

## Supplementary Material

### Differential responses of soil microbial N-cycling functional genes to 35-yr applications of chemical fertilizer and organic manure in wheat field soil on Loess Plateau

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### Supplementary Tables

**Table S1.** Basic information of metagenomic sequencing.

Sample	Raw reads	Clean reads	Percent in raw reads (%)	Contigs	N50(bp)	N90(bp)	ORFs
CK1	74518078	73347560	98.4	588651	557	337	713765

CK2	82203440	80766386	98.3	641506	553	337	777364
CK3	77792976	76523224	98.4	646222	518	334	767785
NF1	97068118	95593222	98.5	853523	562	337	1044869
NF2	71678650	70343720	98.1	513519	534	334	615995
NF3	76053118	74846742	98.4	556979	550	336	675738
OM1	91842924	90429626	98.5	654369	552	335	794524
OM2	94752876	93240514	98.4	724029	566	336	889548
OM3	85329576	83976936	98.4	592182	547	335	717171
NM1	87456992	85732274	98.0	616635	539	334	744038
NM2	82327608	80961138	98.3	575981	543	335	697634
NM3	75858500	74620824	98.4	499066	556	336	608163

**Table S2.** The information of microbial functional genes involved in N cycling processes identified in this study referring to KEGG database.

Classification	KO number	Details for gene function	Gene name
Nitrification	K10944	methane/ammonia monooxygenase subunit A	<i>pmoA-amoA</i>
	K10945	methane/ammonia monooxygenase subunit B	<i>pmoB-amoB</i>
	K10946	methane/ammonia monooxygenase subunit C	<i>pmoC-amoC</i>
	K10535	hydroxylamine dehydrogenase	<i>hao</i>
Denitrification	K00370	nitrate reductase / nitrite oxidoreductase, alpha subunit	<i>narG,narX,nxrA</i>

	K00371	nitrate reductase / nitrite oxidoreductase, beta subunit	<i>narH,narY,nxrB</i>
	K00374	nitrate reductase gamma subunit	<i>narI,narV</i>
	K00368	nitrite reductase (NO-forming)	<i>nirK</i>
	K15864	nitrite reductase (NO-forming) / hydroxylamine reductase	<i>nirS</i>
	K04561	nitric oxide reductase subunit B	<i>norB</i>
	K02305	nitric oxide reductase subunit C	<i>norC</i>
	K00376	nitrous-oxide reductase	<i>nosZ</i>
Dissimilatory	K02567	nitrate reductase (cytochrome)	<i>napA</i>
nitrate reduction	K02568	nitrate reductase (cytochrome), electron transfer subunit	<i>napB</i>
	K00362	nitrite reductase (NADH) large subunit	<i>nirB</i>
	K00363	nitrite reductase (NADH) small subunit	<i>nirD</i>
	K03385	nitrite reductase (cytochrome c-552)	<i>nrfA</i>
	K15876	cytochrome c nitrite reductase small subunit	<i>nrfH</i>
Assimilatory	K00367	ferredoxin-nitrate reductase	<i>narB</i>
nitrate reduction	K00372	assimilatory nitrate reductase catalytic subunit	<i>nasA</i>
	K00360	assimilatory nitrate reductase electron transfer subunit	<i>nasB</i>
	K00366	ferredoxin-nitrite reductase	<i>nirA</i>
Organic nitrogen	K01428	urease subunit alpha	<i>ureC</i>
metabolism	K00034	glucose 1-dehydrogenase	<i>gdh</i>

**Table S3.** Effects of organic manure and chemical N fertilization on soil properties.

Index	Unit	CK	NF	OM	NM	F	<i>p</i>
pH	-	8.27 ± 0.06 a	8.30 ± 0.04 a	8.32 ± 0.05 a	8.28 ± 0.04 a	0.17	0.914
BD	g·cm <sup>-3</sup>	1.19 ± 0.02 a	1.22 ± 0.04 a	1.13 ± 0.02 a	1.12 ± 0.05 a	1.89	0.210
SWC	%	18.84 ± 0.23 b	18.68 ± 0.28 b	21.36 ± 0.76 a	20.95 ± 0.22 a	10.15	<b>0.004</b>
C:N	-	7.13 ± 0.33 b	8.40 ± 0.39 a	8.19 ± 0.23 a	8.26 ± 0.19 a	4.04	0.051
SOC	g·kg <sup>-1</sup>	7.20 ± 0.39 b	7.62 ± 0.11 b	10.62 ± 0.31 a	11.38 ± 0.40 a	41.72	<b>&lt;0.001</b>
POC	g·kg <sup>-1</sup>	2.09 ± 0.20 a	2.54 ± 0.17 a	3.18 ± 0.59 a	2.64 ± 0.27 a	1.67	0.250
PCM	mg·kg <sup>-1</sup>	183.60 ± 2.08 b	216.00 ± 4.16 b	309.60 ± 12.47 a	320.40 ± 18.71 a	35.05	<b>&lt;0.001</b>
MBC	mg·kg <sup>-1</sup>	223.41 ± 5.43 b	246.34 ± 6.45 b	354.63 ± 17.81 a	327.32 ± 17.16 a	23.23	<b>&lt;0.001</b>

