Roles of microbial community and keystone taxa in rice productivity under green manuring in south China

Table S1 Primers and PCR conditions used for quantitative PCR

Functional gene	Primer	Sequence (5′-3′)	Amplicon length (bp)	Efficiency (%)	Reference
nifH	PolyF/PolyR	TGCGAYCCSAARGCBGACTC /ATSGCCATCATYTCRCCGGA	360	84.10	(Shu et al., 2012)
AOA *amoA*	26F/417R	GACTACATMTTCTAYACWGA YTGGGC/GGKGTCATRTATGGWGG YAAYGTTGG	415	83.50	(Park et al., 2008)
AOB *amoA*	1F/2R	GGGGTTTCTACTGGTGGT/ CCCCTCKGSAAAGCCTTCTTC	500	80.70	(Rotthauwe et al., 1997)
narG	1962m2F/2050m2R	TAYGTSGGGCAGGARAAACTG/ CGTAGAAGAAGCTGGTGCTGTT	110	83.60	(Kandeler et al., 2006)
nirS	cd3aF/R3cd	GTSAACGTSAAGGARACSGG/ GASTTCGGRTGSGTCTTGA	425	85.30	(Kandeler et al., 2006)
nirK	F1aCu/R3Cu	ATCATGGTSCTGCCGCG/GCCTCGA TCAGRTTGTGGTT	473	81.80	(Levy-Booth et al., 2014)
nosZ	1126F/1381R	GGGCTBGGGCCRTTGCA/GAAGCG RTCCTTSGARAACTTG	252	84.20	(Chen et al., 2012)

Table S2 Good's coverage for each sample (%)

Repetition	16S rRNA for bacteria				ITS for fungi			
	GQ_GM	GQ_WF	NX_GM	NX_WF	GQ_GM	GQ_WF	NX_GM	NX_WF
Repetition 1	96.30	96.41	96.23	96.33	99.98	99.97	99.99	99.99
Repetition 2	96.05	96.83	96.91	97.49	99.98	99.99	99.98	99.99
Repetition 3	95.80	96.99	96.21	98.68	99.98	99.98	99.98	99.99
Repetition 4	97.12	96.57	96.13	99.60	99.99	99.97	99.99	100.00
Repetition 5	96.92	96.49	96.80	96.24	99.99	99.97	99.99	100.00
Repetition 6	97.03	96.68	98.35	97.92	99.99	99.99	99.99	100.00

GQ, Gaoqiao site; NX, Nanxian site; GM, green manure treatment; WF, winter fallow treatment.

Numbers after the repetitions of each treatment. GQ_GM, green manure treatment at Gaoqiao site;

GQ_WF, winter fallow treatment at Gaoqiao site; NX_GM, green manure treatment at Nanxian site;

NX_WF, winter fallow treatment at Nanxian site.

Table S3 Soil properties in different treatments at the two sites in 2019

Treatment	pH	SOM (g kg^{-1})	TN (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	NH$_4^+$-N (mg kg^{-1})	NO$_3^-$-N (mg kg^{-1})	DOC (mg kg^{-1})	DON (mg kg^{-1})
GQ_GM	5.43	21.93	1.51	21.27	109.66	3.43	8.30	35.59	4.76
	(0.16)	(1.82)	(0.16)	(3.84)	(20.62)	(1.00)	(0.44)	(9.58)	(1.53)
GQ_WF	5.33	23.28	1.48	24.40	97.00	2.73	10.60	17.39	2.99
	(0.14)	(1.68)	(0.09)	(4.81)	(20.86)	(0.74)	(0.54)	(1.99)	(0.38)
P value	0.139	0.222	0.789	0.421	0.244	0.604	**0.008****	0.079	0.228
NX_GM	8.04	53.96	2.45	11.09	57.84	50.10	16.01	18.38	5.63
	(0.03)	(7.41)	(0.10)	(2.11)	(1.25)	(8.32)	(2.98)	(2.49)	(0.55)
NX_WF	8.09	41.50	2.15	10.00	62.17	34.59	17.58	21.75	5.17
	(0.02)	(4.13)	(0.12)	(0.95)	(0.89)	(3.12)	(1.71)	(3.52)	(0.65)
P value	0.150	0.074	0.101	0.690	0.063	0.084	0.606	0.331	0.490

