Long-term effects of mineral and manure fertilization on rice grain yield, yield stability and bacterial community in a double rice-cropping system

Jin Li[1,2#], Kai-lou Liu[3#], Jiang Xie[2], Yu Jiang[4], Guo-qiang Deng[2], Da-ming Li[3], Xian-jiao Guan[2], Xi-huang Liang[2,5], Xian-mao Chen[2,5], Cai-fei Qiu[2], Yin-fei Qian[2], Wen-jian Xia[2], Jia Liu[2], Chun-rui Peng[2], Stephen M. Bell[6], Ji Chen[7,8,9*], Jin Chen[2,5*]

[1]*Nanchang Normal University, Nanchang, 330032, China*

[2]*Soil and Fertilizer & Resources and Environment Institute, Jiangxi Academy of Agricultural Sciences / Key Laboratory of Crop Ecophysiology and Farming System for the Middle and Lower Reaches of the Yangtze River, Ministry of Agriculture and Rural Affairs / National Engineering and Technology Research Center for Red Soil Improvement/National Agricultural Experimental Station for Agricultural Environment of Yichun, Nanchang, 330200, China*

[3]*Jiangxi Institute of Red Soil/National Engineering and Technology Research Center for Red Soil Improvement, Nanchang, 330046, China*

[4] *Jiangsu Collaborative Innovation Center for Modern Crop Production/National Engineering and Technology Center for Information Agriculture/Key Laboratory of Crop Physiology and Ecology in Southern China, Nanjing Agricultural University, Nanjing, 210095, China*

[5]*Jinggangshan Institute of Red Soil / Jinggangshan Branch of Jiangxi Academy of Agricultural Sciences, Ji'an, 343016, China*

[6] *Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain*

[7] *Department of Agroecology, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark*

[8] *Aarhus University Centre for Circular Bioeconomy, Aarhus University, 8830 Tjele, Denmark.*

[9] *iCLIMATE Interdisciplinary Centre for Climate Change, Aarhus University, 4000 Roskilde, Denmark.*

Correspondence Chen Jin, Tel: +86-791-87832356, E-mail: chenjin2004777@163.com

Correspondence Chen Ji, Tel: +45-71374531, E-mail: ji.chen@agro.au.dk

 # These authors contributed equally to this study.

Supplementary information.

Table S1. Linear mixed-effects models of the effects of mineral and manure fertilization, year and their interactive effects on rice grain yield production and yield stability.

Variable	Treat	numDF	denDF	F	p
	Intercept	1	288	3732.906	<.0001
	Treat	3	6	656.155	<.0001
	Year	36	288	82.668	<.0001
Grain Yield	Treat:Year	108	288	7.676	<.0001
	Intercept	1	6	3754.533	<.0001
Yield stability	Treat	3	6	23.818	0.001
Annual grain yield					
	Intercept	1	6	6239.273	<.0001
1981	Treat	3	6	464.048	<.0001
	Intercept	1	6	3651.322	<.0001
1982	Treat	3	6	375.680	<.0001
	Intercept	1	6	7187.887	<.0001
1983	Treat	3	6	185.282	<.0001
	Intercept	1	6	243602.600	<.0001
1984	Treat	3	6	2504.350	<.0001
	Intercept	1	6	3329.482	<.0001
1985	Treat	3	6	187.695	<.0001
	Intercept	1	6	2685.992	<.0001
1986	Treat	3	6	66.113	<.0001
	Intercept	1	6	786.944	<.0001
1987	Treat	3	6	113.589	<.0001
	Intercept	1	6	2536.643	<.0001
1988	Treat	3	6	141.381	<.0001
	Intercept	1	6	1669.506	<.0001
1989	Treat	3	6	39.490	<.0001
	Intercept	1	6	4806.172	<.0001
1990	Treat	3	6	99.035	<.0001
	Intercept	1	6	11936.521	<.0001
1991	Treat	3	6	792.431	<.0001
	Intercept	1	6	243.842	<.0001
1992	Treat	3	6	78.247	<.0001
	Intercept	1	6	6238.813	<.0001
1993	Treat	3	6	79.390	<.0001
	Intercept	1	6	2777.719	<.0001
1994	Treat	3	6	96.992	<.0001
	Intercept	1	6	820.269	<.0001
1995	Treat	3	6	116.208	<.0001
1996	Intercept	1	6	6664.571	<.0001

