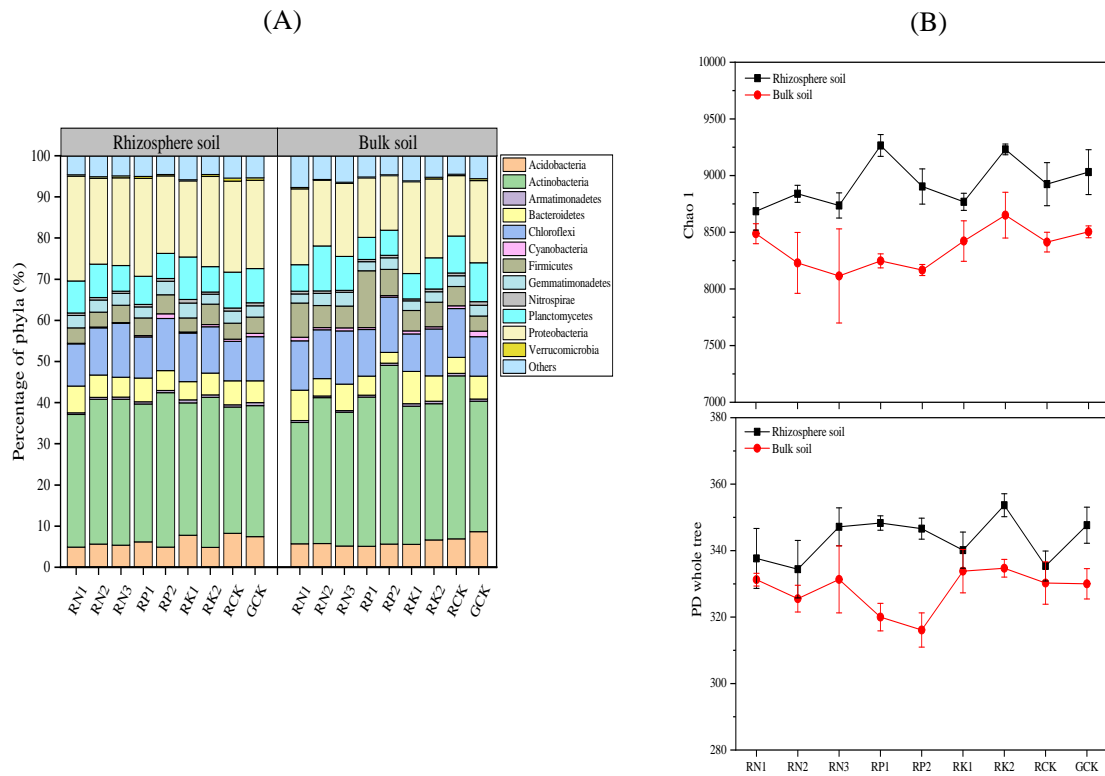


Fig. S4. Composition and diversity of bacterial communities in rhizosphere and bulk soils under individual N, P, or K fertilization. (A) Percentage of bacterial phyla (>0.5% in any sample). (B) Bacterial α -diversity that represented by Chao1 and PD whole tree index.