SOM, soil organic matter; TN, total nitrogen; AP, available phosphorus, AK, available potassium; DOC, dissolved organic carbon; DON, dissolved organic nitrogen. Values in the bracket represent standard error. Table S2 shows treatment abbreviations.

Table S4 Topological properties of bacterial and fungal co-occurrence networks

Network indexes	Bacterial community				Fungal community			
	GQ_GM	GQ_WF	NX_GM	NX_WF	GQ_GM	GQ_WF	NX_GM	NX_WF
Empirical networks								
Number of nodes	860	669	852	879	179	124	119	85
Number of edges	1687	972	2779	3807	673	218	232	135
Positive correlations (%)	72.38	56.28	54.7	75.36	50.52	55.96	46.12	54.07
Negative correlations (%)	27.62	43.72	45.3	24.64	49.48	44.04	53.88	45.93
R^2 of power-law	0.783	0.797	0.828	0.696	0.458	0.644	0.61	0.689
Average connectivity	3.923	2.906	6.523	8.662	7.52	3.516	3.899	3.176
Average clustering coefficient (*avgCC*)	0.425	0.477	0.273	0.506	0.536	0.324	0.304	0.29
Average path length (GD)	8.215	8.542	5.098	6.206	5.285	6.049	4.74	5.541
Transitivity	0.512	0.56	0.23	0.581	0.498	0.465	0.324	0.386
Connectedness	0.472	0.241	0.866	0.717	0.913	0.875	0.902	0.908
Modularity & (the number of modules)	0.856 (98)	0.923 (130)	0.558 (48)	0.751 (63)	0.616 (10)	0.763 (12)	0.673 (9)	0.723 (9)
Random networks								
avgCC ± SD	0.007 ± 0.002	0.005 ± 0.003	0.023 ± 0.003	0.035 ± 0.003	0.077 ± 0.008	0.028 ± 0.010	0.036 ± 0.013	0.036 ± 0.016
GD ± SD	4.756 ± 0.030	5.731 ± 0.068	3.627 ± 0.019	3.313 ± 0.017	2.859 ± 0.029	3.847 ± 0.086	3.562 ± 0.047	3.820 ± 0.110
Modularity ± SD	0.526 ±	0.654 ±	0.358 ±	0.291 ±	0.301 ±	0.514 ±	0.477 ±	0.526 ±

0.004	0.005	0.003	0.003	0.007	0.014	0.012	0.016

Table S2 shows treatment abbreviations.

Table S5 Keystone ASVs identified in the combined bacterial-fungal networks documented with taxonomy assignments and the degree of co-occurrence values

	Phylum	Class	Order	Family	Genus	Degree	ASV ID
GQ_GM							
Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	uncultured	uncultured bacterium	8	bASV1
	Actinobacteria	Acidimicrobiia	uncultured	unclassified_uncultured	unclassified_uncultured	4	bASV2
	Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	uncultured bacterium	9	bASV3
	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	14	bASV4
						9	bASV5
					Leptolinea	33	bASV6
			RBG-13-54-9	uncultured Anaerolineaceae bacterium	uncultured Anaerolineaceae bacterium	11	bASV7
				unclassified_RBG-13-54-9	unclassified_RBG-13-54-9	33	bASV8
						14	bASV9
	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured	9	bASV10

