

Table S1. Illumina MiSeq sequenced bacterial data (at 97% sequence similarity) based on the 16S rRNA gene.

Sample	Sequences	Bases(bp)	AverageLength(bp)	OTU
N0_1	51025	19233244	376.94	3314
N0_2	49889	18800111	376.84	3286
N0_3	56869	21432675	376.88	3385
N1_1	58245	21952210	376.89	3507
N1_2	52106	19640197	376.93	3350
N1_3	47758	18002357	376.95	3299
N2_1	42656	16077789	376.92	3283
N2_2	44601	16807312	376.84	3290
N2_3	47879	18043097	376.85	3343
N3_1	48645	18336209	376.94	3357
N3_2	45727	17228439	376.77	3025
N3_3	41102	15495430	377	3111
N4_1	41352	15589396	376.99	3181
N4_2	55213	20813666	376.97	3391
N4_3	48155	18153711	376.98	3352

N0, N1, N2, N3, and N4 represent N fertilizer was used at a level of 0, 90, 150, 210, and 270 kg ha⁻¹, respectively.

Table S2. Relative abundance (%) at bacterial phyla level under different N gradients.

	Treatment	Proteobacteria	Gemmatimonadetes	Bacteroidetes	Nitrospirae	Firmicutes	Deinococcus-Thermus	Omnitrophicaeota
	N0	27.08	4.48	3.22	0.93	0.46	0.04	0.03
	N1	26.56	4.97	2.78	1.12	0.59	0.01	0.02
	N2	28.05	5.30	3.11	1.40	0.64	0.06	0.04
	N3	28.83	4.20	2.26	1.20	1.55	0.01	0.03
	N4	25.20	5.09	2.63	1.36	0.75	0.05	0.05
<i>P</i> -value	N0 vs N1	ns ^a	ns	ns	ns	ns	ns	0.013
	N0 vs N2	ns	8.77E-03	ns	0.044	ns	ns	ns
	N0 vs N3	ns	ns	0.031	ns	0.025	0.038	ns
	N0 vs N4	0.032	8.78E-04	ns	ns	4.47E-03	ns	ns

^a ns, not significant; *P* at 95% confidence intervals, Student's *t*-test, equal variance.

N0, N1, N2, N3, and N4 represent N fertilizer was used at a level of 0, 90, 150, 210, and 270 kg ha⁻¹, respectively.

Table S3. The statistical analysis base on Weighted-Unifrac for distance of bacterial community beta diversity among different N gradients

Treatment	<i>t</i> statistic	Parametric <i>P</i> -value
N0 vs. N1	-2.413	0.037
N0 vs. N2	-3.851	0.003
N0 vs. N3	-2.427	0.036
N0 vs. N4	-3.878	0.003
N1 vs. N2	-3.265	0.009
N1 vs. N3	-1.812	0.100
N1 vs. N4	-2.893	0.016
N2 vs. N3	-3.182	0.010
N2 vs. N4	-6.655	0.000
N3 vs. N4	-1.792	0.103

The tests of significance were performed using a two-sided Student's two-sample *t*-test.

Table S4. The statistical analysis and LDA score of different genera under five N fertilizer levels

