Organic-inorganic fertilization builds higher stability of soil and root microbial networks than exclusive mineral or organic fertilization

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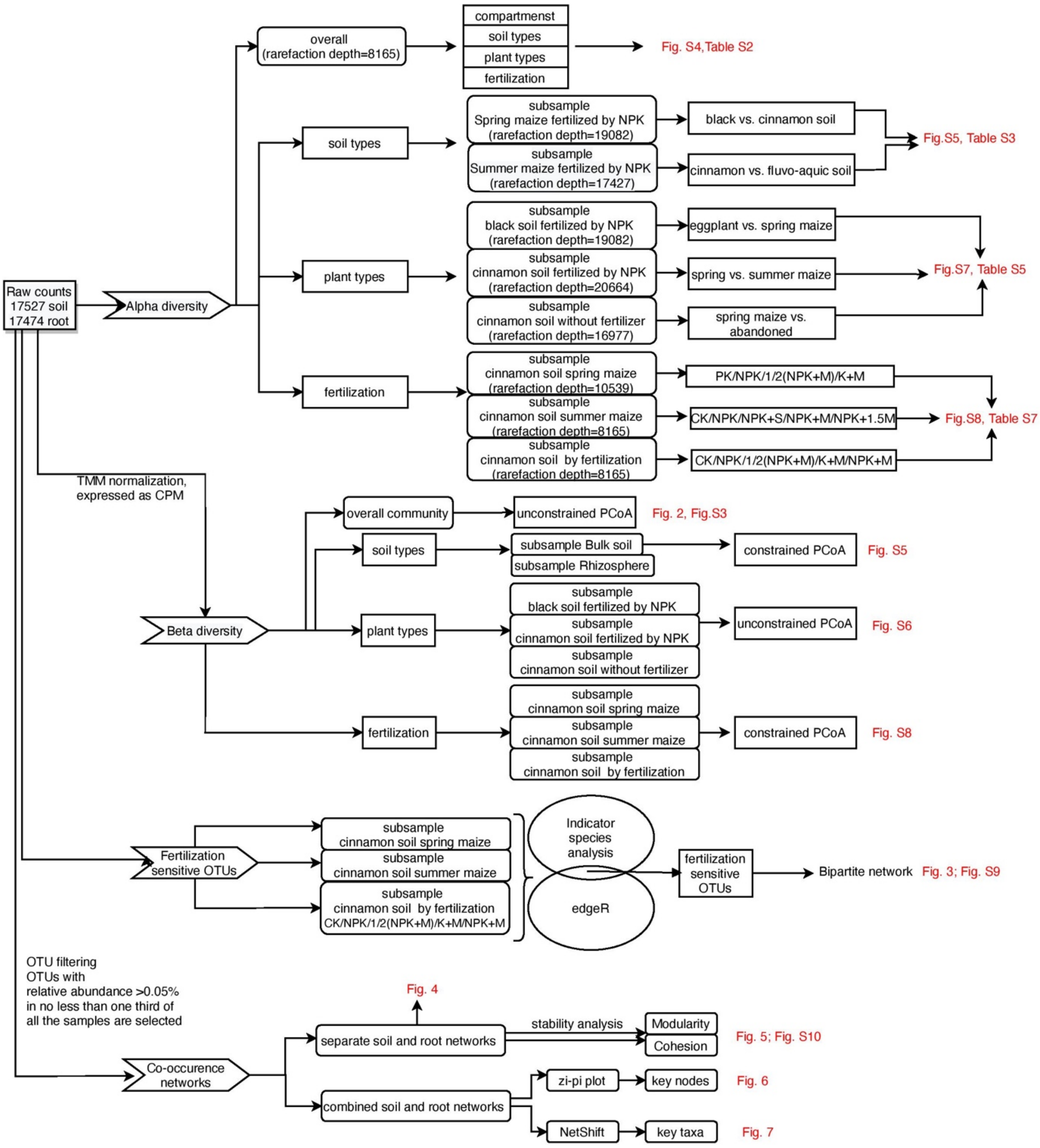

Fig. S1 Schematic flow diagram of analysis steps in this study. The figures and tables generated as the output from each step are indicated in red.