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	Patescibacteria	Parcubacteria	Candidatus Jorgensenbacteria	Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9	Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9	13	bASV11
		Gamma proteobacteria	Betaproteobacteriales	Gallionellaceae	Candidatus Nitrotoga	33	bASV12
					Sideroxydans	6	bASV13
	Proteobacteria	Deltaproteobacteria	Myxococcales	mle1-27	uncultured Sorangiineae bacterium	33	bASV14
				Haliangiaceae	Haliangium	33	bASV15
		Alphaproteobacteria	Acetobacterales	Acetobacteraceae	uncultured	11	bASV16
			Rhizobiales	Beijerinckiaceae	Methylocystis	11	bASV17
		Gamma proteobacteria	Betaproteobacteriales	Gallionellaceae	unclassified_Gallionellaceae	6	bASV18
Fungi	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Mollisia	32	fASV1
		Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Issatchenkia	32	fASV2
		Sordariomycetes	Sordariales	Sordariaceae	Neurospora	32	fASV3
	unclass	unclassif	unclassifie	unclassified_Fungi	unclassified_Fung	32	fASV4

	ified_Fungi	ied_Fungi	d_Fungi		i	33	fASV5
						33	fASV6
						10	fASV7
GQ_WF							
Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	uncultured	uncultured bacterium	9	bASV19
	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolinea	11	bASV20
					Leptolinea	10	bASV21
					uncultured	9	bASV22
		KD4-96	uncultured bacterium	uncultured bacterium	uncultured bacterium	10	bASV23
			unclassified_KD4-96	unclassified_KD4-96	unclassified_KD4-96	8	bASV24
						4	bASV25
	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured	9	bASV26
	Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	Singulisphaera	9	bASV27
	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Pajaroellobacter	7	bASV28
			Sva0485	uncultured delta proteobacterium	uncultured delta proteobacterium	8	bASV29

Kingdom	Phylum	Class	Order	Family	Genus		ASV
			Syntrophobacterales	Syntrophobacteraceae	uncultured	5	bASV30
Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	10	fASV8
NX_GM							
Bacteria	Nitrospinae	P9X2b3D02	uncultured bacterium	uncultured bacterium	uncultured bacterium	22	bASV31
	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter	22	bASV32
		Gammaproteobacteria	CCD24	unclassified_CCD24	unclassified_CCD24	22	bASV33
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Zopfiella	22	fASV9
NX_WF							
Bacteria	Acidobacteria	Subgroup 17	unclassified_Subgroup 17	unclassified_Subgroup 17	unclassified_Subgroup 17	6	bASV34
		Subgroup 6	unclassified_Subgroup 6	unclassified_Subgroup 6	unclassified_Subgroup 6	6	bASV35
	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	12	bASV36
				unclassified_Ignavibacteriales	unclassified_Ignavibacteriales	26	bASV37

	Chloro flexi	KD4-96	uncultured bacterium	uncultured bacterium	uncultured bacterium	6	bASV38
	GAL15	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium	8	bASV39
	Gemm atimon adetes	Gemmat imonade tes	Gemmatim onadales	Gemmatimonadac eae	uncultured	11	bASV40
	Nitros pirae	Nitrospi ra	Nitrospiral es	Nitrospiraceae	Nitrospira	8	bASV41
					Leptospirillum	10	bASV42
		Thermo desulfov ibrionia	uncultured	uncultured bacterium	uncultured bacterium	26	bASV43
	Proteo bacteri a	Alphapr oteobact eria	Rhizobiales	Methyloligellaceae	uncultured	5	bASV44
		Deltapro teobacter ia	Desulfuro monadales	Geobacteraceae	Geobacter	16	bASV45
			NB1-j	unclassified_NB1-j	unclassified_NB1-j	20	bASV46
				uncultured bacterium	uncultured bacterium	8	bASV47
Fu ng	Ascom ycota	Eurotio mycetes	Eurotiales	Aspergillaceae	Aspergillus	18	fASV10

i		Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	16	fASV11
	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	11	fASV12