Taxonomy	Treatment	LDA score	<i>P</i> value
Bacteria.Proteobacteria.Gammaproteobacteria.Betaproteobacteriales.Burkholderiaceae.Comamonas	N1	2.875	0.032
Bacteria.Firmicutes.Bacilli.Bacillales.Bacillaceae.Bacillus	N3	3.192	0.017
Bacteria.Proteobacteria.Deltaproteobacteria.Bdellovibrionales	N4	2.783	0.013
Bacteria.Deinococcus_Thermus.Deinococci.Deinococcales.Deinococcaceae.Deinococcus	N2	2.527	0.039
Bacteria.Deinococcus_Thermus	N2	2.545	0.036
Bacteria.Proteobacteria.Gammaproteobacteria.Betaproteobacteriales.Nitrosomonadaceae.MND1	N4	3.354	0.048
Bacteria.Proteobacteria.Deltaproteobacteria.Myxococcales.Haliangiaceae	N4	2.844	0.038
Bacteria.Verrucomicrobia.Verrucomicrobiae.Opitutales.Opitutaceae	N4	2.587	0.025
Bacteria.Gemmatimonadetes	N2	3.884	0.036
Bacteria.Planctomycetes.Planctomycetacia.Pirellulales	N4	2.937	0.014
Bacteria.Chloroflexi.Chloroflexia.Thermomicrobiales.JG30_KF_CM45	N1	3.101	0.042
Bacteria.Deinococcus_Thermus.Deinococci.Deinococcales	N2	2.552	0.036
Bacteria.Bacteroidetes.Bacteroidia.Sphingobacteriales.env_OPS_17	N4	2.580	0.031
Bacteria.Verrucomicrobia.Verrucomicrobiae.Opitutales.Opitutaceae.Opitutus	N4	2.456	0.038
Bacteria.Proteobacteria.Gammaproteobacteria.Xanthomonadales.Xanthomonadaceae.Luteimonas	N3	2.577	0.036
Bacteria.Proteobacteria.Gammaproteobacteria.R7C24	N0	2.760	0.031
Bacteria.Verrucomicrobia	N2	2.900	0.049
Bacteria.Firmicutes.Bacilli.Bacillales.Bacillaceae	N3	3.420	0.017
Bacteria.Deinococcus_Thermus.Deinococci.Deinococcales.Deinococcaceae	N2	2.522	0.039
Bacteria.Firmicutes	N3	3.738	0.021
Bacteria.Planctomycetes.Planctomycetacia.Pirellulales.Pirellulaceae.Pirellula	N4	2.979	0.050
Bacteria.Proteobacteria.Deltaproteobacteria.Myxococcales.Haliangiaceae.Haliangium	N4	2.903	0.038

Bacteria.Firmicutes.Bacilli	N3	3.761	0.021
Bacteria.Gemmatimonadetes.Gemmatimonadetes.Gemmatimonadales.Gemmatimonadaceae	N2	3.795	0.032
Bacteria.Gemmatimonadetes.Gemmatimonadetes.Gemmatimonadales	N2	3.795	0.032
Bacteria.Verrucomicrobia.Verrucomicrobiae	N2	2.906	0.049
Bacteria.Acidobacteria.Acidobacteriia	N2	3.222	0.028
Bacteria.Deinococcus_Thermus.Deinococci	N2	2.522	0.036
Bacteria.Chloroflexi.Chloroflexia.Thermomicrobiales.AKYG1722	N4	2.635	0.043
Bacteria.Patescibacteria.Saccharimonadia.Saccharimonadales	N4	2.613	0.042
Bacteria.Gemmatimonadetes.Gemmatimonadetes.Gemmatimonadales.Gemmatimonadaceae.Gemmatimonas	N2	3.245	0.031
Bacteria.Proteobacteria.Gammaproteobacteria.Diplorickettsiales.Diplorickettsiaceae.Aquicella	N4	2.842	0.034
Bacteria.Verrucomicrobia.Verrucomicrobiae.Opitutales	N4	2.657	0.025
Bacteria.Acidobacteria.Blastocatellia_Subgroup_4.Blastocatellales.Blastocatellaceae.Stenotrophobacter	N0	2.696	0.033
-er			
Bacteria.Gemmatimonadetes.Gemmatimonadetes	N2	3.817	0.032
Bacteria.Actinobacteria.Acidimicrobiia.Microtrichales	N1	3.234	0.035
Bacteria.Patescibacteria	N4	2.719	0.027
Bacteria.Proteobacteria.Alphaproteobacteria.Micavibrionales.Micavibrionaceae	N4	2.491	0.037
Bacteria.Firmicutes.Bacilli.Bacillales	N3	3.741	0.021
Bacteria.Bacteroidetes.Bacteroidia.Chitinophagales.Chitinophagaceae.Segetibacter	N2	2.726	0.033
Bacteria.Planctomycetes.Planctomycetacia.Pirellulales.Pirellulaceae	N4	3.059	0.014
Bacteria.Patescibacteria.Saccharimonadia	N4	2.597	0.042
Bacteria.Chloroflexi.Ktedonobacteria	N4	3.038	0.043

LDA score > 2.0. *P* at 95% confidence intervals tested by one-way ANOVA .