Heterogeneity of soil bacterial and bacteriophage communities in three rice agroecosystems and potential impacts on nutrient cycling

Yajiao Wang[1,2], Yu Liu[1], Yuxing Wu[2], Nan Wu[1], Wenwen Liu[1], Xifeng Wang[1]

[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[2] Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding 071000, China

*Correspondence authors: Xifeng Wang (wangxifeng@caas.cn)

Table S1 Chemical applications (apps.) on farms using three rice cropping systems. GZ: Rice double cropping ecosystems in Guangzhou; JMS: Rice single cropping ecosystems in Jiamusi; NJ: Rice-wheat rotation cropping ecosystems in Nanjing.

System	Crop	Type of pesticide	Chemical	Growth period of app.	Dose /growing season (g/hectare)
GZ	Rice (double cropping / year)	insecticide	Imidacloprid	seedling	30
			Thiamethoxam+lambda-cyhalothrin	tillering	22.5
			Abamectin	heading	6
		fungicide	Isoprothiolane	tillering	600
		bactericide	Bismerthiazol	3 leaf stage	375
		herbicide	Bensulfuron methyl	sowing	30
JMS	Rice (single cropping / year)	insecticide	Thiamethoxam+lambda-cyhalothrin	heading	22.5
		fungicide	Fludioxonil	seed pelleting	6
			Isoprothiolane	heading	600
		herbicide	Bensulfuron methyl	sowing	180
			Pyrazosulfuron-ethyl	3 leaf stage	60
	Rice (single cropping / year)	insecticide	Imidacloprid	tillering	60
			Thiamethoxam+lambda-cyhalothrin	heading	22.5
		fungicide	Isoprothiolane	tillering	600
			Pyraclostrobin	heading	90
		herbicide	Bensulfuron methyl	sowing	180
			Pyrazosulfuron-ethyl	3 leaf stage	30
NJ	Wheat (single cropping / year)	insecticide	Imidacloprid	seed pelleting	22.5
			Lambda-cyhalothrin	reviving	15
		fungicide	Fludioxonil	seed pelleting	6
			Carbendazim	reviving	600
			Tebuconazole	flowering	75
		herbicide	Bensulfuron-methyl	sowing	30
			Tribenuron-methyl+bensulfuron methyl	reviving	45

Table S2 Means ± standard deviation for environmental factors variables at the three agroecosystem sites in China.

System	AK (mg/kg)	AN (mg/kg)	SOC (g/kg)	AP (mg/kg)	AAT (℃)	AP (mm)
JMS	121.51 ± 62.03 a	75.25 ± 1.43 a	5.032 ± 0.13 a	108.44 ± 4.81 b	3200	518
GZ	41.778 ± 1.28 c	57.167 ± 1.65 c	3.038 ± 0.19 b	247.61 ± 4.15 a	7500	1800
NJ	62.287 ± 1.56 b	64.167 ± 3.30 b	3.147 ± 0.08 b	64.78 ± 1.20 c	5400	1047

Different lowercase letters within a column indicate a significant difference in a variable among sites ($P < 0.05$) according to Tukey's test. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation cropping in Nanjing; SOC: soil organic carbon; AK: available potassium, AN: available nitrogen; AP: available phosphorus; AAT: annual accumulated temperature; AP: annual precipitation.

Table S3 Percentage distribution of the dominant genera in soils of three rice cropping agroecosystems. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation in Nanjing.

Genus	GZ (%)	JMS (%)	NJ (%)
Nocardioides	0.86	0.74	3.38
Gemmatimonas	2.16	1.21	2.53
Solirubrobacter	0.94	0.65	2.43
Conexibacter	0.89	0.50	2.43
Mycobacterium	1.28	0.44	2.40
Gemmatirosa	1.17	0.86	2.14
Streptomyces	1.13	1.14	1.98
Phycicoccus	0.32	0.77	1.91
Sphingomonas	0.61	0.59	1.52
Candidatus_Solibacter	1.64	2.43	1.49
Bradyrhizobium	1.28	2.62	1.44
Rhodoplanes	0.89	0.44	1.10
Pseudolabrys	1.31	0.14	1.04
Anaeromyxobacter	2.04	2.36	0.88
Anaerolinea	1.37	1.85	0.84
Ardenticatena	0.75	1.19	0.61
Longilinea	0.86	1.38	0.53
Levilinea	0.77	1.21	0.46
Nitrospira	1.11	0.27	0.47