ASV ID only used in this analysis. bASV, the bacterial ASV, fASV, the fungal ASV. Table S2 shows
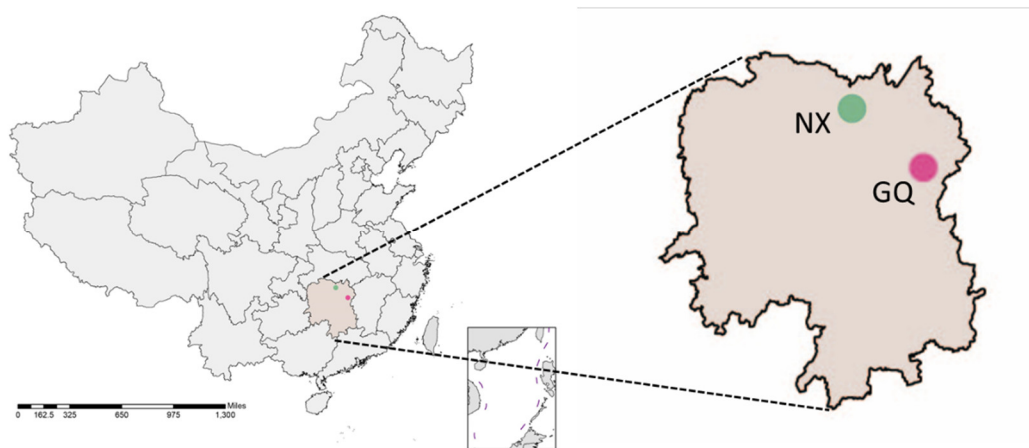
treatment abbreviations.

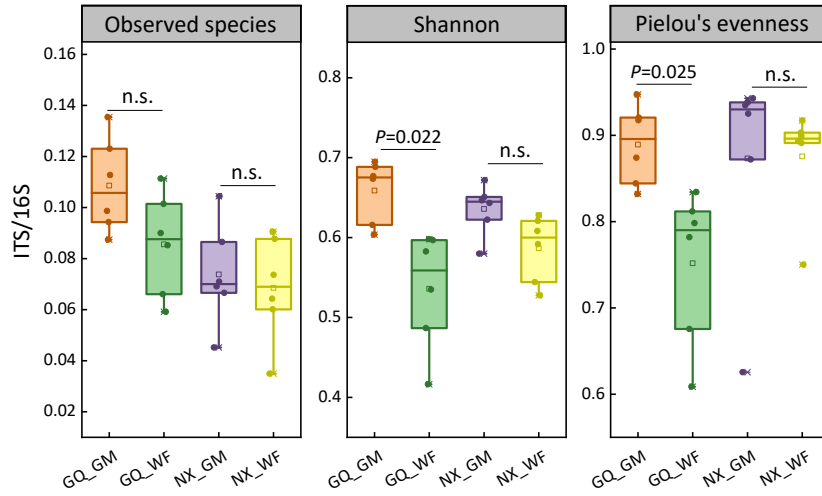**Figure S1** field experiment map of two sites. GQ, Gaoqiao site; NX, Nanxian Site.