	Treat	3	6	133.910	<.0001
	Intercept	1	6	3677.617	<.0001
1997	Treat	3	6	500.585	<.0001
	Intercept	1	6	2202.641	<.0001
1998	Treat	3	6	599.371	<.0001
	Intercept	1	6	1834.578	<.0001
1999	Treat	3	6	99.031	<.0001
	Intercept	1	6	2030.083	<.0001
2000	Treat	3	6	89.557	<.0001
	Intercept	1	6	1084.485	<.0001
2001	Treat	3	6	28.961	0.001
	Intercept	1	6	2415.187	<.0001
2002	Treat	3	6	246.450	<.0001
	Intercept	1	6	9180.642	<.0001
2003	Treat	3	6	185.903	<.0001
	Intercept	1	6	5187.608	<.0001
2004	Treat	3	6	130.523	<.0001
	Intercept	1	6	9563.534	<.0001
2005	Treat	3	6	65.385	<.0001
	Intercept	1	6	4634.341	<.0001
2006	Treat	3	6	126.818	<.0001
	Intercept	1	6	8673.201	<.0001
2007	Treat	3	6	155.352	<.0001
	Intercept	1	6	6406.287	<.0001
2008	Treat	3	6	159.365	<.0001
	Intercept	1	6	2043.649	<.0001
2009	Treat	3	6	145.909	<.0001
	Intercept	1	6	7093.342	<.0001
2010	Treat	3	6	116.179	<.0001
	Intercept	1	6	202.463	<.0001
2011	Treat	3	6	40.532	<.0001
	Intercept	1	6	6989.801	<.0001
2012	Treat	3	6	157.752	<.0001
	Intercept	1	6	3304.528	<.0001
2013	Treat	3	6	68.097	<.0001
	Intercept	1	6	2553.496	<.0001
2014	Treat	3	6	64.234	<.0001
	Intercept	1	6	1089.135	<.0001
2015	Treat	3	6	43.236	<.0001
	Intercept	1	6	2670.645	<.0001
2016	Treat	3	6	41.697	<.0001
	Intercept	1	6	1550.818	<.0001
2017	Treat	3	6	84.541	<.0001

numDF, numerator degree of freedom. denDF, denominator degree of freedom. Linear mixed-effects

models were conducted when all year observations are pooled together or separately for each year. For all observations, mineral and manure fertilization, year and their interactions were considered as fixed factors, while block and plot were considered as random factors. For observations from each year, mineral and manure fertilization was considered as fixed factors, while block and plot were considered as random factors.

Table S2. Linear mixed-effects models of the effects of mineral and manure fertilization on soil pH, soil EC, available nitrogen, available phosphorus, available potassium, ammonium, nitrate, and ammonium:nitrate.

Variable	Treat	numDF	denDF	F	p
	Intercept	1	6	31726.400	<.0001
Soil pH	Treat	3	6	10.250	0.009
	Intercept	1	6	588.000	<.0001
Soil EC	Treat	3	6	11.783	0.006
	Intercept	1	6	5200.900	<.0001
Available nitrogen	Treat	3	6	35.666	<.0001
	Intercept	1	6	8395.121	<.0001
Available phosphorus	Treat	3	6	3456.947	<.0001
	Intercept	1	6	9244.472	<.0001
Available potassium	Treat	3	6	145.946	<.0001
	Intercept	1	6	638.556	<.0001
Ammonium	Treat	3	6	83.250	<.0001
	Intercept	1	6	1318.078	<.0001
Nitrate	Treat	3	6	132.861	<.0001
	Intercept	1	6	60.802	<.0001
Ammonium:Nitrate	Treat	3	6	35.617	<.0001

numDF, numerator degree of freedom. denDF, denominator degree of freedom. Linear mixed-effects models were conducted, mineral and manure fertilization was considered as fixed factor, while block and plot were considered as random factor.