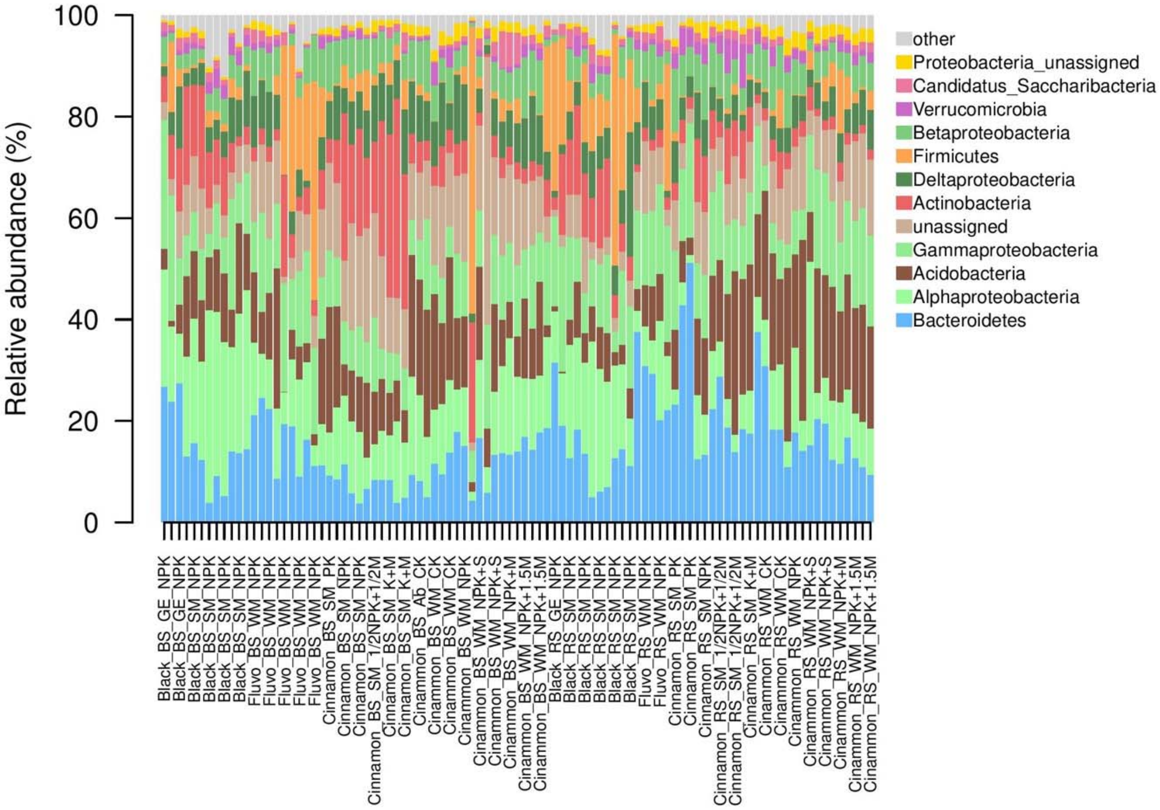

Fig. S2 Taxonomic profiles of bacteria communities at phylum level. Bacteria phyla with relative abundances lower than 1% were summarized with ‘other’. (BS: bulk soil; RS: rhizosphere; GE: green eggplant; SM: spring maize; WM: wheat maize rotation; Ab: fallow fields)

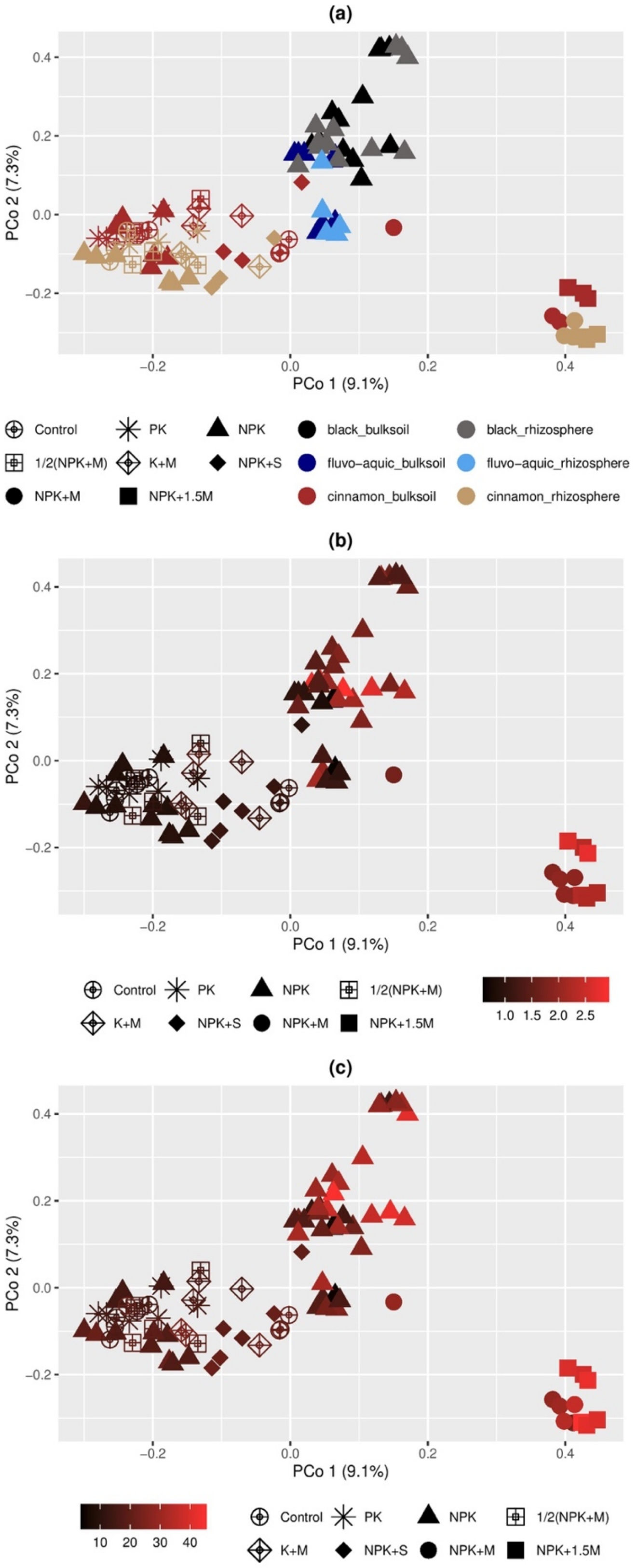

Fig. S3 Unconstrained PCoA ordinations of bacteria. Percentage of variation given on each axis refers to the explained fraction of total variation in the community. Symbols refer to the different fertilization treatments. Figure (a) are colored by soil types and compartments. Figure (b) and (c) are colored by the total nitrogen (TN) values and soluble organic matter (SOM) values respectively.

