Supplementary Material for

High manure load reduces bacterial diversity and network complexity in a paddy soil under crop rotations

Haiyang Liu[a, b], Xing Huang[a], Wenfeng Tan[b], Hongjie Di[a], Jianming Xu[a], Yong Li[a]*

[a] Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Zhejiang University, Hangzhou, 310058, China

[b] Key Laboratory of Horticultural Plant Biology, The Ministry of Education, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China

This file includes:

Supplementary Table S1 and S2

Supplementary Figure S1 and S2

Table S1 Pearson correlations – R values – between alpha-diversity and edaphic variables

		Chao 1	Shannon	pH	TC	TN	AP	AK	NO_3^--N	NH_4^+-N	C/N	C/P	N/P
Rice	**Chao 1**	-	0.721[b]	0.845	0.746	0.872	0.866	0.64	0.445	0.222	0.637	0.975	0.961
	Shannon	0.115[a]	-	**0.016**	**0.001**	**0.012**	**<0.001**	0.901	0.181	0.097	0.279	**<0.001**	**0.001**
	pH	0.063	**-0.674***	-	**<0.001**	**0.004**	**<0.001**	0.742	0.061	0.135	0.18	**<0.001**	**0.001**
	TC	0.105	**-0.832****	**0.869****	-	**<0.001**	**<0.001**	0.935	0.065	0.29	0.143	**<0.001**	**0.001**
	TN	-0.052	**-0.697***	**0.766****	**0.855****	-	**0.002**	0.657	0.109	0.392	**0.001**	**0.011**	**0.047**
	AP	-0.055	**-0.872****	**0.851****	**0.959****	**0.789****	-	0.832	0.2	0.272	0.248	**<0.001**	**<0.001**
	AK	-0.151	0.04	-0.106	0.026	0.143	0.069	-	0.445	0.292	0.539	0.323	0.252
	NO_3^--N	0.244	-0.414	0.555	0.549	0.486	0.398	-0.244	-	0.222	0.283	0.119	0.16
	NH_4^+-N	0.381	-0.501	0.458	0.333	0.272	0.345	-0.332	0.381	-	0.691	0.127	0.132
	C/N	0.152	0.34	-0.415	-0.449	**-.843****	-0.362	-0.197	-0.338	-0.128	-	0.356	0.692
	C/P	0.01	**0.850****	**-.0862****	**-.0891****	**-.0704***	**-.0906****	0.312	-0.474	-0.466	0.292	-	**<0.001**
	N/P	-0.016	**0.822****	**-.0821****	**-.0846****	**-.0582***	**-.0876****	0.359	-0.433	-0.46	0.128	**0.986****	-

Continued table S1

		Chao 1	Shannon	pH	TC	TN	AP	AK	NO_3^--N	NH_4^+-N	C/N	C/P	N/P
Rape	**Chao 1**	-	**0.005**	**0.003**	**0.002**	**0.004**	**0.001**	**0.017**	0.528	**0.026**	0.351	**<0.001**	**<0.001**
	Shannon	**0.749****	-	**0.006**	**0.012**	**0.022**	**0.009**	0.17	0.625	0.089	0.494	**0.004**	**0.003**
	pH	**0.772****	**0.744****	-	**<0.001**	**0.001**	**0.001**	**0.002**	0.157	0.125	0.081	**<0.001**	**<0.001**
	TC	**0.793****	**0.694***	**0.857****	.-	**<0.001**	**<0.001**	**0.001**	0.625	**0.018**	**0.014**	**<0.001**	**<0.001**
	TN	**0.760****	**0.650***	**0.844****	**0.996****	-	**<0.001**	**0.001**	0.62	**0.025**	**0.005**	**<0.001**	**<0.001**
	AP	**0.835****	**0.712****	**0.836****	**0.972****	**0.961****	-	**0.002**	0.805	**0.009**	**0.036**	**<0.001**	**<0.001**
	AK	**0.670***	0.423	**0.797****	**0.811****	**0.814****	**0.795****	-	0.184	**0.045**	0.06	**0.004**	**0.004**
	NO_3^--N	0.203	0.157	0.436	0.158	0.16	0.08	0.411	-	0.855	0.468	0.402	0.407
	NH_4^+-N	**0.637***	0.511	0.468	**0.667***	**0.641***	**0.714****	**0.587***	-0.059	-	0.405	**0.046**	**0.044**
	C/N	-0.295	-0.219	-0.523	**-0.684***	**-0.747****	**-0.609***	-0.557	-0.232	-0.265	-	0.05	0.063
	C/P	**-0.849****	**-0.767****	**-0.944****	**-0.931****	**-0.917****	**-0.939****	**-0.766****	-0.267	**-0.585***	0.576	-	**<0.001**
	N/P	**-0.856****	**-0.774****	**-0.945****	**-0.926****	**-0.909****	**-0.936****	**-0.762****	-0.264	**-0.588***	0.551	**1.000****	-