Table S3. Linear mixed-effects models of the effects of mineral and manure fertilization on microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC:MBN, soil organic carbon (SOC), soil total nitrogen (TN), and SOC:TN.

Variable	Treat	numDF	denDF	F	p
	Intercept	1	6	3770.705	<.0001
Microbial biomass carbon	Treat	3	6	107.626	<.0001
	Intercept	1	6	396.506	<.0001
Microbial biomass nitrogen	Treat	3	6	3.706	0.081
	Intercept	1	6	303.800	<.0001
MBC:MBN	Treat	3	6	8.044	0.016
	Intercept	1	6	5342.673	<.0001
Soil organic carbon	Treat	3	6	31.604	0.001
	Intercept	1	6	16905.457	<.0001
Soil total nitrogen	Treat	3	6	366.784	<.0001
	Intercept	1	6	6325.676	<.0001
SOC:TN	Treat	3	6	1.506	0.306

numDF, numerator degree of freedom. denDF, denominator degree of freedom. Linear mixed-effects models were conducted, mineral and manure fertilization was considered as fixed factor, while block and plot were considered as random factor.

Table S4. Linear mixed-effects models of the effects of mineral and manure fertilization on soil bacterial richness, diversity and community composition.

Variable	Treat	numDF	denDF	F	p
	Intercept	1	6	235.061	<.0001
OTU	Treat	3	6	2.489	0.158
	Intercept	1	6	197.466	<.0001
Chao1	Treat	3	6	2.549	0.152
	Intercept	1	6	50238.150	<.0001
Shannon	Treat	3	6	1.770	0.252
	Intercept	1	6	81508496.000	<.0001
Simpson	Treat	3	6	4.000	0.057
	Intercept	1	6	1562.572	<.0001
Proteobacteria	Treat	3	6	0.973	0.465
	Intercept	1	6	595.911	<.0001
Acidobacteria	Treat	3	6	0.157	0.921
	Intercept	1	6	1799.097	<.0001
Chloroflexi	Treat	3	6	0.657	0.608
	Intercept	1	6	174.652	<.0001
Nitrospirae	Treat	3	6	2.122	0.199
	Intercept	1	6	12.746	0.012
Firmicutes	Treat	3	6	1.540	0.298
	Intercept	1	6	116.352	<.0001
Bacteroidetes	Treat	3	6	4.285	0.062
	Intercept	1	6	221.061	<.0001
Actinobacteria	Treat	3	6	17.901	0.002
	Intercept	1	6	930.577	<.0001
Chlorobi	Treat	3	6	0.802	0.537
	Intercept	1	6	88.579	<.0001
Gemmatimonadetes	Treat	3	6	10.538	0.008
	Intercept	1	6	175.346	<.0001
Verrucomicrobia	Treat	3	6	0.299	0.825

numDF, numerator degree of freedom. denDF, denominator degree of freedom. Linear mixed-effects models were conducted, mineral and manure fertilization was considered as fixed factor, while block and plot were considered as random factors.

Table S5. Mantel analysis of the relationship between bacterial community structure and soil characteristics, grain yield and yield stability.