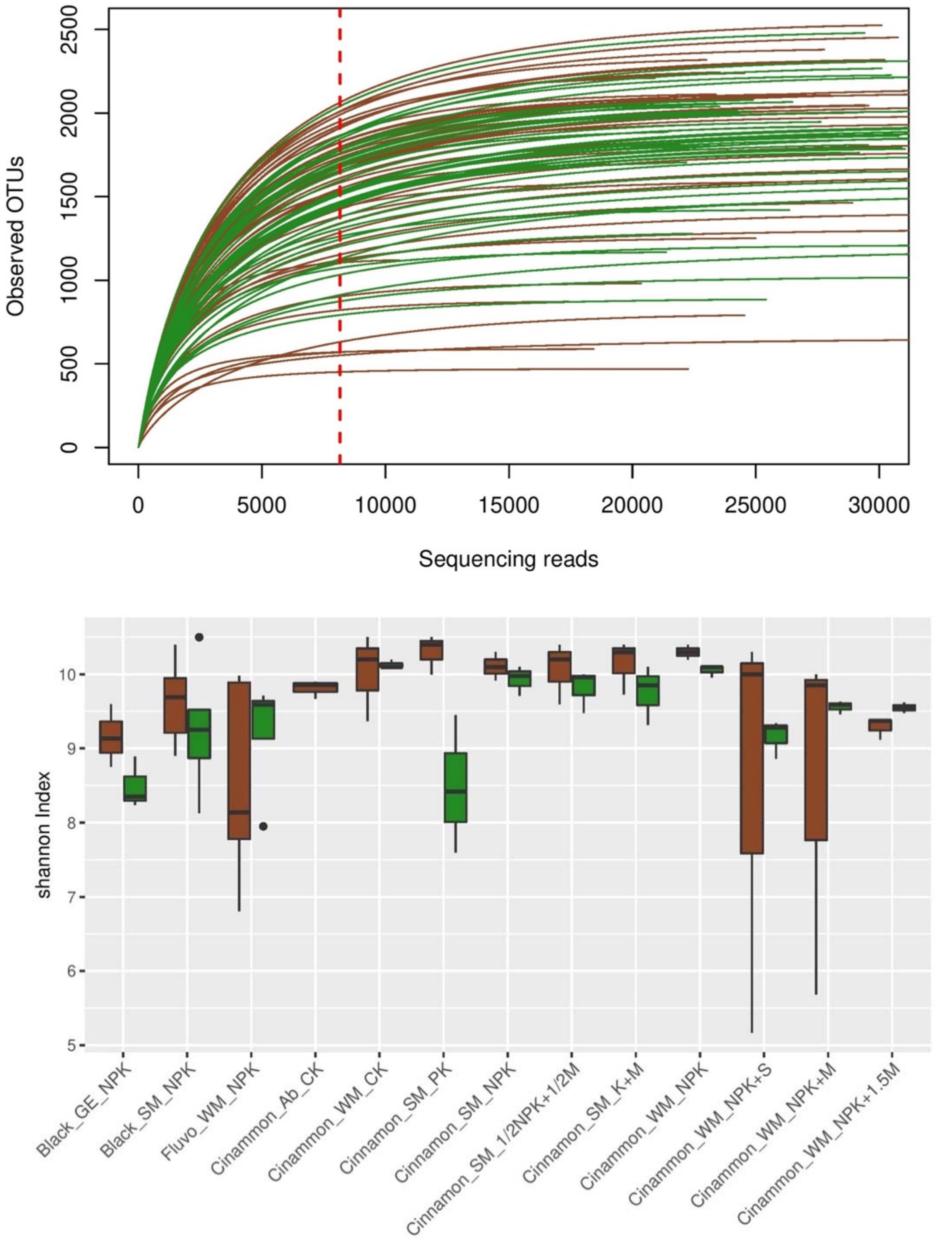

Fig. S4 a).Rarefaction curves for observed OTU richness. Brown lines indicate soil samples, and green lines indicate root samples. The dashed red line indicates the selected rarefaction depth used to generate the box plots below the curve, 8165 sequences per sample. b).The boxplots show the Shannon index at the rarefaction depth. X axis labels indicate the soil type, plant type and fertilization treatment of each box, which are colored by compartment. Results of the statistical tests are given in Table S2. (GE: Greenhouse Eggplant; SM: Spring Maize; WM: Wheat Maize rotation; Ab: Fallow fields)