Treatments were CK, control; NF, chemical N fertilization; OM, organic manure application; and NM, combination of chemical N fertilizer and organic manure application. Soil properties were pH; BD, bulk density; SWC, soil water content; C: N, the ratio of soil organic C to total N; SOC, soil organic C; POC, particulate organic C; PCM, potential C mineralization; and MBC, microbial biomass C. Values are means ± standard errors (n = 3). Different lowercase letters in a row indicate significant difference (*p* < 0.05) among treatments.

**Table S4.** The abundances of genes involved in soil N cycling under different fertilization treatments.

Classification	Gene	CK	NF	OM	NM	F	<i>p</i>
Nitrification	<i>amoA</i>	4.30 ± 0.44 ab	5.23 ± 1.19 ab	3.27 ± 0.79 b	6.51 ± 1.09 a	2.22	0.163
	<i>amoB</i>	17.12 ± 0.93 a	18.78 ± 1.59 a	18.13 ± 1.30 a	18.61 ± 1.28 a	0.33	0.804
	<i>amoC</i>	5.75 ± 0.42 b	9.17 ± 0.42 a	9.99 ± 0.14 a	9.51 ± 1.07 a	9.732	<b>0.005</b>
	<i>hao</i>	0.49 ± 0.07 b	3.14 ± 1.02 a	0.55 ± 0.29 ab	3.16 ± 1.23 a	3.505	0.069

Denitrification	<i>narG</i>	60.22 ± 2.04 b	61.52 ± 3.22 b	67.79 ± 1.93 b	78.44 ± 3.89 a	8.3	<b>0.008</b>
	<i>narH</i>	26.71 ± 2.31 c	30.79 ± 2.01 bc	35.41 ± 0.50 ab	39.29 ± 3.92 a	4.798	<b>0.034</b>
	<i>narI</i>	4.32 ± 0.71 b	4.54 ± 0.13 b	5.37 ± 1.01 b	9.65 ± 0.64 a	12.727	<b>0.002</b>
	<i>nirK</i>	83.31 ± 3.59 b	84.69 ± 1.94 b	95.82 ± 5.08 a	96.45 ± 0.99 a	4.555	<b>0.038</b>
	<i>nirS</i>	5.80 ± 0.47 a	6.00 ± 1.18 a	5.35 ± 0.73 a	6.23 ± 1.25 a	0.151	0.926
	<i>norB</i>	55.63 ± 3.55 a	50.65 ± 2.60 a	53.87 ± 1.87 a	54.39 ± 4.34 a	0.433	0.735
	<i>norC</i>	3.40 ± 0.65 a	2.70 ± 0.06 a	3.21 ± 0.18 a	3.98 ± 0.58 a	1.392	0.314
	<i>nosZ</i>	26.95 ± 1.73 c	28.30 ± 1.10 bc	30.88 ± 0.68 b	40.19 ± 0.60 a	28.277	<b>&lt;0.001</b>
Dissimilatory	<i>napA</i>	32.36 ± 2.59 b	32.50 ± 3.02 b	38.45 ± 2.06 ab	43.77 ± 3.01 a	4.105	<b>0.049</b>
nitrate reduction	<i>napB</i>	4.34 ± 0.62 b	3.69 ± 0.51 b	8.06 ± 1.45 a	8.95 ± 0.74 a	8.383	<b>0.007</b>
	<i>nirB</i>	157.42 ± 1.27 ab	144.38 ± 4.68 b	164.13 ± 3.27 a	157.95 ± 6.40 ab	3.669	0.063
	<i>nirD</i>	43.01 ± 2.52 a	36.07 ± 0.79 a	39.15 ± 1.69 a	42.28 ± 3.24 a	1.996	0.193
	<i>nrfA</i>	53.36 ± 1.74 a	52.68 ± 5.49 a	58.71 ± 2.45 a	60.85 ± 2.65 a	1.394	0.313
	<i>nrfH</i>	18.18 ± 1.17 ab	17.74 ± 1.18 b	19.57 ± 0.34 ab	21.17 ± 0.94 a	2.546	0.129
Assimilatory	<i>narB</i>	6.83 ± 0.60 a	5.22 ± 1.14 a	5.54 ± 0.72 a	7.03 ± 0.42 a	1.406	0.310
nitrate reduction	<i>nasA</i>	107.93 ± 2.54 b	106.64 ± 5.42 b	120.31 ± 2.61 a	123.57 ± 1.23 a	6.664	<b>0.014</b>
	<i>nasB</i>	8.26 ± 1.47 a	6.95 ± 0.92 a	8.79 ± 1.07 a	7.38 ± 1.45 a	0.444	0.728
	<i>nirA</i>	47.40 ± 1.49 a	49.01 ± 1.69 a	51.51 ± 1.35 a	47.08 ± 2.30 a	1.34	0.328
Organic nitrogen	<i>ureC</i>	155.51 ± 3.54 a	149.04 ± 5.64 a	154.92 ± 1.69 a	154.71 ± 3.52 a	0.613	0.625
metabolism	<i>gdh</i>	108.38 ± 4.88 a	100.99 ± 2.11 ab	94.86 ± 0.99 b	92.54 ± 2.79 b	5.421	<b>0.025</b>