Figure S2 Fungi-to-bacteria ratios (ITS/16S ratios) of observed species, Shannon index, and Pielou's evenness. GQ_GM, green manure treatment at the Gaoqiao site; GQ_WF, winter fallow treatment at the Gaoqiao site; NX_GM, green manure treatment at the Nanxian site; NX_WF, winter fallow treatment at the Nanxian site. The solid line and hollow block within each box represent the median and mean values, respectively. Top and bottom edges represent 75 and 25 percentiles, respectively. The solid circle represents value of each sample.
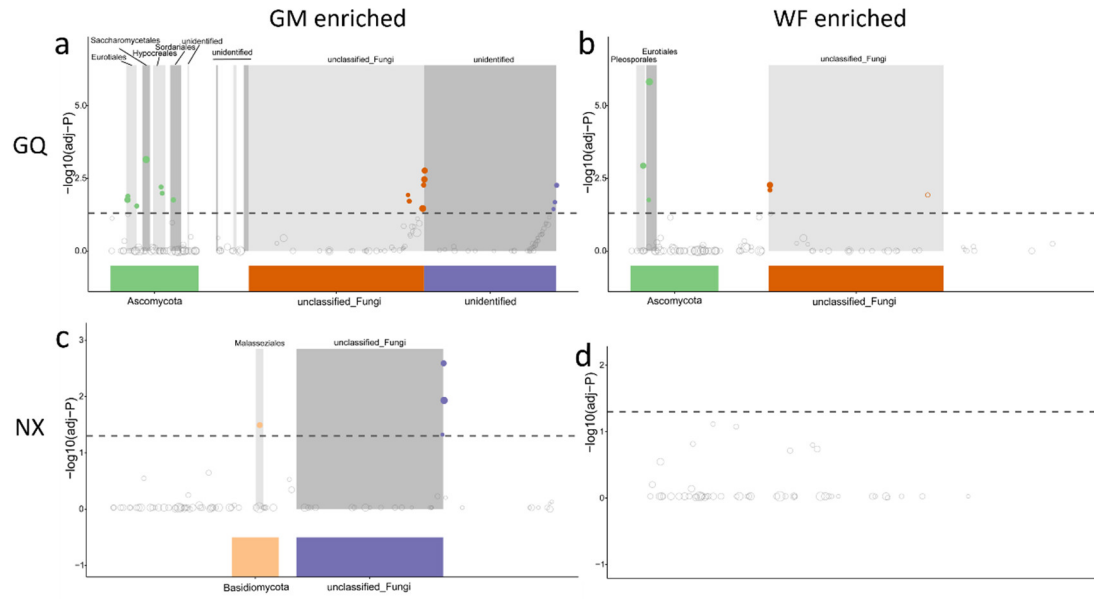
Figure S3 Manhattan plots based on the MetagenomeSeq analysis of bacterial community showing GM-enriched ASVs at GQ (a), WF-enriched ASVs at GQ (b), GM-enriched ASVs at NX (c), and WF-enriched ASVs at NX (d) of bacteria. ASVs that are significantly enriched are depicted as full circles, and others are depicted as hollow circle. The dashed line separates the significantly enriched ASVs from others. The color of each full circle represents the different taxonomic affiliations of ASVs (phylum level), and the size represents the relative abundance of each ASV. Gray boxes are used to denote different taxonomic groups (phylum level).
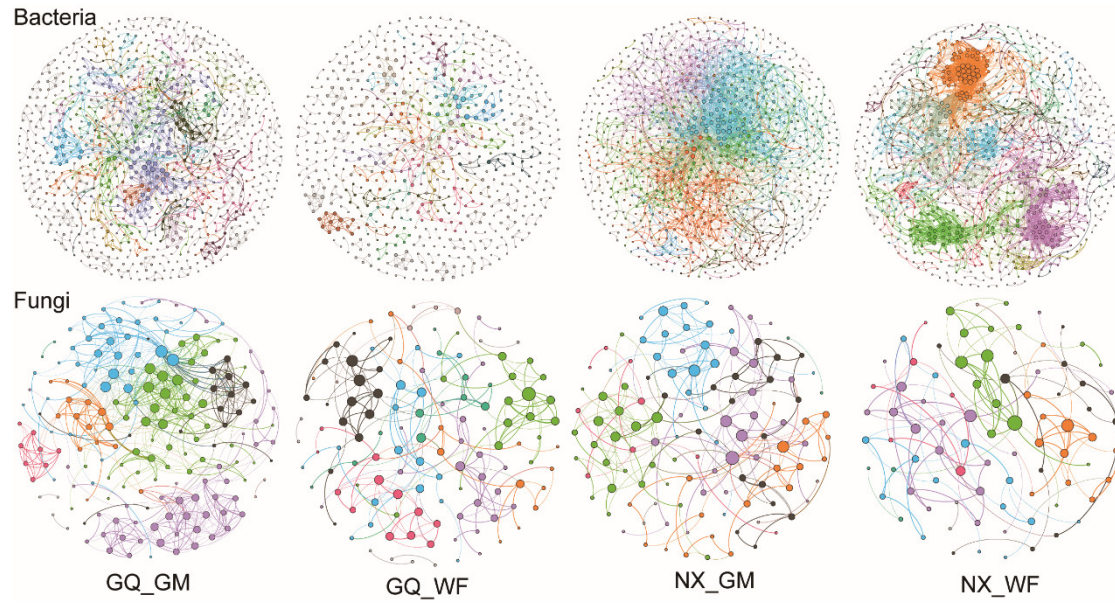
Figure S4 Manhattan plots based on the MetagenomeSeq analysis of fungal community showing GM-enriched ASVs at GQ (a), WF-enriched ASVs at GQ (b), GM-enriched ASVs at NX (c), and WF-enriched ASVs at NX (d) of bacteria. ASVs that are significantly enriched are depicted as full circles, and others are depicted as hollow circle. The dashed line separates the significantly enriched ASVs from others. The color of each full circle represents the different taxonomic affiliations of ASVs (phylum level), and the size represents the relative abundance of each ASV. Gray boxes are used to denote different taxonomic groups (phylum level).