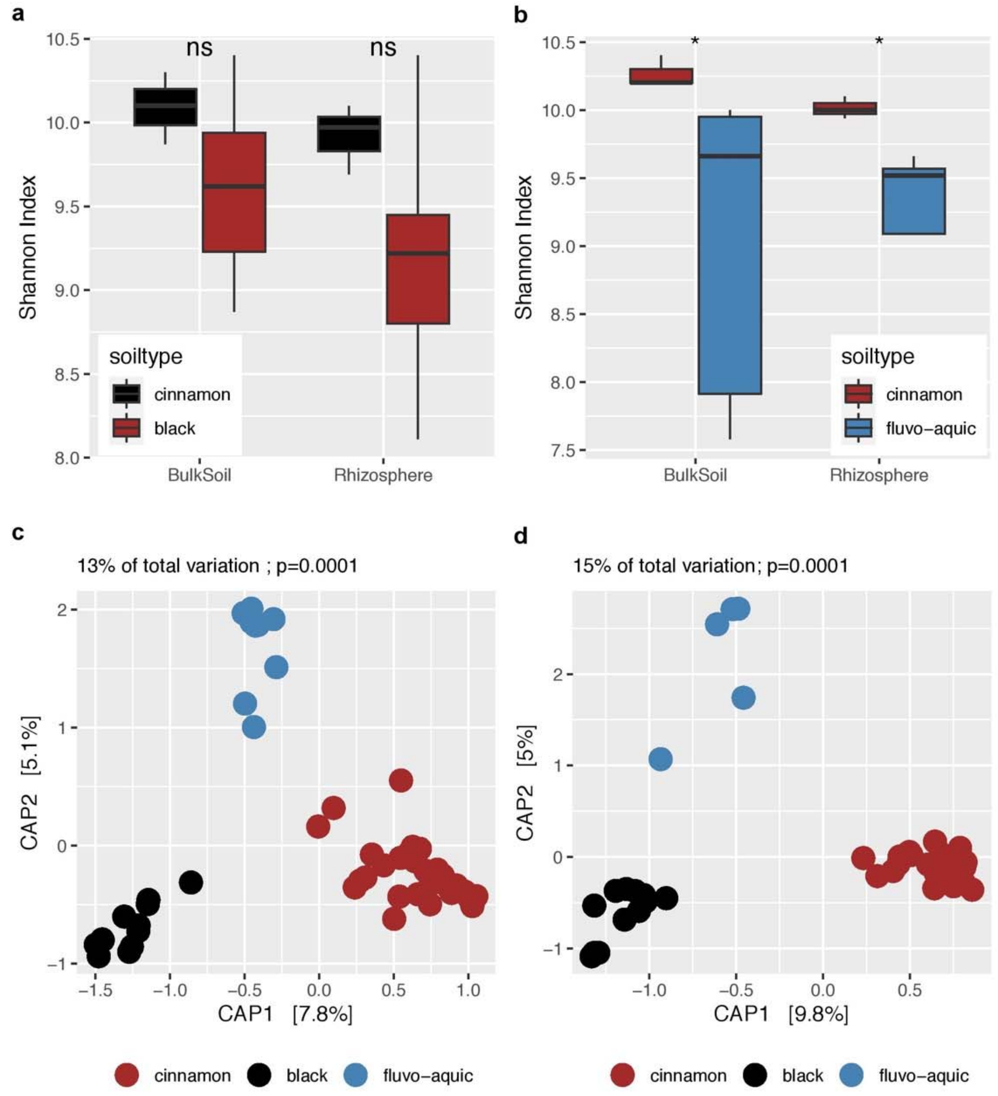

Fig. S5 The alpha diversity and beta diversity of soil types in soil and root samples. The selected samples in each figure were with the same crops and fertilization treatments. Samples were rarified at the depth of the minimum sequences within the samples for alpha diversity comparison (ns: not significant, *:$p<0.05$). CAP analyses were constrained by the factor “soil types”. The explained fraction of the total variance is indicated above the plots. Percentage of variation given on each axis refers to the explained fraction of total variation.

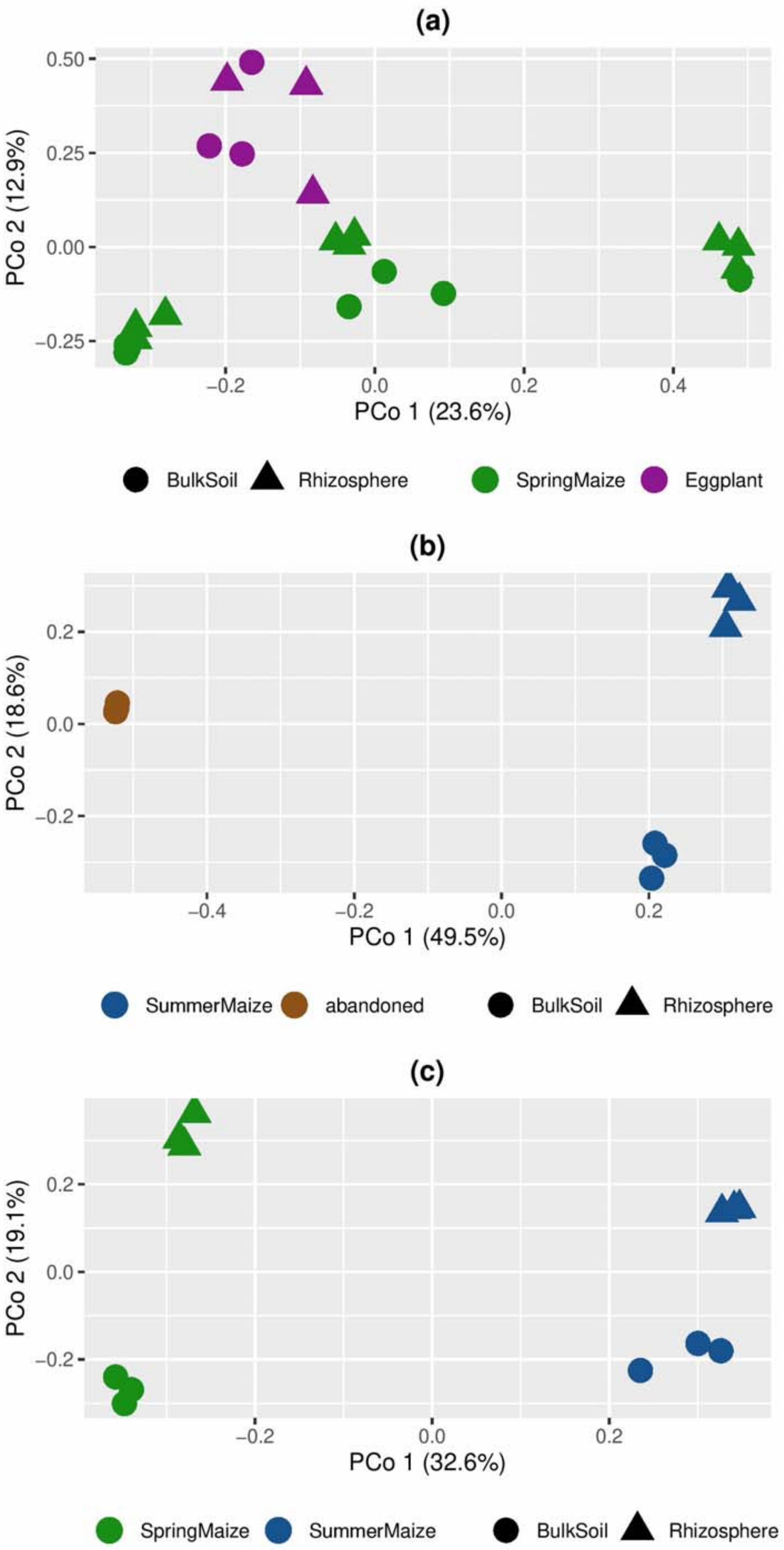

Fig. S6 PCoA plots depicted the plant type effects on soil and root bacterial communities.