Variable	OTU		Shannon		Simpson		Chao1		Community	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
AN	0.080	0.279	0.021	0.413	-0.086	0.674	0.110	0.203	0.073	0.306
NH_4^+	0.006	0.419	0.174	0.187	-0.059	0.543	0.106	0.226	-0.101	0.657
NO_3^-	-0.027	0.520	0.120	0.242	0.053	0.312	0.033	0.383	-0.085	0.653
NH_4^+:NO_3^-	0.055	0.336	0.140	0.228	-0.060	0.520	0.161	0.165	-0.108	0.637
AP	0.186	0.118	0.162	0.147	-0.111	0.769	0.235	0.057	-0.027	0.523
AK	0.171	0.131	0.145	0.164	-0.043	0.553	0.143	0.149	-0.081	0.286
MBC	0.094	0.221	0.157	0.141	-0.039	0.544	0.111	0.182	0.167	0.126
MBN	0.425	0.023	0.532	0.009	-0.157	0.804	0.282	0.050	0.016	0.400
MBC:MBN	0.077	0.611	-0.054	0.547	-0.200	0.915	0.032	0.368	0.006	0.436
pH	-0.032	0.550	-0.203	0.979	-0.143	0.900	0.078	0.237	0.148	0.138
EC	0.115	0.257	0.101	0.278	-0.120	0.705	0.098	0.243	-0.100	0.641
SOC	0.283	0.033	0.102	0.210	-0.040	0.547	0.262	0.039	0.174	0.102
TN	0.228	0.076	0.181	0.123	-0.062	0.612	0.248	0.049	0.096	0.247
SOC:TN	0.072	0.303	-0.220	0.884	0.245	0.120	-0.134	0.817	0.543	0.006
Yield	0.393	0.019	-0.107	0.693	-0.052	0.556	0.354	0.013	0.118	0.235
Yield stability	0.228	0.095	-0.149	0.803	0.236	0.083	0.236	0.083	-0.057	0.579

AN, available nitrogen. AP, available nitrogen. AK, available nitrogen. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen. SOC, soil organic carbon. TN, total nitrogen.

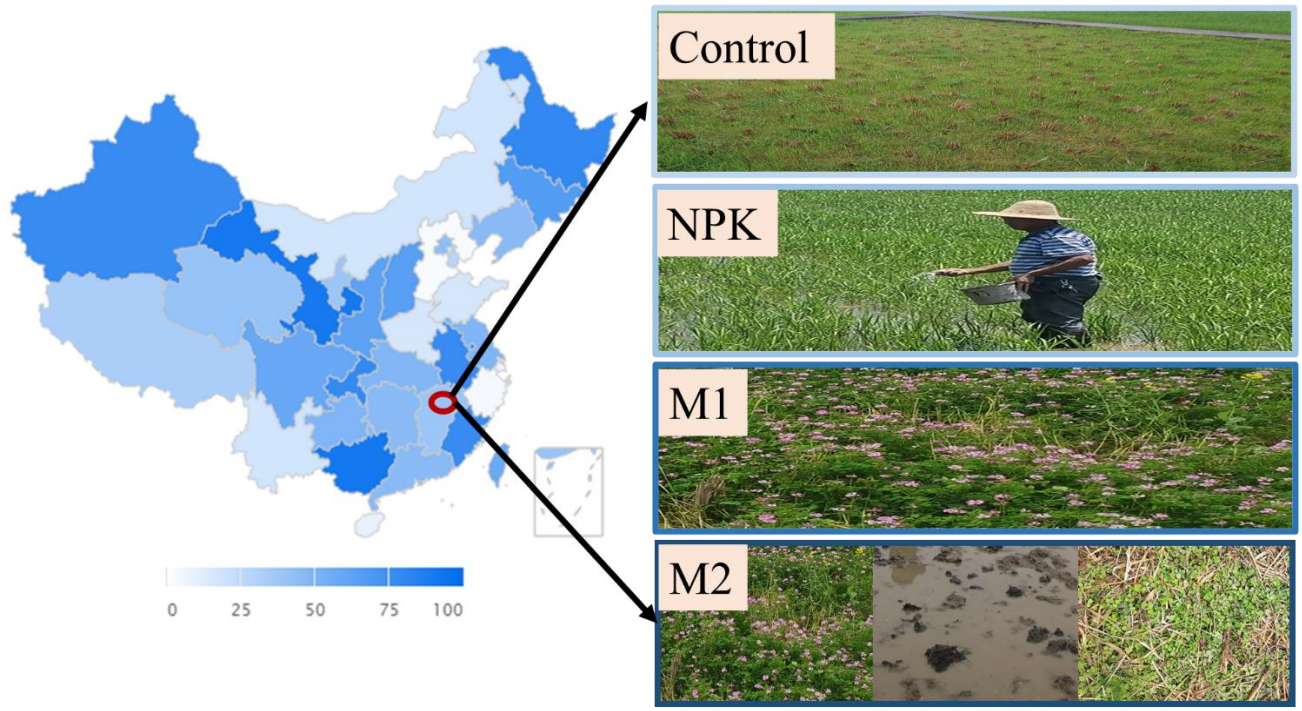

Figure S1. The study site and the experimental design.