Treatments were CK, control; NF, chemical N fertilization; OM, organic manure application; and NM,

combination of chemical N fertilizer and organic manure application. Values are means  $\pm$  standard errors

(n = 3). Different lowercase letters in a row indicate significant difference ( $p < 0.05$ ) among treatments.

**Table S5.** Relative abundances of microbial dominant phyla (relative abundance > 1%) involved in soil N cycling under different fertilization treatments.

Phyla	CK	NF	OM	NM	F	<i>p</i>
<i>Proteobacteria</i>	33.5 $\pm$ 1.15 ab	33.0 $\pm$ 0.44 b	36.0 $\pm$ 0.66 a	35.6 $\pm$ 0.56 a	3.84	0.057
<i>Actinobacteria</i>	26.6 $\pm$ 0.33 a	26.8 $\pm$ 1.01 a	26.2 $\pm$ 0.31 a	26.1 $\pm$ 1.07 a	0.19	0.901
<i>Thaumarchaeota</i>	7.8 $\pm$ 0.42 a	6.9 $\pm$ 0.13 a	7.0 $\pm$ 0.24 a	6.8 $\pm$ 0.35 a	2.19	0.166
<i>Acidobacteria</i>	5.2 $\pm$ 0.09 a	5.5 $\pm$ 0.01 a	5.3 $\pm$ 0.25 a	5.3 $\pm$ 0.22 a	0.71	0.573
<i>Unclassified</i>	3.4 $\pm$ 0.04 a	3.5 $\pm$ 0.13 4	3.5 $\pm$ 0.25 a	3.7 $\pm$ 0.07 a	0.782	0.537
<i>Others</i>	3.4 $\pm$ 0.21 a	3.6 $\pm$ 0.23 a	3.4 $\pm$ 0.53 a	3.5 $\pm$ 0.20 a	0.14	0.933
<i>Nitrospirae</i>	3.0 $\pm$ 0.15 a	3.2 $\pm$ 0.21 a	3.2 $\pm$ 0.15 a	3.4 $\pm$ 0.17 a	0.79	0.535
<i>Verrucomicrobia</i>	3.0 $\pm$ 0.13 a	3.0 $\pm$ 0.46 a	3.1 $\pm$ 0.23 a	2.8 $\pm$ 0.11 a	0.30	0.828
<i>Cyanobacteria</i>	2.9 $\pm$ 0.04 a	3.1 $\pm$ 0.05 a	2.4 $\pm$ 0.17 b	2.4 $\pm$ 0.06 b	14.50	<b>0.001</b>
<i>Chloroflexi</i>	2.7 $\pm$ 0.20 a	2.8 $\pm$ 0.17 a	2.3 $\pm$ 0.12 a	2.6 $\pm$ 0.22 a	1.43	0.303
<i>Firmicutes</i>	2.7 $\pm$ 0.19 a	2.5 $\pm$ 0.07 ab	2.1 $\pm$ 0.07 c	2.2 $\pm$ 0.07 bc	6.94	<b>0.013</b>
<i>Crenarchaeota</i>	1.6 $\pm$ 0.11 a	1.7 $\pm$ 0.15 a	1.6 $\pm$ 0.29 a	1.7 $\pm$ 0.05 a	0.07	0.974
<i>Bacteroidetes</i>	1.6 $\pm$ 0.03 a	1.8 $\pm$ 0.28 a	1.4 $\pm$ 0.02 a	1.3 $\pm$ 0.10 a	1.73	0.239
<i>Planctomycetes</i>	1.4 $\pm$ 0.11 a	1.4 $\pm$ 0.18 a	1.5 $\pm$ 0.14 a	1.6 $\pm$ 0.13 a	0.28	0.841
<i>Candidatus_Rokubacteria</i>	1.0 $\pm$ 0.11 a	1.1 $\pm$ 0.07 a	1.0 $\pm$ 0.04 a	0.9 $\pm$ 0.08 a	0.37	0.777