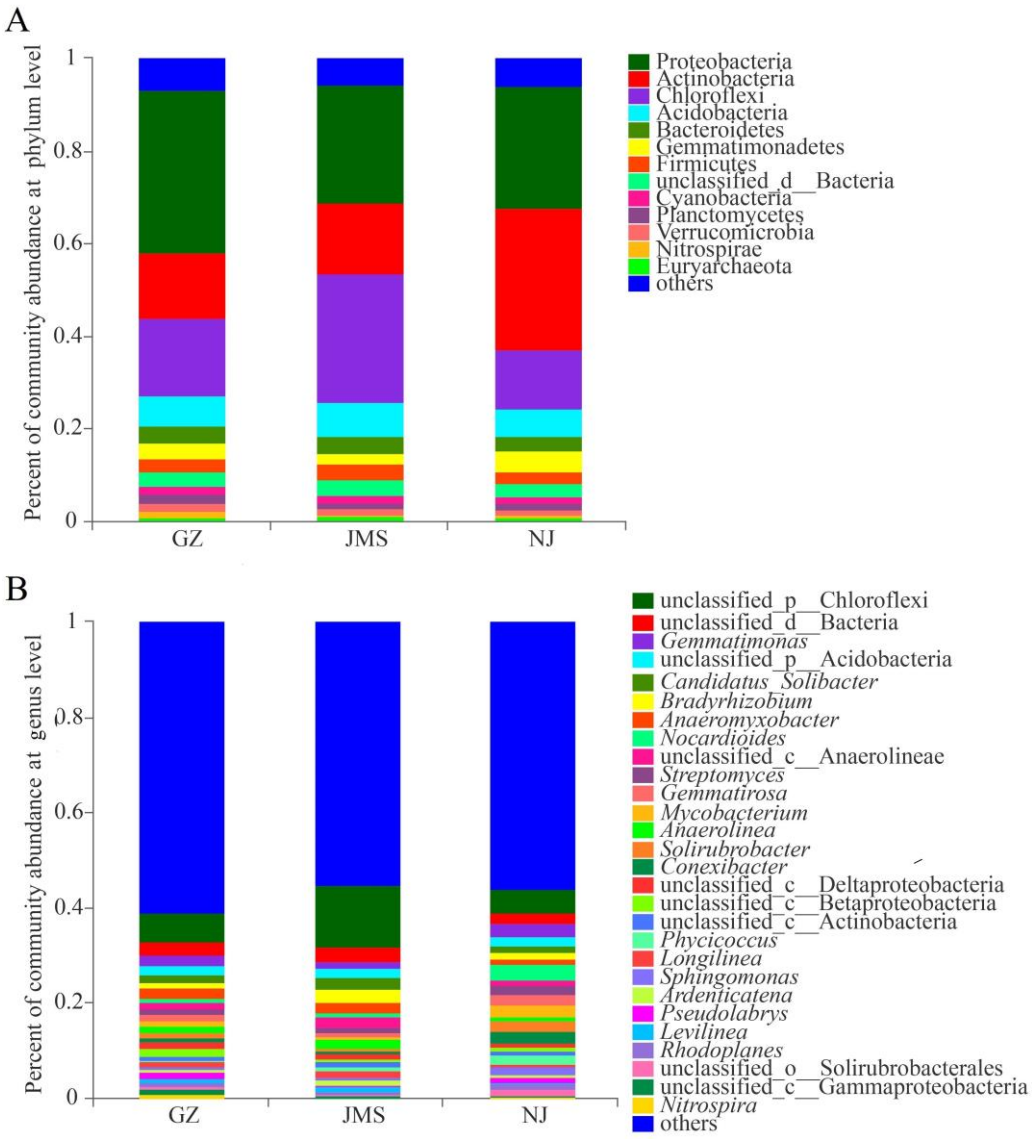

Fig. S1. Relative abundance of main microbial phyla (A) and genera (B) in soils of three rice cropping agroecosystems. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation in Nanjing.

