Supplementary material

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Table S1 Primers for quantitative PCR.

Primer	Sequence 5′–3′	Specificity	Reference
Arch-amoAF	STAATGGTCTGGCTTAGACG	AOA *amoA*	Francis et al. (2005)
Arch-amoAR	GCGGCCATCCATCTGTATGT	AOA *amoA*	Francis et al. (2005)
amoA-1F	GGGG TTTCTACTGGTGGT	AOB *amoA*	Rotthauwe et al. (1997)
amoA-2R	CCCCTCKGSAAAGCCTTCTTC	AOB *amoA*	Rotthauwe et al. (1997)
515F	GTGCCAGCMGCCGCGG	16S rRNA	Stubner (2002)
907R	CCGTCAATTCMTTTRAGTTT	16S rRNA	Stubner (2002)

Table S2 Diversity of the 16S rRNA gene-based bacterial communities.

Treatments	No. of observed OTUs	Chao1	Shannon
N0	5244 ± 229 ab	6647 ± 324 ab	10.2 ± 0.57 a
N135	5404 ± 257 a	6823 ± 215 ab	10.4 ± 0.18 a
N180	5427 ± 244 a	6968 ± 135 a	10.6 ± 0.04 a
N225	4901 ± 289 b	6297 ± 496 b	10.4 ± 0.17 a
N360	5444 ± 139 a	6869 ± 168 ab	10.5 ± 0.02 a

Note: The data represents the mean ± standard deviation (n = 3). Different letters in the same column mean significant difference at $P < 0.05$ among the five treatments. N0 (without nitrogen fertilizer), N135 (with a rate of 135 kg N ha^{-1}), N180 (with a rate of 180 kg N ha^{-1}), N225(with a rate of 225 kg N ha^{-1}) and N360 (with a rate of 360 kg N ha^{-1}).

Table S3 Relationship between soil properties and α-diversity under different N fertilizer levels.

soils properties	OTUs		Chao1		Shannon	
	PCC	*P*	PCC	*P*	PCC	*P*
pH	0.114	0.685	0.200	0.475	0.568	0.027*
SOM (g kg^{-1})	0.082	0.771	0.225	0.420	0.089	0.752
TN (g kg^{-1})	-0.440	0.101	-0.332	0.226	-0.349	0.203
NO_3-N (mg kg^{-1})	0.314	0.254	0.325	0.237	0.507	0.054
NH_4-N (mg kg^{-1})	-0.166	0.553	-0.039	0.889	0.274	0.324
AN (mg kg^{-1})	-0.140	0.619	0.018	0.949	-0.068	0.809
TP (g kg^{-1})	-0.129	0.648	-0.261	0.348	0.339	0.216
AP (mg kg^{-1})	-0.496	0.060	-0.475	0.074	-0.407	0.132
TK (g kg^{-1})	0.157	0.576	0.213	0.447	0.318	0.248
AK (mg kg^{-1})	-0.357	0.191	-0.389	0.152	-0.300	0.277

Note: SOM, soil organic matter; NO_3-N, nitrate N; NH_4-N, ammonium N; TP, total P; TN, total N; TK, total K, AN, available N; AK, available K; AP, available P. PCC is the Spearman's correlation coefficient * $P < 0.05$.

Table S4 The results of Monte Carlo permutation test in the RDA.

Test: F=0.5, *P*=0.906				
Soil properties	RDA1	RDA2	r^2	*P* value
pH	0.7249	-0.6889	0.2038	0.255
SOM	0.0033	-1.0000	0.0610	0.704
TN	0.2120	0.9773	0.5770	0.002**
NO_3-N	-0.3694	0.9293	0.0183	0.896
NH_4-N	0.4992	0.8665	0.1913	0.277
AN	0.8084	0.5887	0.1958	0.235
TP	-0.9993	0.0378	0.1209	0.454
AP	-0.0403	0.9992	0.6316	0.003**
TK	0.1124	0.9937	0.0692	0.651
AK	-0.2655	0.9641	0.2476	0.140

Note: r^2 represents the coefficients of determination of soil properties that were related to OTUs. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. SOM (soil organic matter), NO_3-N (nitrate N), NH_4-N (ammonium N), TP (total P), TN (total N), TK (total K), AN (available N), AK (available K) and AP (available P).