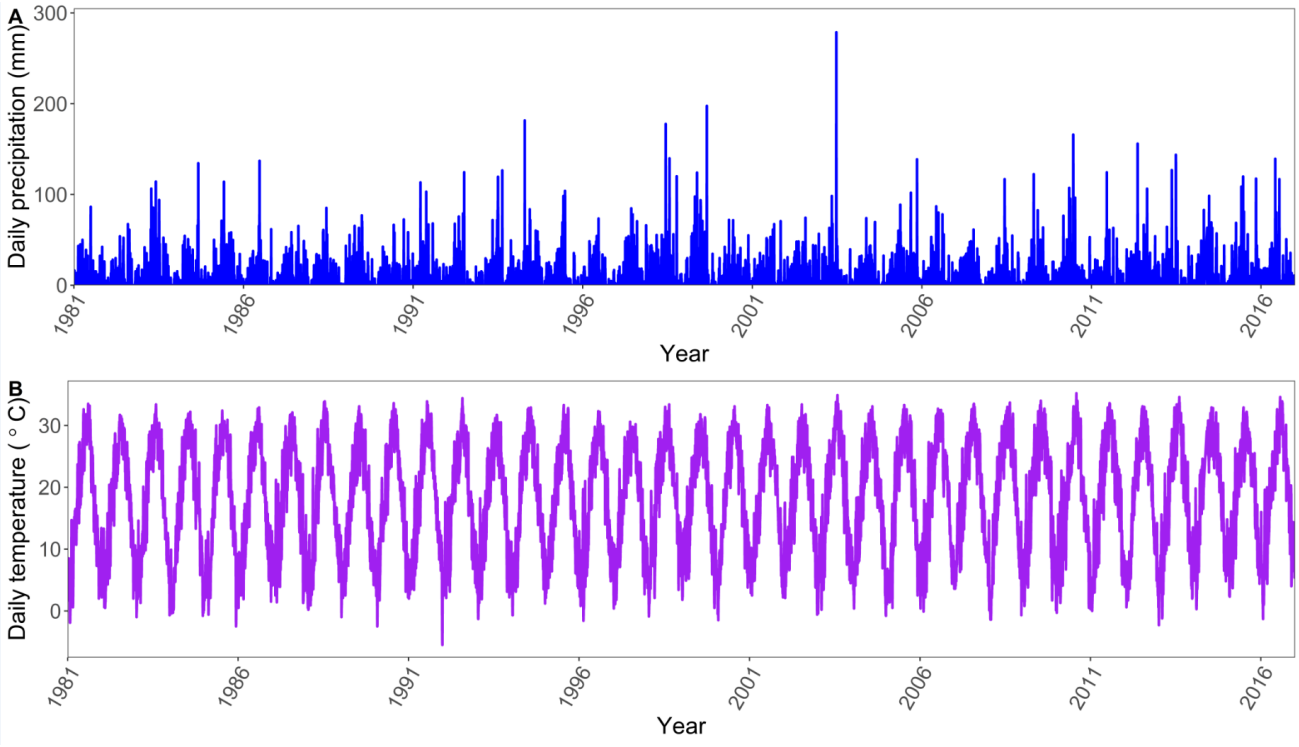

Figure S2. Daily (A) precipitation and (B) temperature at the study site from 1981 to 2017.