Figure S5 Co-occurrence networks of bacterial and fungal communities. ASVs with relative abundance > 0.01% (1986 ASVs for bacteria and 1088 ASVs for fungi) are included in the construction of network. Co-occurrence networks are colored by module. The size of each node is proportional to degree. Figure S1 shows treatment abbreviations.
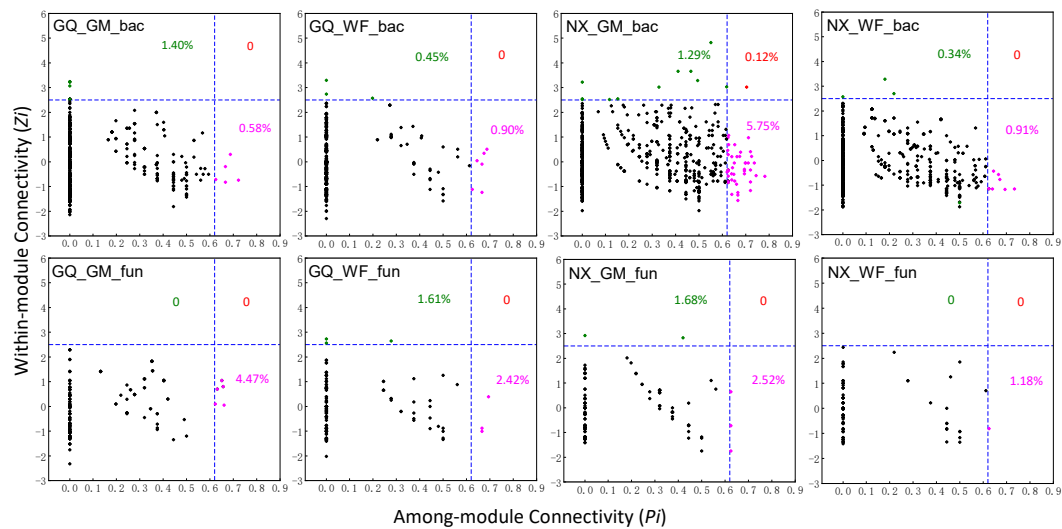
Figure S6 Network roles of analyzing feature at the ASV level determined in accordance with within- (Zi)

and among-module (Pi) connectivity values. Figure S1 shows treatment abbreviations. bac represents

bacterial network, whereas fun represents fungal network.

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