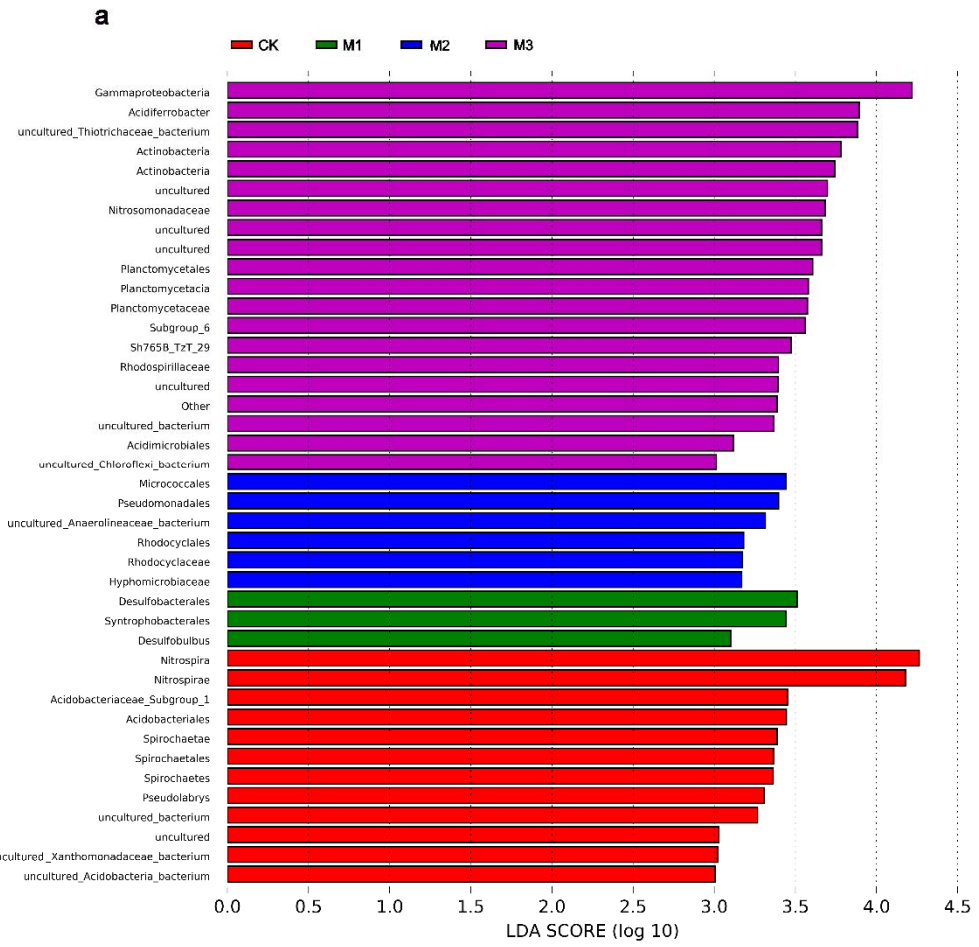
[a]Pearson correlations—r values (left-bottom half part of the table). Significant ($P < 0.05$) correlations are indicated in bold.

[b] P-values for the same correlations (right-top half of the table). Significant ($P< 0.05$) correlations are indicated in bold.

Table S2 Network topological characteristics calculated by NetworkAnalyzer tool in Cytoscape v.3.6.1.

Network topological characteristics	CK	M1	M2	M3	Rice	Rape
Number of nodes	693	1052	360	302	351	409
Number of edges	796	1022	253	218	330	645
Network density	0.003	0.002	0.002	0.005	0.005	0.008
Network heterogeneity	1.479	1.008	0.539	0.524	0.878	1.025
Clustering coefficient	0.242	0.229	0.032	0.116	0.057	0.122
Connected components	176	264	121	105	85	60
Network diameter	14	25	3	7	12	15
Network radius	1	1	1	1	1	1
Network centralization	0.092	0.035	0.013	0.012	0.026	0.039
Modularity(no. ofmodules)	0.952 (34)	0.965 (72)	0.901 (9)	0.935(13)	0.924 (7)	0.769 (11)
Shortest paths	39516	97972	6014	938	18936	72392
Characteristic path length	4.654	7.191	1.202	2.021	5.378	4.921
Avg. number of neighbors	2.297	1.943	1.406	1.444	1.880	3.154