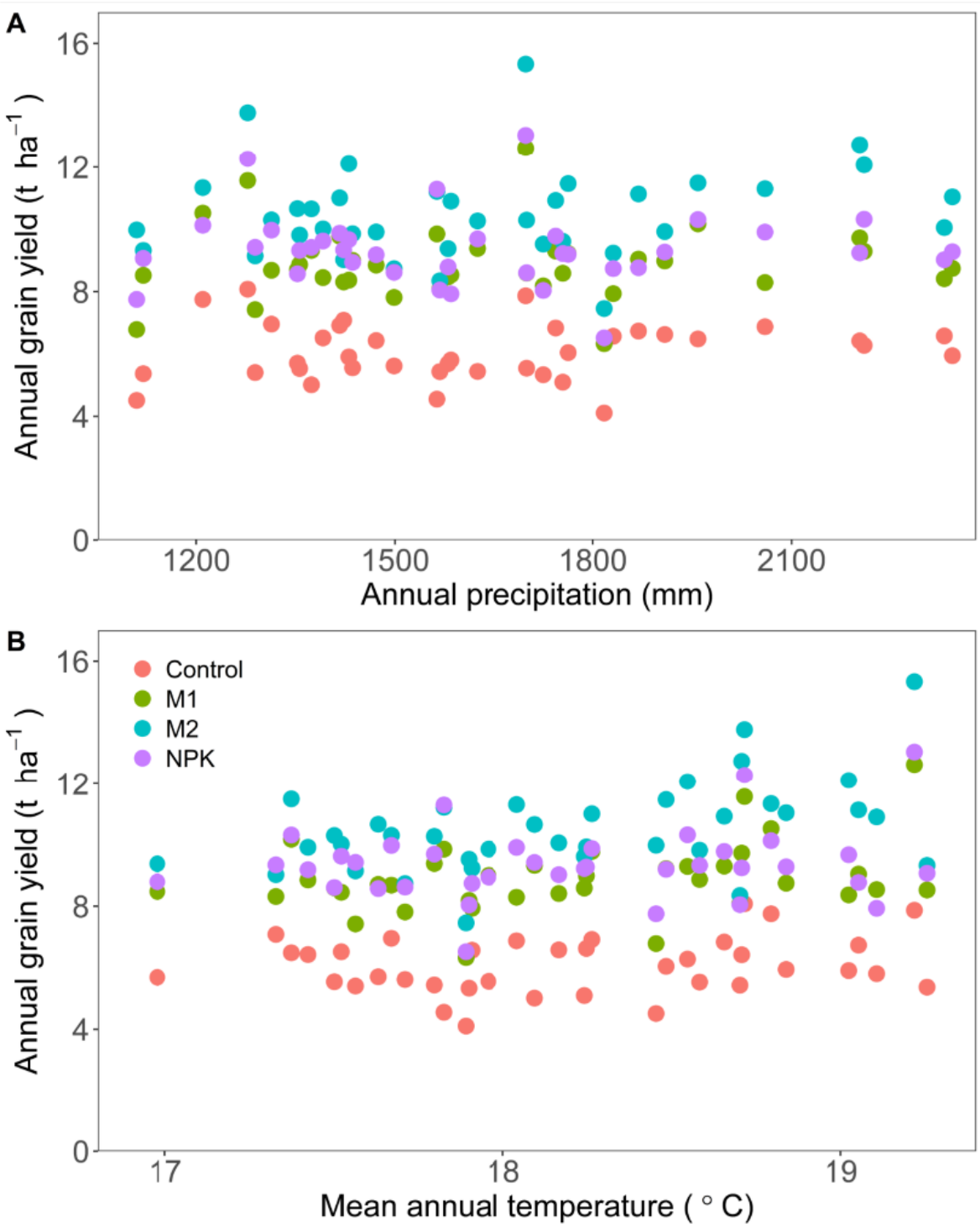

Figure S3. Relationship between annual grain yield and (A) annual precipitation and (B) mean annual temperature. There were four treatments, no application of fertilizer (Control), application of nitrogen-phosphorus-potassium fertilizer in early rice (NPK), NPK plus green manure in early rice (M1), NPK plus green manure in early rice and farmyard manure in late rice and rice straw return in winter (M2).