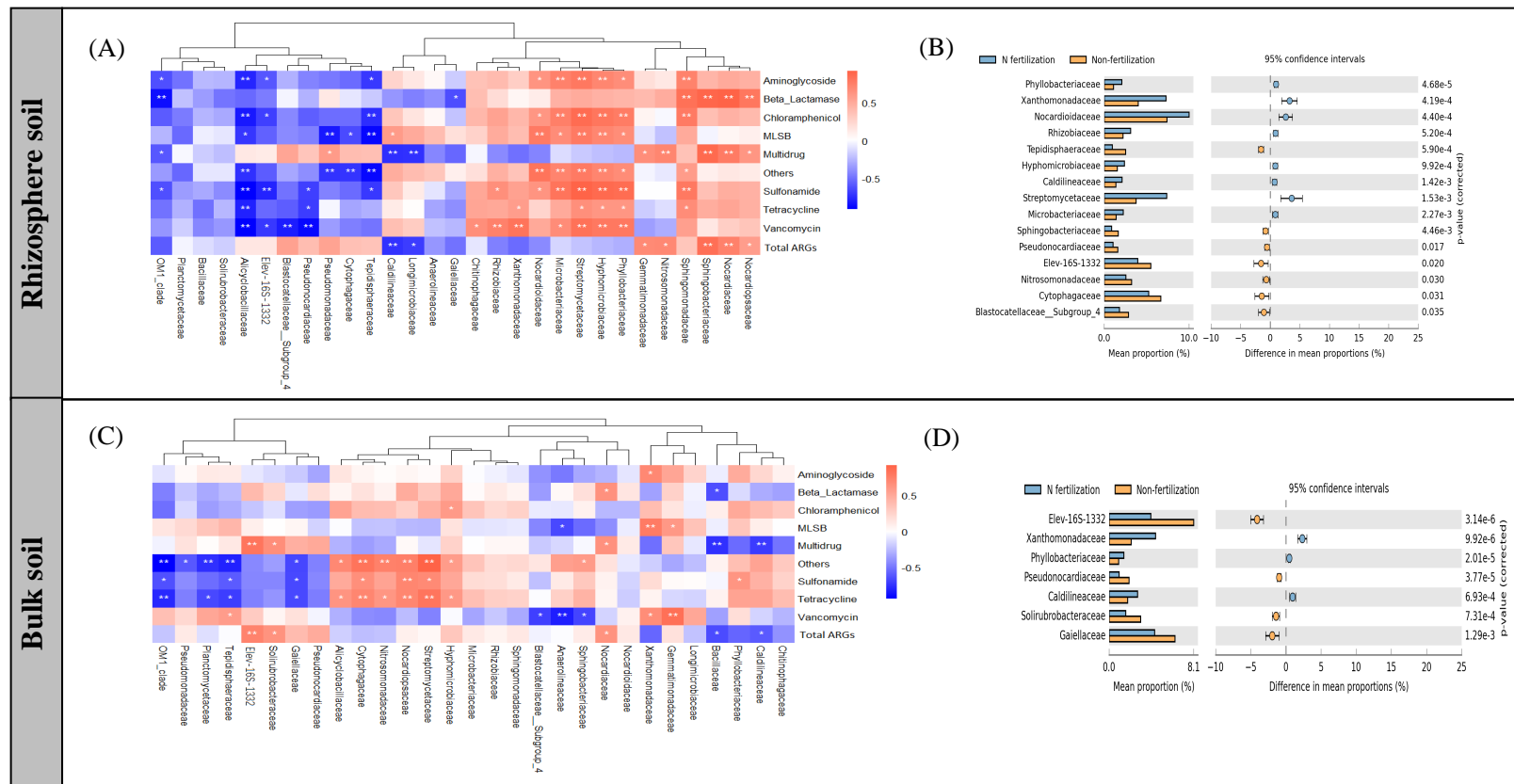


Fig. S5. (A and C) Spearman's **rank** correlation between bacterial family (>1% in any sample) and antibiotic resistance genes (ARGs) in rhizosphere and bulk soil **under N fertilization**. (B and D) Bacterial families (>1% in any sample) in rhizosphere and bulk soil showing significant differences in abundance **between N fertilized and unfertilized soils**.

Table S1: Properties of groundwater and reclaimed water.

Items□	Groundwater	Reclaimed water
pH (pH-H ₂ O)	8.07	8.10
EC (m _s /cm)	1.99	1.66
COD (mg/L)	104.00	80.00
Total N (mg/L)	0.55	9.43
Total P (mg/L)	-	1.78
Ca (g/L)	0.06	1.23
Mg (g/L)	0.12	1.28
Total Cu (µg/L)	2.45	7.00
Total Zn (mg/L)	0.02	0.05
Total Pb (µg/L)	0.65	13.20
Total Cd (µg/L)	0.05	4.10

Table S2: Effects of individual N, P, or K fertilization on tomato yield and soil physicochemical properties.

Treatments	Tomato yield (kg/plot)	Rhizosphere soil			Bulk soil		
		pH	TN (mg/g)	TP (mg/g)	pH	TN (mg/g)	TP (mg/g)
RN1	65.08 a	8.06 h	1.92 a	0.74 c	8.16 f	1.44 bc	0.69 c
RN2	63.85 a	8.91 b	1.36 c	0.67 cd	8.91 a	1.24 cde	0.72 cd
RN3	56.86 a	8.34 g	1.44 bc	0.63 cd	7.86 g	1.69 a	0.71 c
RP1	69.63 a	7.99 i	1.60 b	1.39 a	8.22 e	1.29 cd	1.45 a
RP2	68.68 a	8.51 e	1.63 b	1.16 b	8.88 bc	1.15 e	1.24 b
RK1	60.98 a	8.82 c	1.53 b	0.62 de	8.74 d	1.34 bc	0.66 cd
RK2	66.50 a	8.63 d	1.32 c	0.61 cd	8.88 ab	1.12 e	0.65 cd
RCK	70.33 a	9.12 a	1.29 c	0.61 cd	8.92 ab	1.15 de	0.64 d
GCK	65.10 a	8.45 f	1.02 d	0.48 e	8.86 c	1.06 e	0.61 d

Note: Means followed by the same letter are not significantly difference at $P < 0.05$.