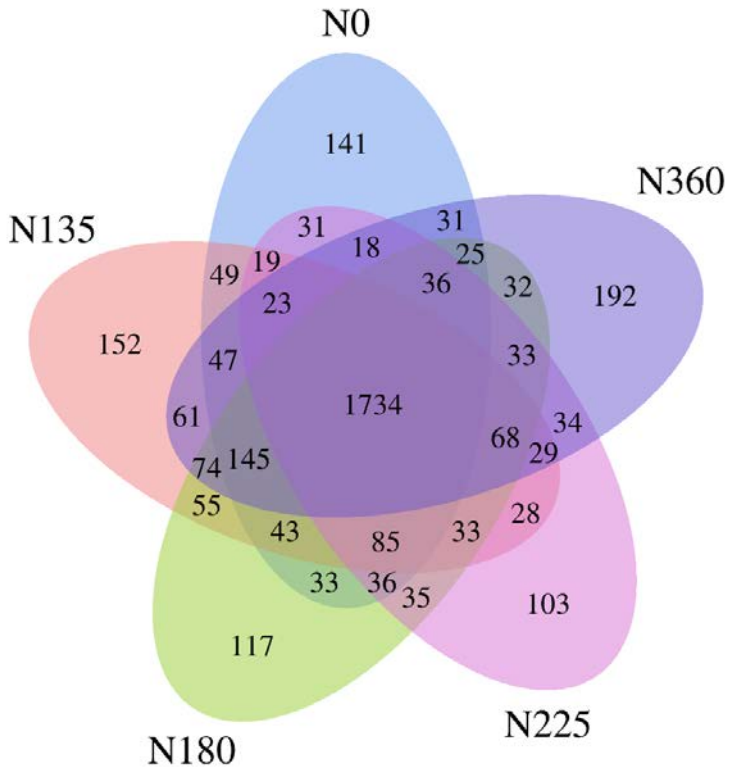

Fig. S1 Venn diagram showing unique and shared operational taxonomic units (OTUs). N0 (without nitrogen fertilizer), N135 (with a rate of 135 kg N ha^{-1}), N180 (with a rate of 180 kg N ha^{-1}), N225(with a rate of 225 kg N ha^{-1}) and N360 (with a rate of 360 kg N ha^{-1}).