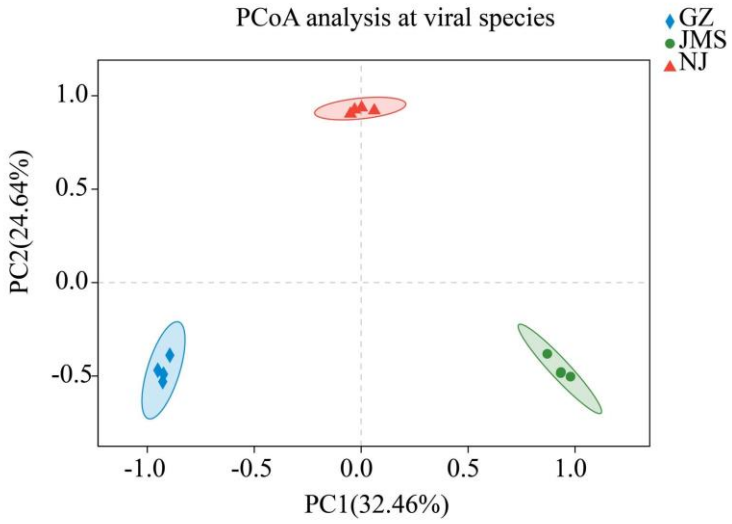

Fig. S2. Principal coordinate analysis (PCoA) of soil microbial community based on Bray–Curtis distance matrices. The percentage represents the explanatory value of the PCoA axes to the difference in sample composition; distances between symbols on the ordination plot reflect relative dissimilarities. GZ: rice double cropping in Guangzhou in blue; JMS: rice single cropping in Jiamusi in red; NJ: rice–wheat rotation cropping in Nanjing.

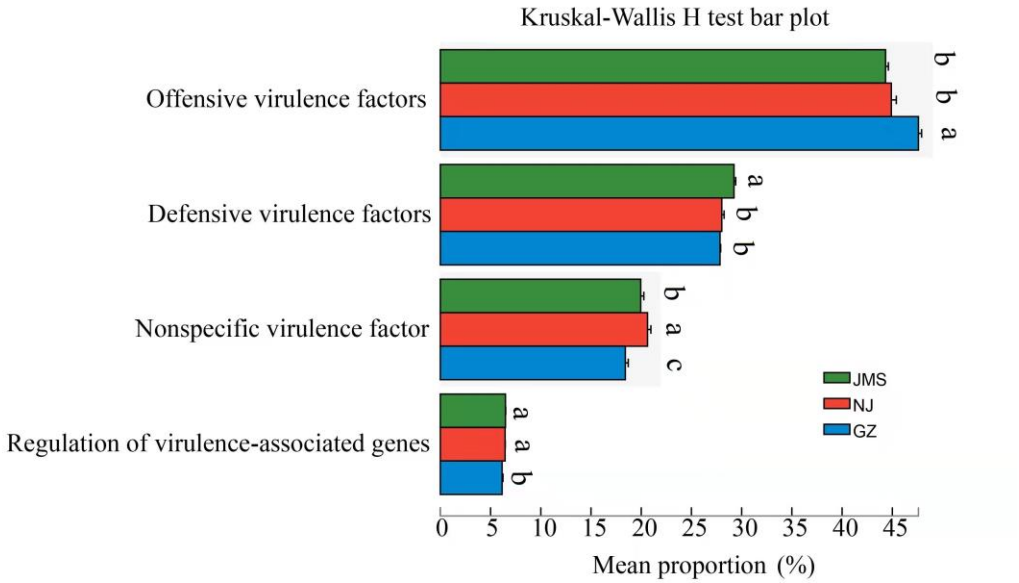

Fig. S3. Relative abundance of genes related to xenobiotics biodegradation and metabolism in viruses and bacteria in soils of three rice agroecosystems. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation in Nanjing. Different letters above the bars indicate a significant difference ($p < 0.05$) according to Kruskal-Wallis H test.