Note: CK, M1, M2, M3 Rice and Rape correspond to Fig.7a, 7b, 7c ,7d, Fig S2a and Fig S2b, respectively.


a
CK
M1
M2
M3
Gammaproteobacteria
Acidiferrobacter
uncultured_Thiotrichaceae_bacterium
Actinobacteria
Actinobacteria
uncultured
Nitrosomonadaceae
uncultured
uncultured
Planctomycetales
Planctomycetacia
Planctomycetaceae
Subgroup_6
Sh765B_TzT_29
Rhodospirillaceae
uncultured
Other
uncultured_bacterium
Acidimicrobiales
uncultured_Chloroflexi_bacterium
Micrococcales
Pseudomonadales
uncultured_Anaerolineaceae_bacterium
Rhodocyclales
Rhodocyclaceae
Hyphomicrobiaceae
Desulfobacterales
Syntrophobacterales
Desulfobulbus
Nitrospira
Nitrospirae
Acidobacteriaceae_Subgroup_1
Acidobacteriales
Spirochaetae
Spirochaetales
Spirochaetes
Pseudolabrys
uncultured_bacterium
uncultured
uncultured_Xanthomonadaceae_bacterium
uncultured_Acidobacteria_bacterium
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
LDA SCORE (log 10)

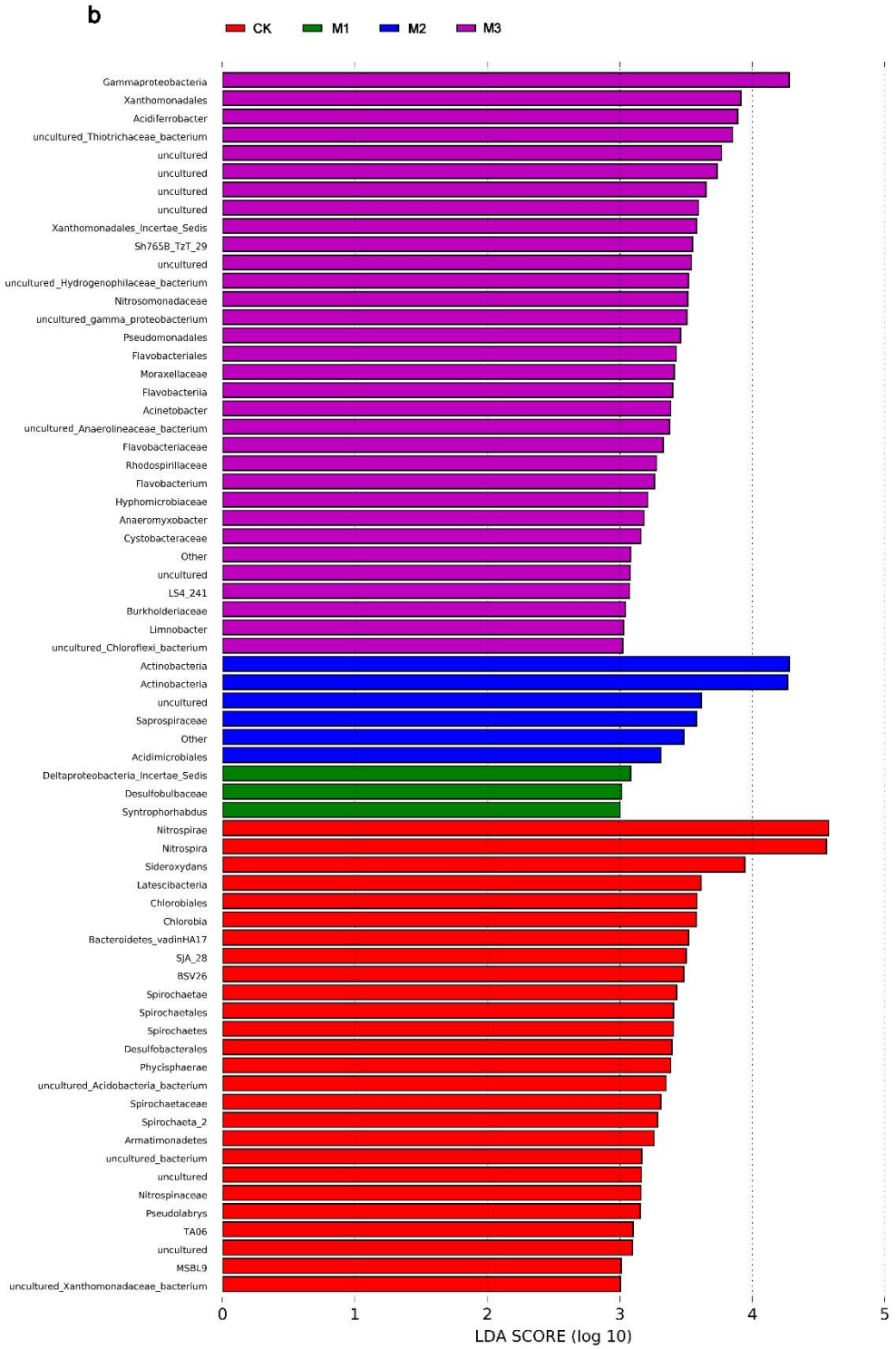


Fig. S1. Key phylotypes of bacterial communities responding to different rates of manure application in rice (a) and rape (b) seasons identified using LEfSe. The histogram shows the LDA scores computed for features (on the OTU level) differentially abundant under the four treatments. Only taxa meeting an LDA

significance threshold of 3.0 were shown.

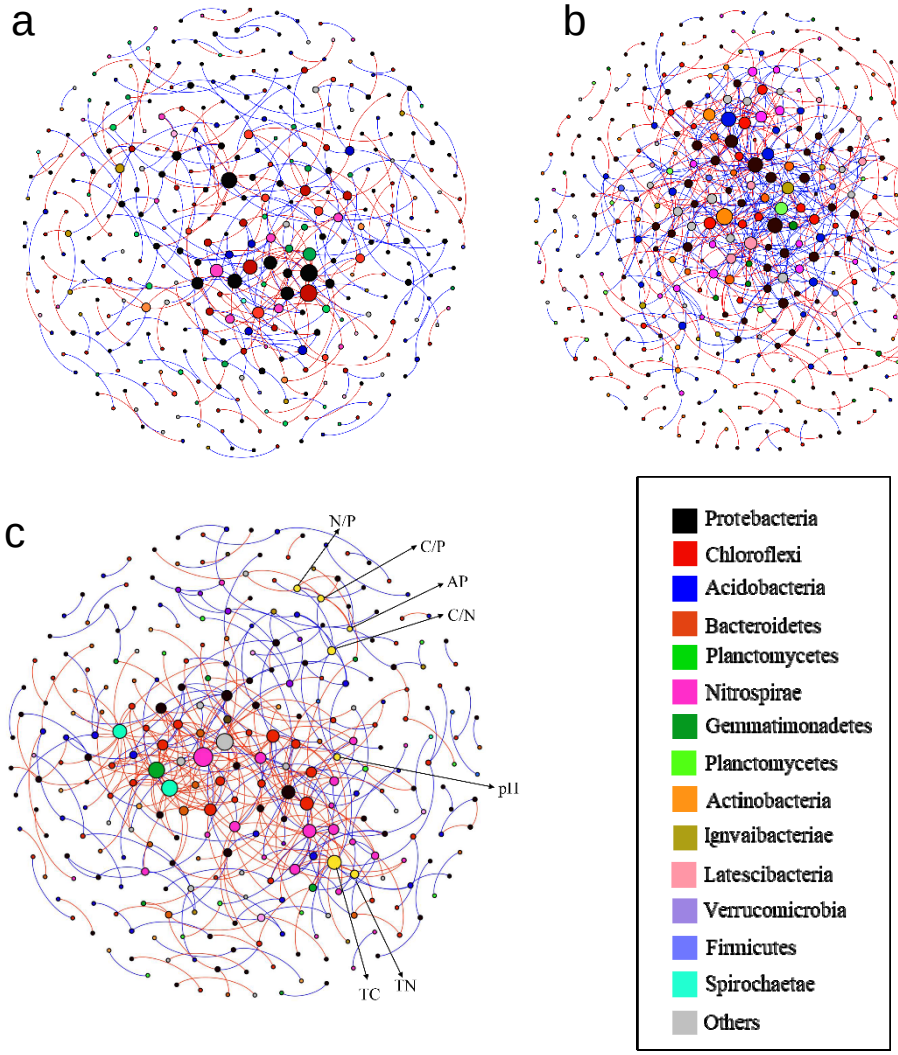


Fig. S2. Network analysis revealing the associations among bacterial OTUs in riceseason (a), rapeseason (b) and soil properties in all treatments (c). Blue line and red line represent strong positive linear ($r > 0.8$) and strong negative linear ($r <= -0.8$) and relationships, respectively. Colored nodes signify corresponding OTUs assigned to major phyla. The size of each node is proportional to the number of connections (that is, degree). The yellow nodes in represent major soil properties.