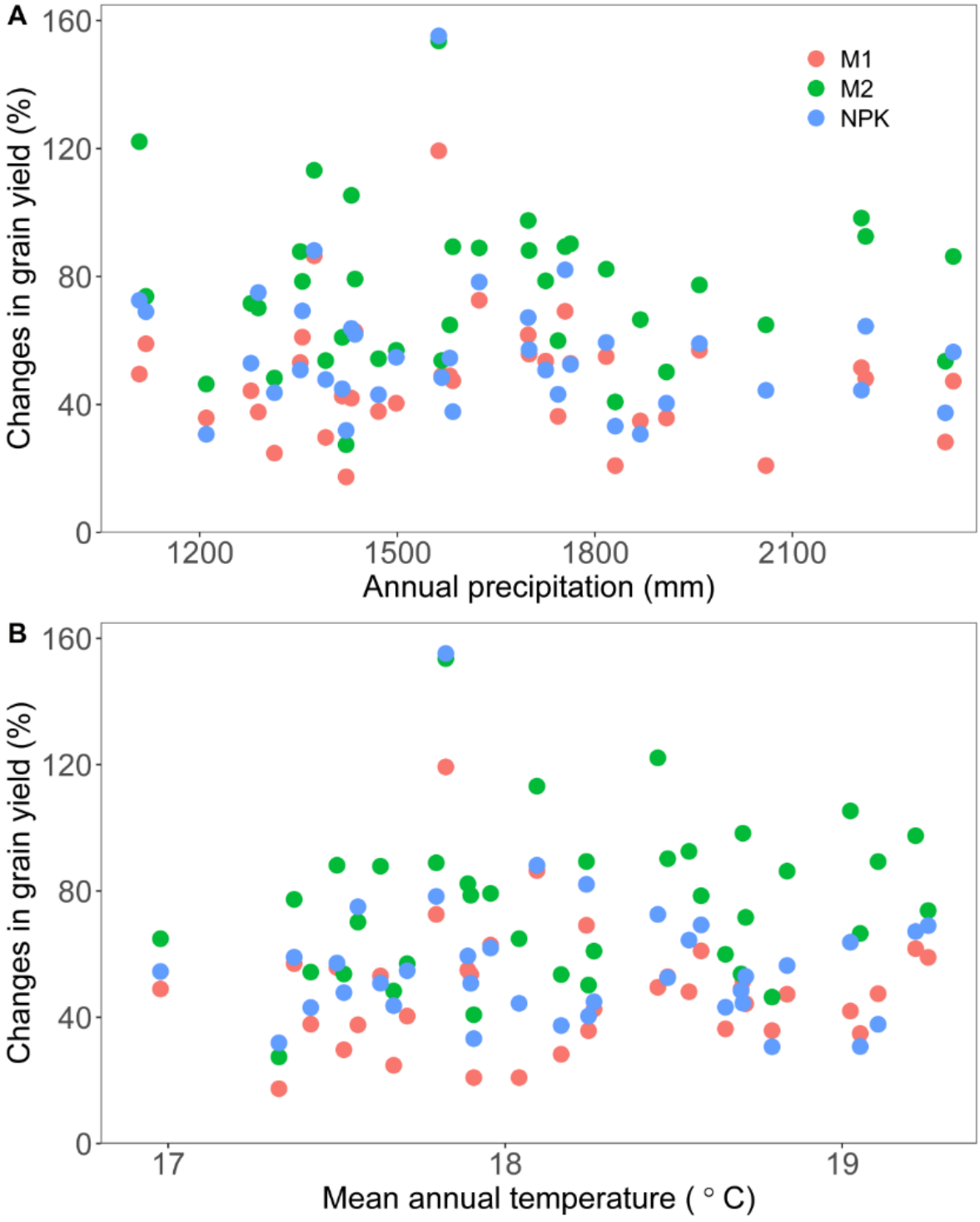

Figure S4. Relationship between fertilization-induced changes in annual grain yield and (A) annual precipitation and (B) mean annual temperature. There were four treatments, no application of fertilizer (Control), application of nitrogen-phosphorus-potassium fertilizer in early rice (NPK), NPK plus green manure in early rice (M1), NPK plus green manure in early rice and farmyard manure in late rice and rice straw return in winter (M2).