Treatments were CK, control; NF, chemical N fertilization; OM, organic manure application; and NM,

combination of chemical N fertilizer and organic manure application. ‘Others’ refers to all phyla with relative abundance less than 1%. Values are means  $\pm$  standard errors ( $n = 3$ ). Different lowercase letters in a row indicate significant difference ( $p < 0.05$ ) among treatments.

**Table S6.** Correlation coefficients of Mantel tests between the functional gene composition responsible for soil N cycling and soil properties.

Environmental attributes	N-cycling		Nitrification		Denitrification		DNRA		ANRA		ONM	
	r	p	r	p	r	p	r	p	r	p	r	p
pH	-0.081	0.764	-0.075	0.711	-0.036	0.573	-0.048	0.627	-0.015	0.474	-0.113	0.872
BD	0.352	<b>0.030</b>	0.070	0.334	-0.031	0.543	0.340	<b>0.028</b>	0.397	<b>0.047</b>	0.495	<b>0.014</b>
SWC	0.310	<b>0.025</b>	-0.186	0.937	0.389	<b>0.012</b>	0.202	0.089	0.087	0.218	0.070	0.259
C:N	-0.009	0.505	0.339	<b>0.010</b>	0.122	0.233	-0.128	0.731	-0.127	0.713	-0.065	0.550
SOC	0.608	<b>0.002</b>	0.044	0.324	0.593	<b>0.001</b>	0.321	<b>0.019</b>	0.373	<b>0.006</b>	0.234	<b>0.037</b>
POC	-0.210	0.937	0.021	0.413	-0.233	0.960	-0.133	0.820	-0.043	0.544	-0.069	0.582
PCM	0.588	<b>0.003</b>	0.183	0.083	0.518	<b>0.002</b>	0.331	<b>0.021</b>	0.368	<b>0.008</b>	0.270	<b>0.018</b>
MBC	0.449	<b>0.009</b>	0.010	0.406	0.418	<b>0.011</b>	0.239	0.057	0.394	<b>0.007</b>	0.113	0.167
STN	0.638	<b>0.001</b>	-0.026	0.519	0.532	<b>0.001</b>	0.472	<b>0.002</b>	0.437	<b>0.005</b>	0.202	<b>0.046</b>