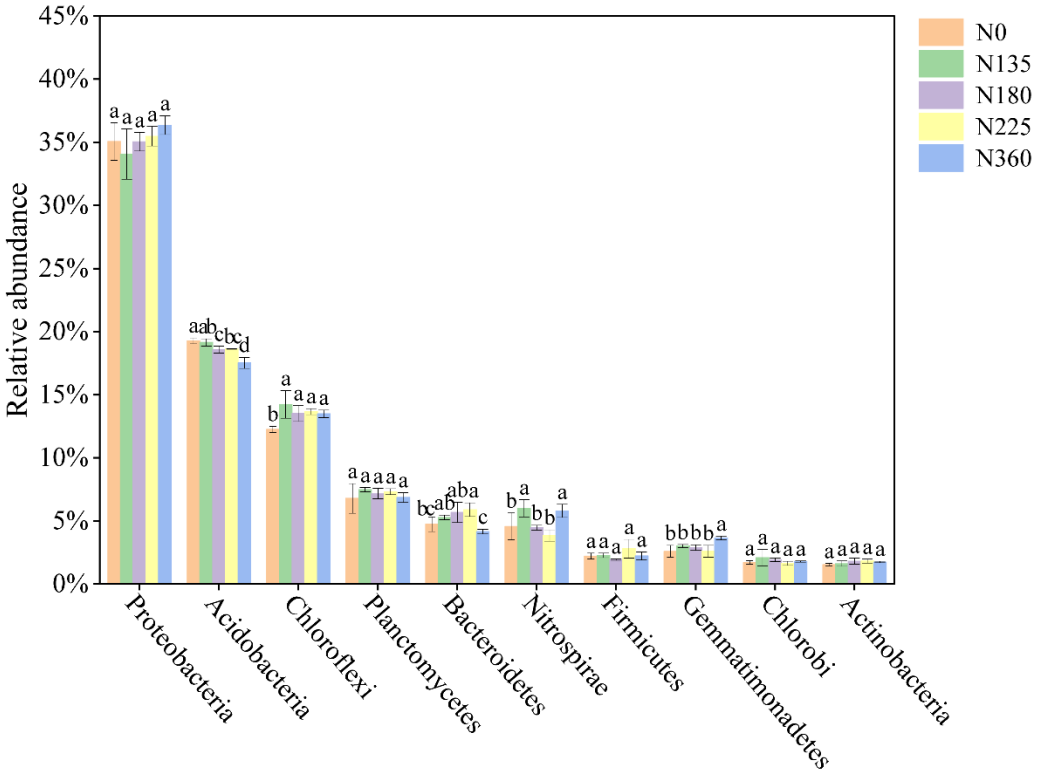

Fig. S2 The relative abundance of bacterial dominance phylum in different treatments. N0 (without nitrogen fertilizer), N135 (with a rate of 135 kg N ha^{-1}), N180 (with a rate of 180 kg N ha^{-1}), N225(with a rate of 225 kg N ha^{-1}), N360 (with a rate of 360 kg N ha^{-1}). Vertical bars represent the standard deviation (n = 3) and lowercase letters indicate significant differences among treatments ($P < 0.05$).

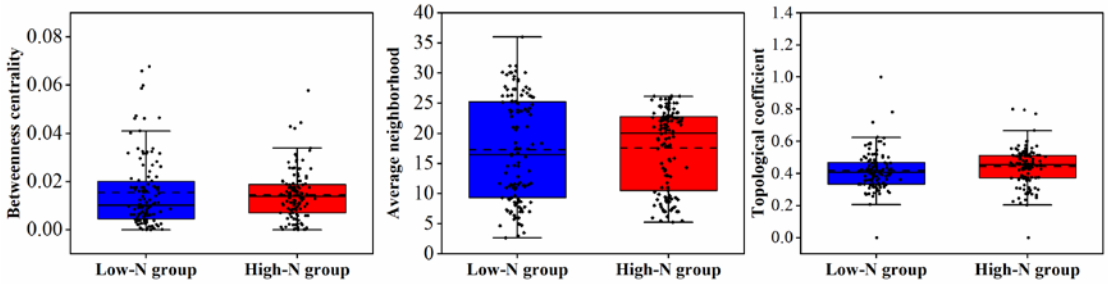

Fig. S3 The differences in topological features of the microbial networks in the soils of the low-N group (N0 and N135) and the high-N group (N225 and N360).

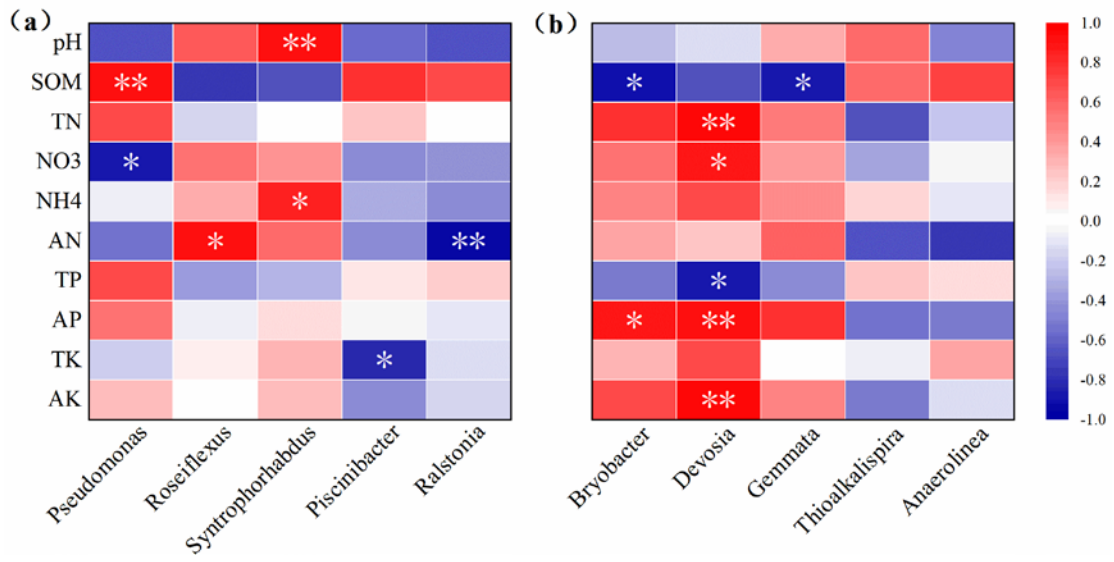

Fig. S4 Pearson's correlations between soil physicochemical characteristics and relative abundances of keystone species under (a) the low-N group (N0 and N135) and (b) the high-N group (N225 and N360). SOM, soil organic matter; NO_3, nitrate N; NH_4, ammonium N; TP, total P; TN, total N; TK, total K; AN, available N; AK, available K; AP, available P. "*" and "**" indicate $P < 0.05$ and $P < 0.01$, respectively.

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