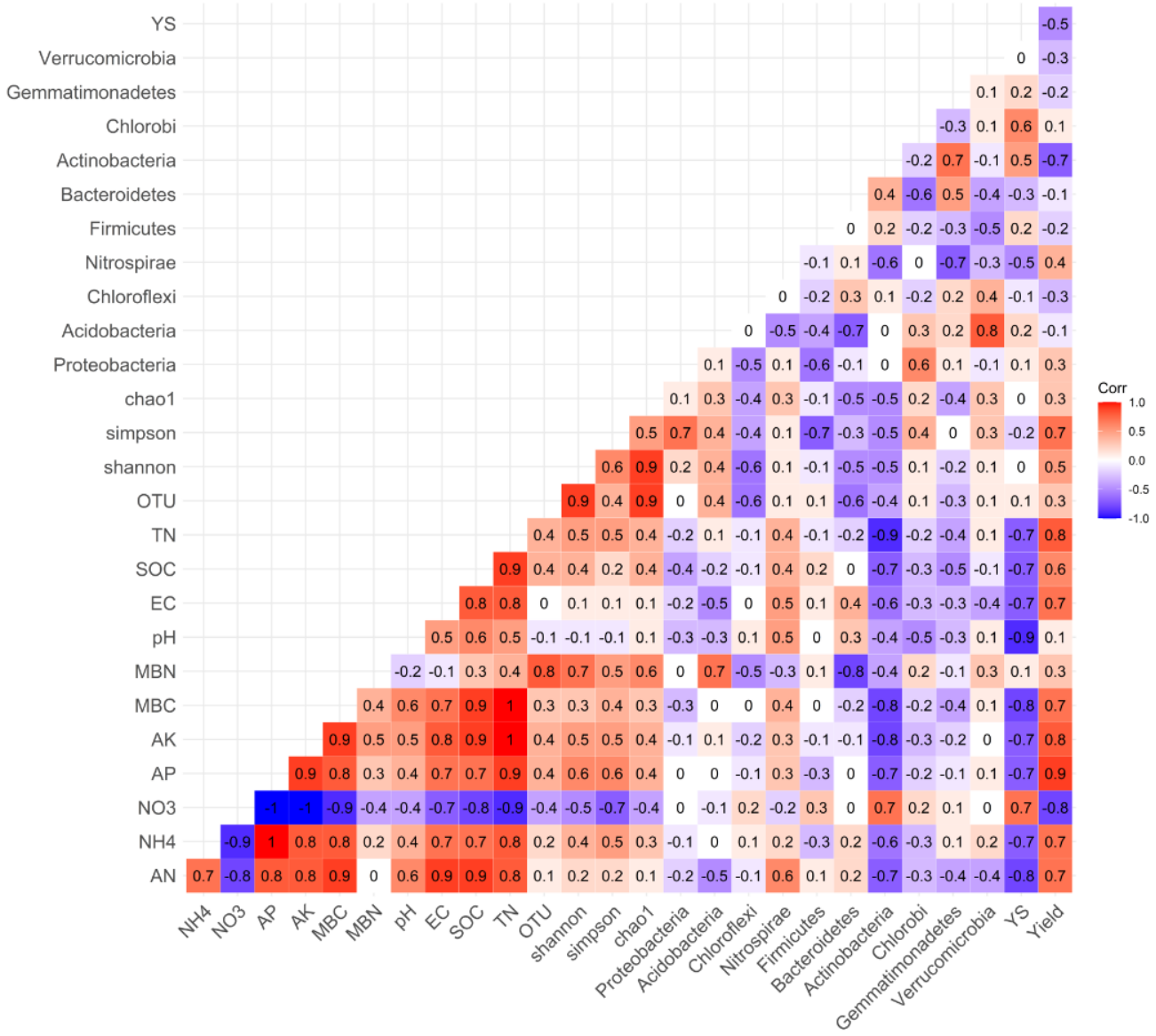

Figure S5. Correlations between mineral- and manure-induced changes between the studied variables. AN, available nitrogen. AP, available nitrogen. AK, available nitrogen. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen. SOC, soil organic carbon. TN, total nitrogen. YS, yield stability.