Table S3: Shannon-Wiener and Simpson **diversity indices** based on abundance of antibiotic resistance genes (ARGs) detected **in unfertilized or individual N, P, or K fertilization**. The diversity index was calculated with R.

Rhizosphere soil	RN1	RN2	RN3	RP1	RP2	RK1	RK2	RCK	GCK
Shannon-Wiener	3.50 a	3.45 a	3.28 a	3.53 a	3.20 a	3.24 a	3.37 a	1.62 b	1.69 b
Simpson	0.95 a	0.95 a	0.94 a	0.95 a	0.92 a	0.94 a	0.95 a	0.55 b	0.54 b
Bulk soil	RN1	RN2	RN3	RP1	RP2	RK1	RK2	RCK	GCK
Shannon-Wiener	3.39 a	3.44 a	3.55 a	2.18 b	1.70 cd	1.49 d	3.42 a	1.65 cd	1.93 bc
Simpson	0.95 a	0.95 a	0.96 a	0.71 b	0.55 c	0.46 d	0.94 a	0.55 c	0.61 c

Note: Means followed by the same letter are not significantly difference at $P < 0.05$.

Table S4: Distribution of antibiotic resistance gene (ARG) types and putative ARG hosts at the phylum level under individual N, P, or K fertilization, as determined by network analysis.

	Percentage of ARGs types			Percentage of potential ARG-associated host bacteria at phylum level	
	Rhizosphere soil	Bulk soil		Rhizosphere soil	Bulk soil
N fertilization					
Aminoglycoside	13.46	0.00	<i>Acidobacteria</i>	5.26	0.00
Beta_Lactamase	19.23	18.18	<i>Actinobacteria</i>	42.11	66.67
Chloramphenicol	3.85	0.00	<i>Bacteroidetes</i>	5.26	16.67
MLSB	11.54	0.00	<i>Chloroflexi</i>	5.26	0.00
Multidrug	28.85	18.18	<i>Firmicutes</i>	5.26	0.00
Sulfonamide	1.92	9.09	<i>Gemmatimonadetes</i>	5.26	16.67
Tetracycline	9.62	45.45	<i>Planctomycetes</i>	5.26	0.00
Vancomycin	7.69	9.09	<i>Proteobacteria</i>	26.32	0.00
Others	3.85	0.00			
P fertilization					
Aminoglycoside	0.00	0.00	<i>Acidobacteria</i>	0.00	0.00
Beta_Lactamase	0.00	0.00	<i>Actinobacteria</i>	0.00	50.00
Chloramphenicol	0.00	0.00	<i>Bacteroidetes</i>	100.00	0.00
MLSB	0.00	0.00	<i>Chloroflexi</i>	0.00	0.00
Multidrug	100.00	83.33	<i>Firmicutes</i>	0.00	0.00
Sulfonamide	0.00	0.00	<i>Gemmatimonadetes</i>	0.00	0.00
Tetracycline	0.00	16.67	<i>Planctomycetes</i>	0.00	25.00
Vancomycin	0.00	0.00	<i>Proteobacteria</i>	0.00	25.00
Others	0.00	0.00			
K fertilization					
Aminoglycoside	14.29	23.08	<i>Acidobacteria</i>	0.00	0.00
Beta_Lactamase	35.71	15.38	<i>Actinobacteria</i>	33.33	20.00
Chloramphenicol	0.00	7.69	<i>Bacteroidetes</i>	0.00	20.00
MLSB	14.29	7.69	<i>Chloroflexi</i>	16.67	0.00
Multidrug	28.57	7.69	<i>Firmicutes</i>	0.00	20.00
Sulfonamide	0.00	15.38	<i>Gemmatimonadetes</i>	0.00	0.00
Tetracycline	0.00	7.69	<i>Planctomycetes</i>	0.00	0.00
Vancomycin	7.14	7.69	<i>Proteobacteria</i>	50.00	40.00
Others	0.00	7.69			