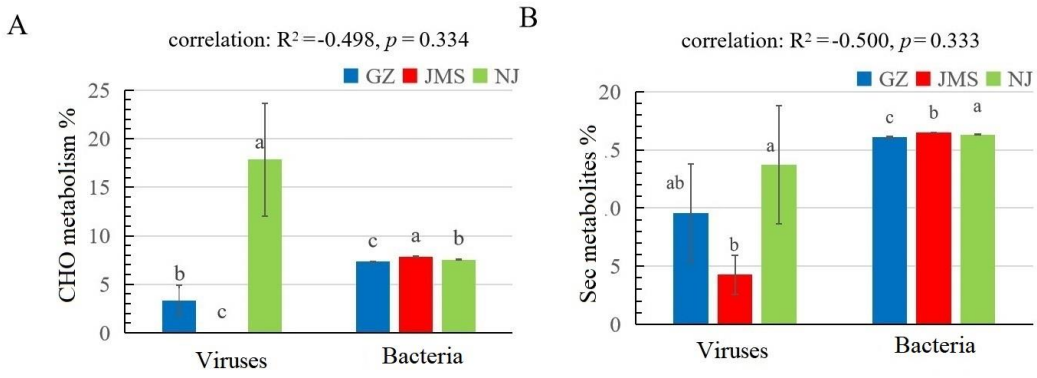

Fig. S4. Relative abundance of genes related to carbohydrate (CHO) metabolism (A) and biosynthesis of secondary (Sec) metabolites (B) in viruses and bacteria in soils of three rice agroecosystems. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation in Nanjing. Different letters above the bars indicate a significant difference ($p < 0.05$) according to Kruskal-Wallis H test.

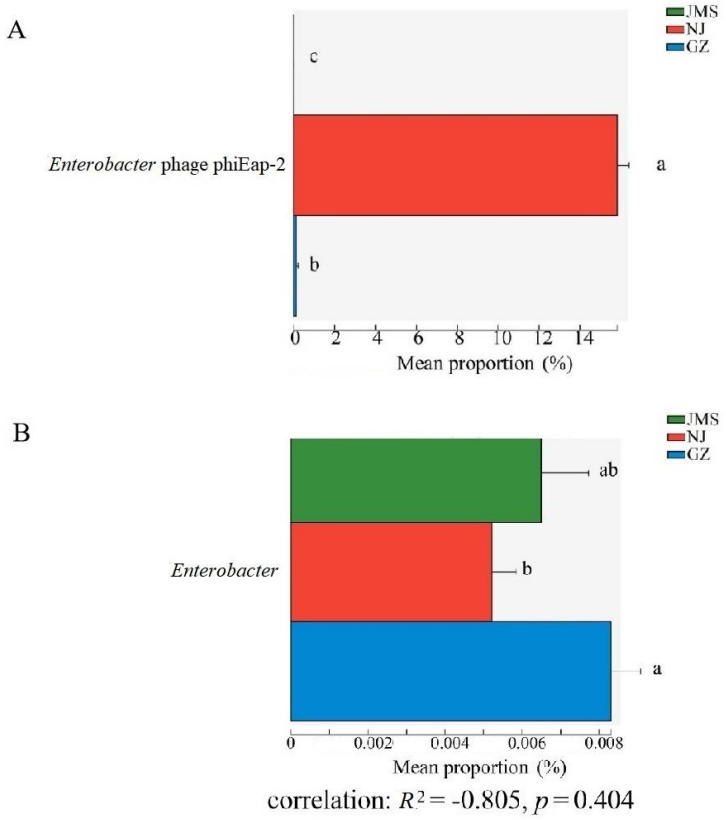

Fig. S5. Relative abundance of *Enterboacter* phage phiEap-2 among soil viruses (A) and relative abundance of *Enterboacter* among soil bacteria (B) in the three rice agroecosystems. GZ: rice double cropping in Guangzhou; JMS: rice single cropping in Jiamusi; NJ: rice–wheat rotation in Nanjing. Different letters above the bars indicate a significant difference ($p < 0.05$) according to Kruskal-Wallis H test.