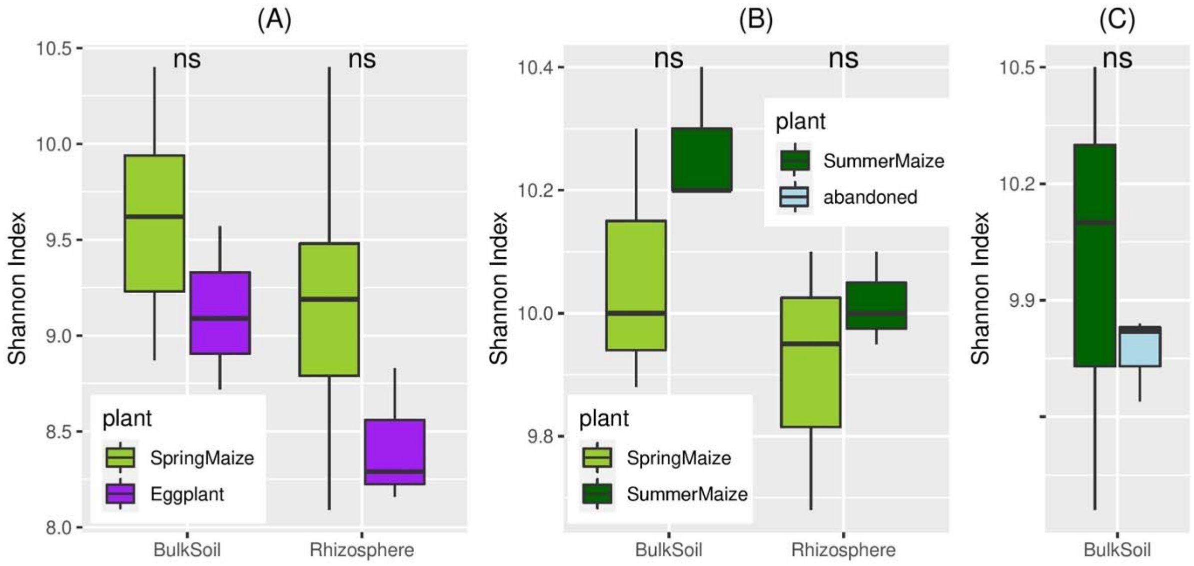

Fig. S7 The comparisons of alpha diversity (Shannon index) between different plant types. The selected samples in each figure were with the same soil type and fertilization treatments. Samples were rarified at the depth of the minimum sequences within the samples.

(A) Soil and root samples from black soil under NPK fertilization. Samples were rarified at 19082 sequences. (B) Soil and root samples from cinnamon soil under NPK fertilization. Samples were rarified at 20664 sequences. (C) Soil samples from cinnamon soil without fertilization. Samples were rarified at 16977 sequences. The detailed statistical results were shown in Table S5. (ns: not significant)

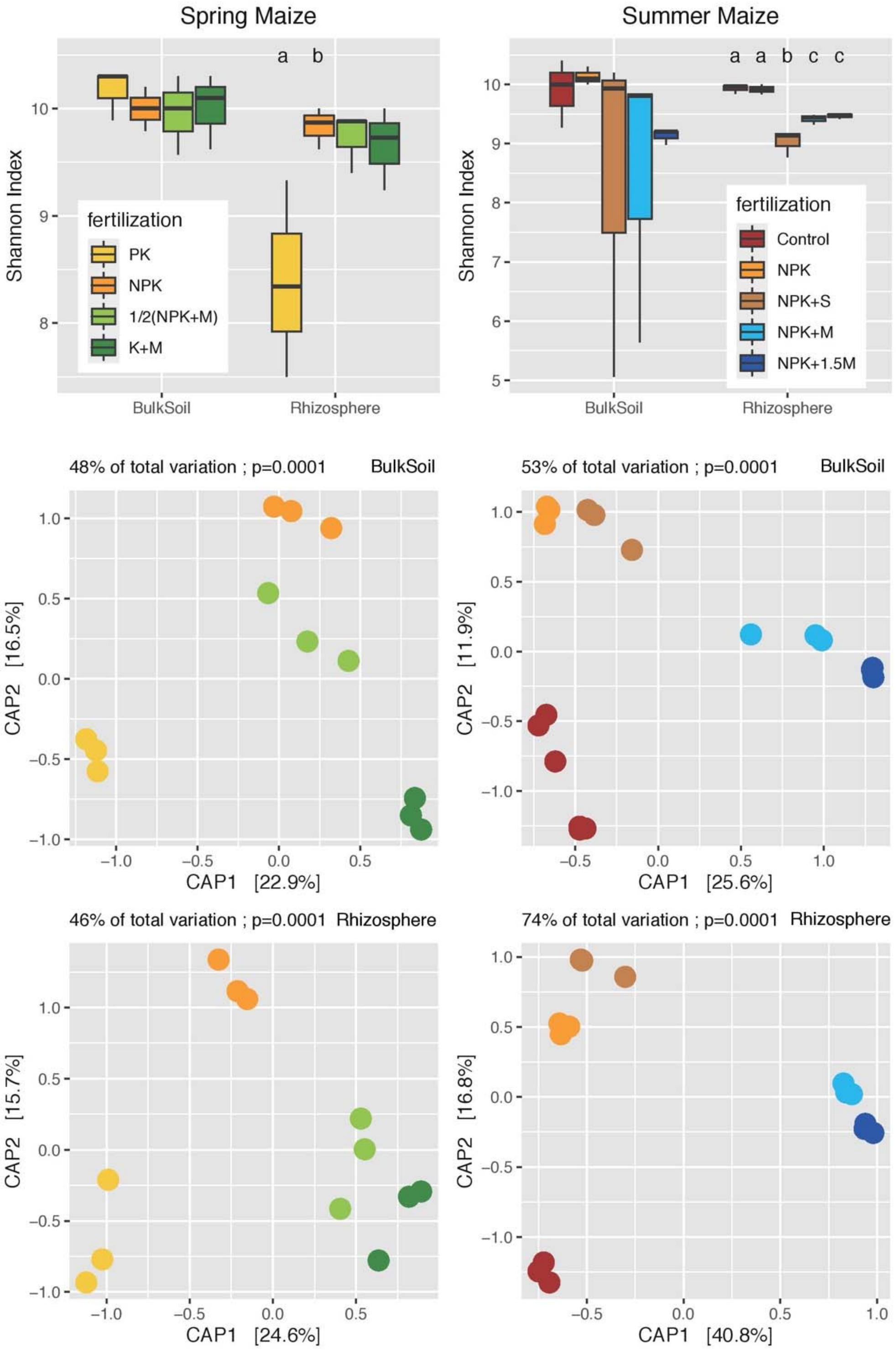

Fig. S8 (C)The alpha diversity and beta diversity of fertilization treatment in soil and root samples. Different letters indicate the groupings of pairwise comparison. Only groups of significant results were labeled. CAP analyses were constrained by the factor “fertilization”. The explained fraction of the total variance is indicated above the plots. Percentage of variation given on each axis refers to the explained fraction of total variation.