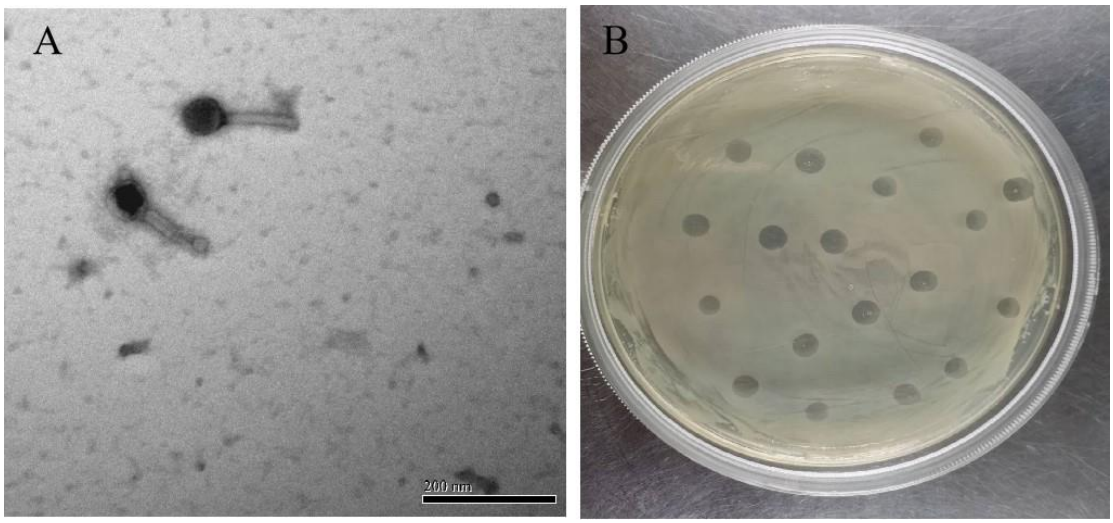

Fig. S6. (A) Transmission electron micrograph of *Enterobacter* phage-NJ. (B) Plate lytic experiment of *Enterobacter* phage-NJ against nitrogen-fixing *Enterobacter cloacae.*

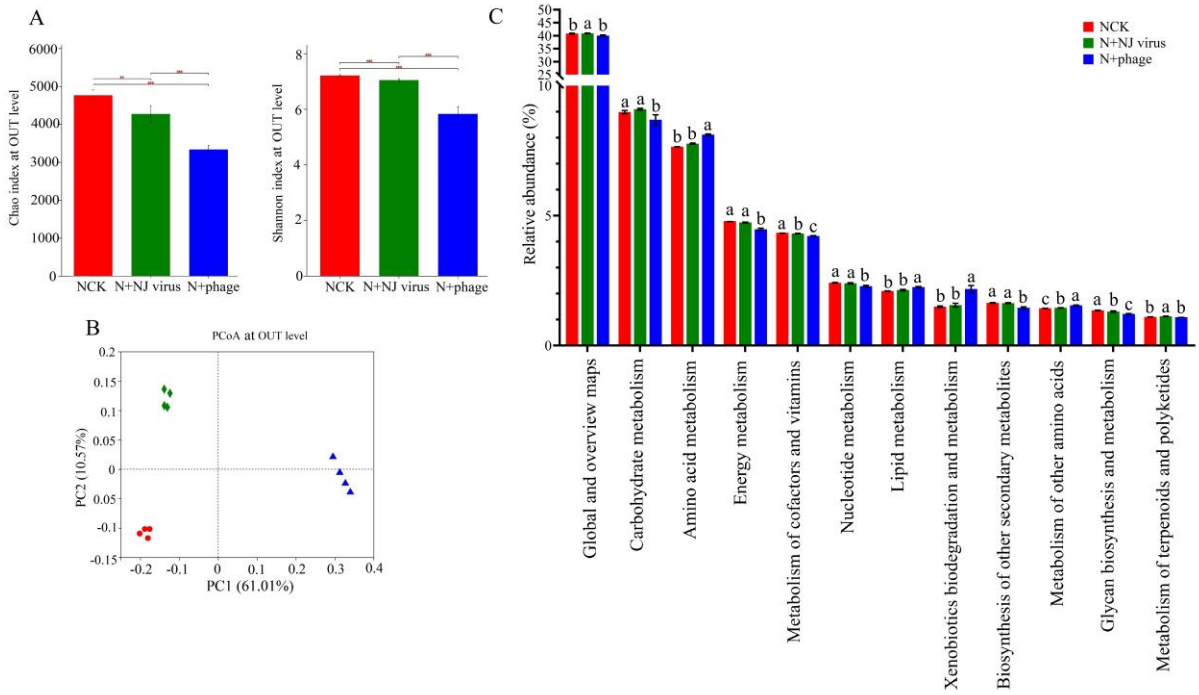

Fig. S7. Paddy soil bacterial diversity and function after addition of *Enterobacter* bacteriophage-NJ to untreated field soil from the Nanjing agroecosystem. (A) Variation in alpha diversity of soil bacterial community based on the Chao and the Shannon indices. Values for the same index with different letters above bars differed significantly ($p < 0.05$) according to Kruskal-Wallis H test. (B) Principal coordinate analysis of soil bacterial community based on Bray–Curtis distance matrices. (C) Paddy soil bacterial function after addition of *Enterobacter* bacteriophage-NJ. The *x*- and *y*-axis represent the two selected principal coordinate axes; the percentage represents the explanatory value of the principal coordinate axes. N+NJ virus: virus isolated from NJ soil was added to untreated field soil; N+phage, *Enterobatcter* bacteriophage inoculation in natural soil; NCK: untreated field soil.