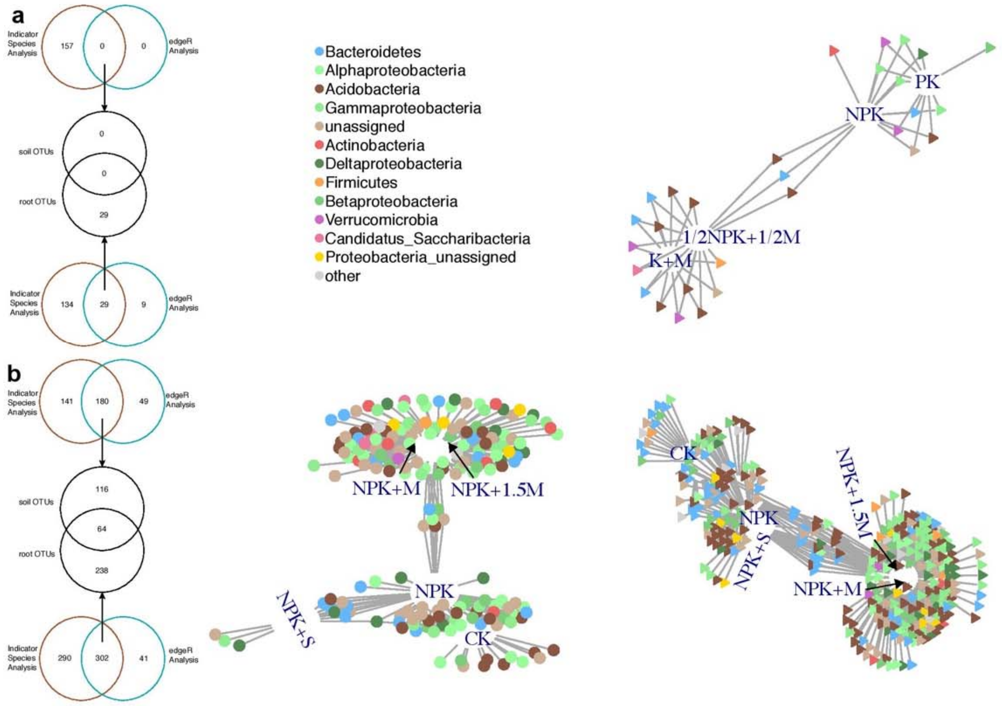

Fig. S9 Defining fertilization sensitive bacteria OTUs in soil and root samples with (a) spring maize, (b) summer maize under different fertilization treatments. Venn diagrams on the left panel showed the number of OTUs responding to fertilization treatments identified with indicator species analysis (brown) and by edgeR (cyan). OTUs identified by both methods (overlap) were defined as fertilization sensitive OTUs. Bipartite networks display fertilization system specific OTUs in the soil and root bacterial communities determined by both methods in the Venn diagram. Circles represent soil bacteria OTUs and triangles represent root bacteria OTUs. OTUs are colored according to their Phylum assignment.

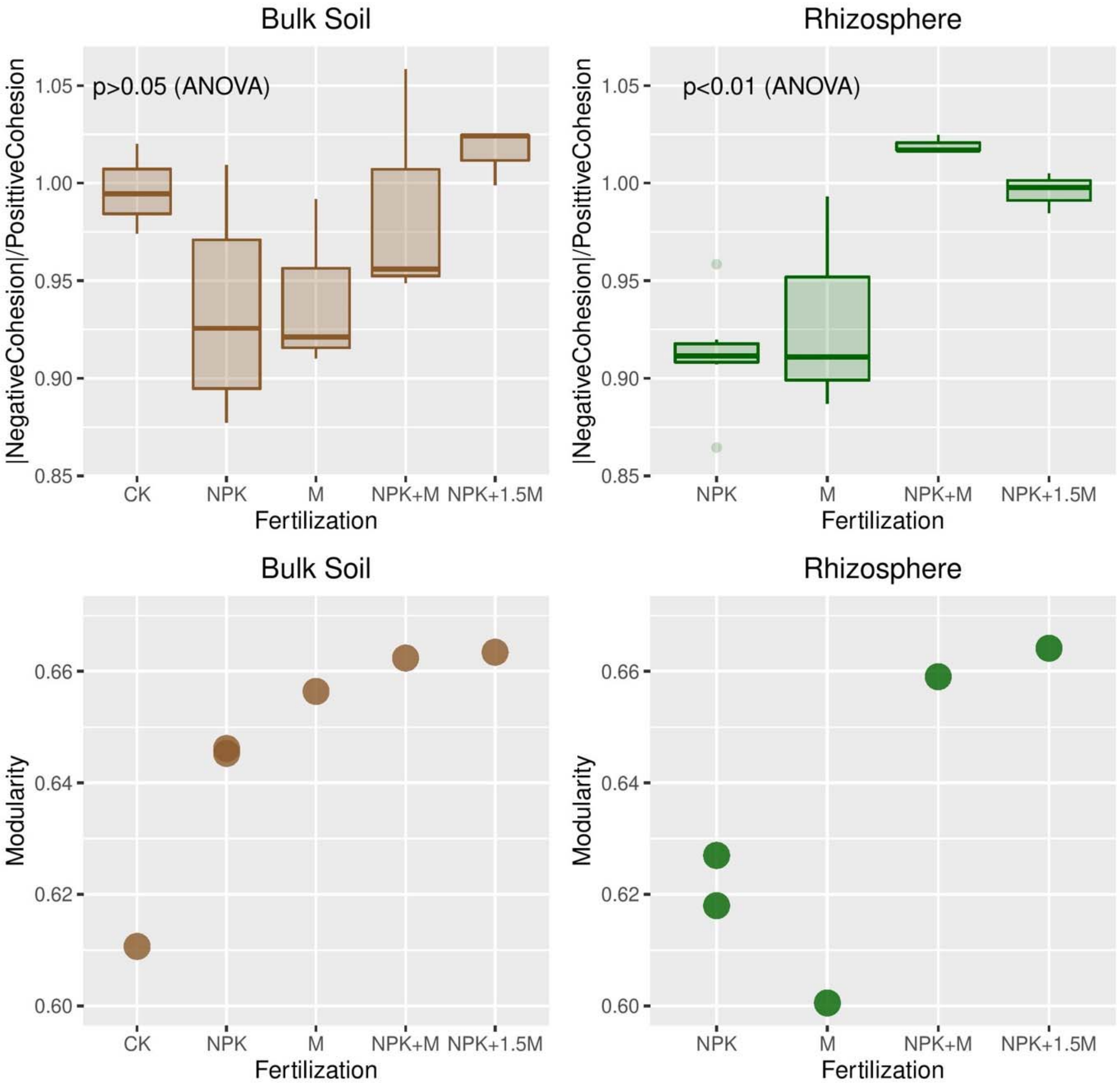

Fig. S10 Characterizing network properties different fertilization regimes. A single value of modularity was calculated for each environment. Box plots show inner quartiles and median negative: positive cohesion.

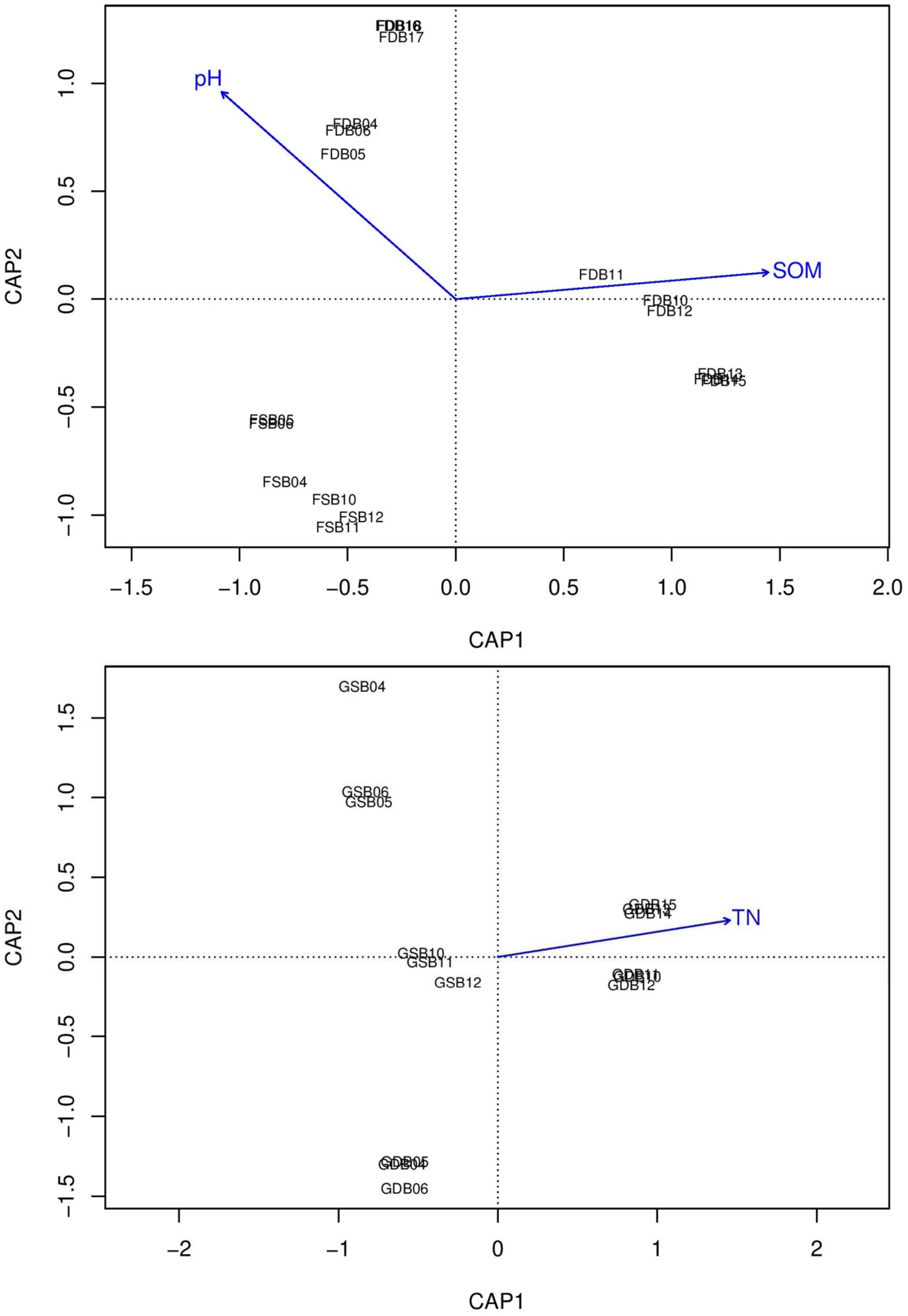

Fig. S11 Canonical correlation analysis of environmental factors responsible for differences of soil and root microbiome under varied fertilization regimes.

SUPPLEMENTARY TABLES

Table S1 Results of PERMANOVA testing the effects of compartment, soil type, plant type and fertilization on bacterial communities.

	F	R^2	P value
Compartment	3.73	0.02	0.0001
Soil type	6.31	0.04	0.0001
Plant type	4.85	0.10	0.0001
Fertilization	4.76	0.22	0.0001

Table S2 Statistic testing results for differences in α-diversity (Shannon index) of bacterial communities in different samples. The soil type effects were tested both in combine and in soil and root separately. Similarly, the fertilization effects were tested in both combine and the two compartments respectively. Fertilization effects were further tested in the cinnamon soil type combined and separately. In addition, fertilization were also tested in the two cropping systems with spring maize and summer maize (wheat maize rotation). Significant effects are indicated in bold (*p<0.05, ***p<0.001).

	Soil	**Root**	
Mean ± SEM	9.35 ± 0.16	9.27± 0.10	
Factors			Methods
Compartment	**W = 1424***		Wilcox
Soil type	**F_{total}=4.58***		ANOVA
	F_{soil}=2.89	**F_{root}=3.51***	
Fertilization	F_{total}=1.53		
	F_{soil}=1.25	F_{root}=2.19	
	$F_{cinnamon}$=1.91		
	$F_{cinnamon\ soil}$=1.24	**$F_{cinnamon\ root}$=7.05*****	
	chi-squared$_{cinnamon\ springmaize}$ = 0.39		Kruskal-Wallis
	$F_{cinnamon\ springmaize\ soil}$=0.30	**$F_{cinnamon\ springmaize\ root}$=5.13***	ANOVA
	$F_{cinnamon\ summermaize}$=1.62		
	$F_{cinnamon\ summermaize\ soil}$=0.67	**$F_{cinnamon\ summermaize\ root}$=26.77*****	
			Pairwise comparison
	Soil	**Root**	
Soil types	Mean ±SEM	Mean ±SEM	
black soil	9.39±0.14 a	**8.93±0.18 ab**	
fluvo-aquic soil	8.57±0.41 a	9.05±0.31 a	
cinnamon soil	9.56±0.22 a	**9.46±0.11 ac**	
Fertilization			
PK	10.11±0.14 a	**8.35±0.52 ab**	Spring Maize
NPK	9.92±0.10 a	**9.76±0.12 ac**	
1/2NPK+1/2M	9.91±0.19 a	9.67±0.15 a	
K+M	10.00±0.21 a	9.63±0.21 a	
Control	9.90± 0.32 a	**9.93±0.02 a**	Summer Maize
NPK	10.13±0.09 a	**9.90±0.06 a**	
NPK+S	8.38±1.68 a	**9.03±0.14 b**	
NPK+M	8.43±1.40 a	**9.39±0.05 c**	
NPK+1.5M	9.15±0.07 a	**9.46±0.02 c**	

Table S3 Results of PERMANOVA testing the effects of soil types on soil and root bacterial communities. Significant effects are indicated in bold (*p<0.05, ***p<0.001). Different letters indicate the groupings of pairwise comparison.

	soil		root	
PERMANVOA test	F	R^2	F	R^2
soil type	__3.53***__	**0.13**	__3.57 ***__	**0.15**
Pairwise Comparisons of soil type				
	black (a)		black (a)	
	fluvo-aquic (b)		fluvo-aquic (b)	
	cinnamon (c)		cinnamon (c)	

Table S4 Statistic testing results for differences in α-diversity (Shannon index) of bacterial communities between different soil types. Soil type effects were tested in the two cropping systems respectively (spring maize and summer maize (wheat maize rotation)). Significant effects are indicated in bold (*: p<0.05). (t indicates t-test, w indicates wilcox test)

Groups		soil		root	
Cropping system	soil type	Mean± SEM	comparison results	Mean± SEM	comparison results
Spring Maize	black	9.60±0.16	t=1.68	9.20±0.21	t=1.90
	cinnamon	10.09±0.12		9.92±0.12	
Summer Maize	fluvo-aquic	9.00±0.43	__w=0*__	9.14±0.33	__w=0*__
	cinnamon	10.27±0.07		10.01±0.05	

Table S5 Results of PERMANOVA testing the effects of plant types on soil and root bacterial communities. (*p<0.05, ***p<0.001).c

soil types	plant types	PERMANOVA tests	
black soil	spring maize vs. eggplants in greenhouse	F	R^2
		__3.16***__	**0.13**
cinnamon soil	spring maize vs. summer maize	__4.78**__	**0.32**
	summer maize vs. abandoned (no crops)	__6.75*__	**0.49**

Table S6 Statistic testing results of α-diversity (Shannon index) comparison of bacterial communities between different plant types. Significant results are indicated in bold (* : p<0.05, t indicates t-test). In this table, none of the testing results are significant.

		soil		root	
soil types	Plant types	Mean± SEM	comparison results	Mean± SEM	comparison results
black	spring maize	9.60±0.16	t=1.53	9.20±0.21	t=1.95
	eggplant	9.13±0.25		8.43±0.21	
cinnamon	spring maize	10.06±0.12	t=1.46	9.91±0.12	t=0.82
	summer maize	10.27±0.09		10.02±0.04	
cinnamon	summer maize	9.99±0.33	t=0.57	9.14±0.33	-
	abandoned	9.77±0.06		10.01±0.05	

Table S7 Statistic testing results of α-diversity (Shannon index) comparison among different fertilization treatments. Significant effects are indicated in bold (*p<0.05, ***p<0.001). Different letters indicate the groupings of pairwise comparison.

	soil	root
Spring Maize		
ANOVA test	F=0.28	**F=5.00***
Pairwise Comparisons of fertilization		
PK	10.16±0.14 (a)	**8.39±0.53 (ac)**
NPK	10.00±0.12 (a)	**9.83±0.11 (bc)**
1/2NPK+1/2M	9.96±0.21 (a)	9.72±0.16 (ac)
M	10.01±0.20 (a)	9.66±0.22 (ac)
Summer Maize		
ANOVA test	F=0.70	**F=29.49*****
Pairwise Comparisons of fertilization		
Control	9.89±0.33 (a)	**9.93±0.05 (a)**
NPK	10.13±0.09 (a)	**9.92±0.05 (a)**
NPK+S	8.40±1.67 (a)	**9.03±0.13 (b)**
NPK+M	8.43±1.40 (a)	**9.41±0.05 (c)**
NPK+1.5M	9.13±0.07 (a)	**9.47±0.02 (c)**
Combined		
ANOVA test	F=1.60	**F=3.30***
Pairwise Comparisons of fertilization		
Control	10.02±0.34 (a)	**10.13±0.03 (a)**
NPK	10.20±0.07 (a)	9.99±0.06 (ac)
1/2NPK+1/2M	10.07±0.24 (a)	9.81±0.17 (a)
M	10.14±0.21 (a)	9.76±0.23 (a)
NPK+M	8.51±1.42 (a)	**9.56±0.05 (b)**