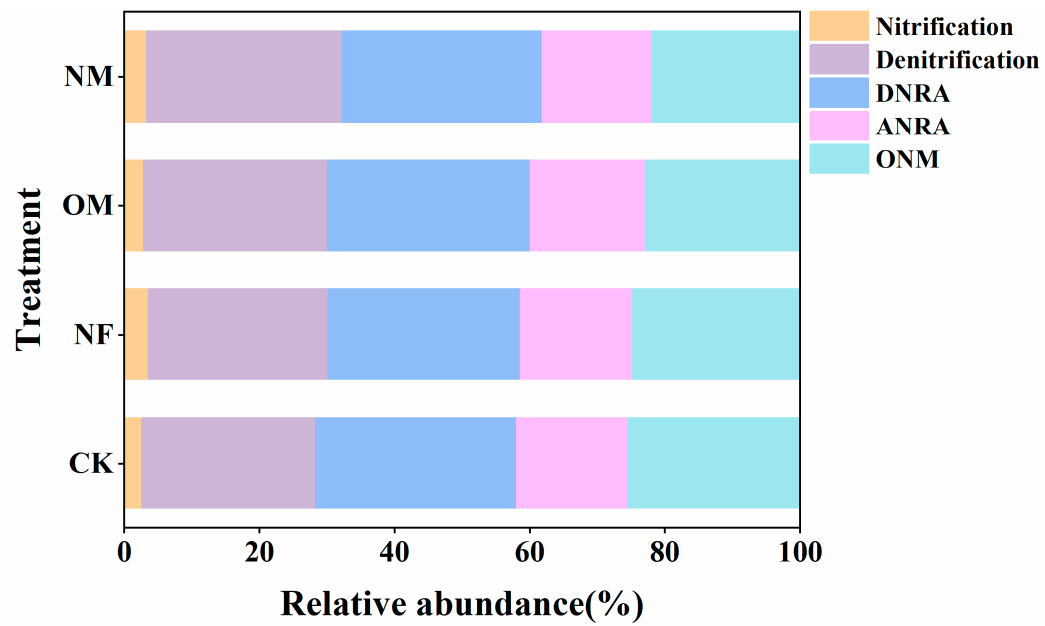


PON	0.118	0.225	0.201	0.081	0.122	0.217	-0.146	0.825	0.267	0.072	0.132	0.217
PNM	0.376	<b>0.014</b>	-0.066	0.648	0.543	<b>0.002</b>	0.063	0.334	0.101	0.243	0.114	0.251
MBN	0.610	<b>0.002</b>	0.128	0.189	0.678	<b>0.002</b>	0.274	0.052	0.141	0.197	0.285	0.055
NH <sub>4</sub> <sup>+</sup> -N	0.292	<b>0.027</b>	-0.031	0.542	0.127	0.165	0.423	<b>0.003</b>	0.137	0.188	0.128	0.200
NO <sub>3</sub> <sup>-</sup> -N	0.154	0.168	-0.060	0.639	-0.133	0.820	0.426	<b>0.006</b>	0.120	0.201	0.172	0.189

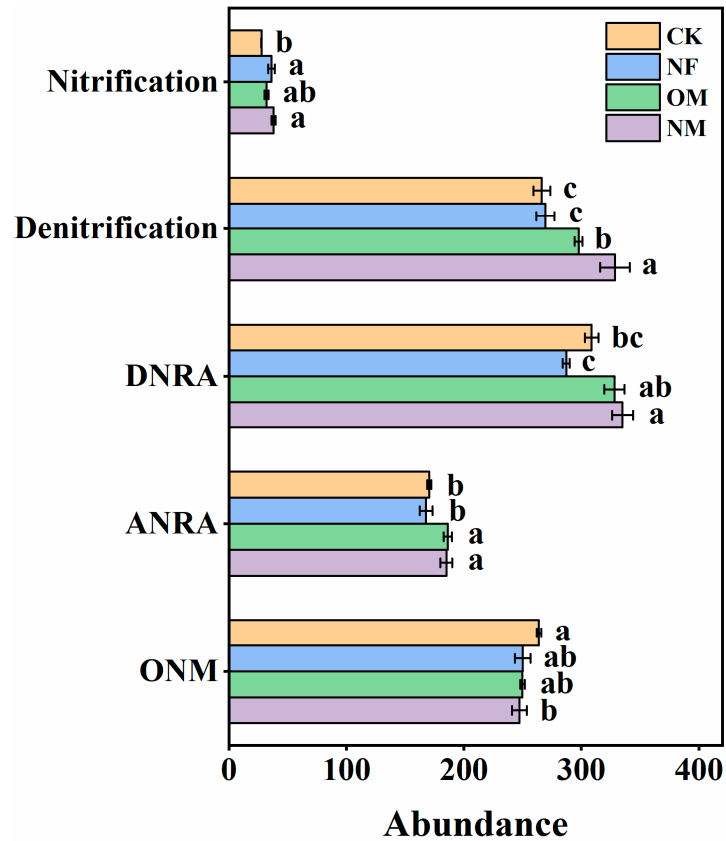
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Soil properties were pH; BD, bulk density; SWC, soil water content; C: N, the ratio of soil organic C to total N; SOC, soil organic C; POC, particulate organic C; PCM, potential C mineralization; MBC, microbial biomass C; STN, soil total N; PON, particulate organic N; PNM, potential N mineralization; MBN, microbial biomass N; NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N. Significant ( $p < 0.05$ ) correlations are shown in bold.

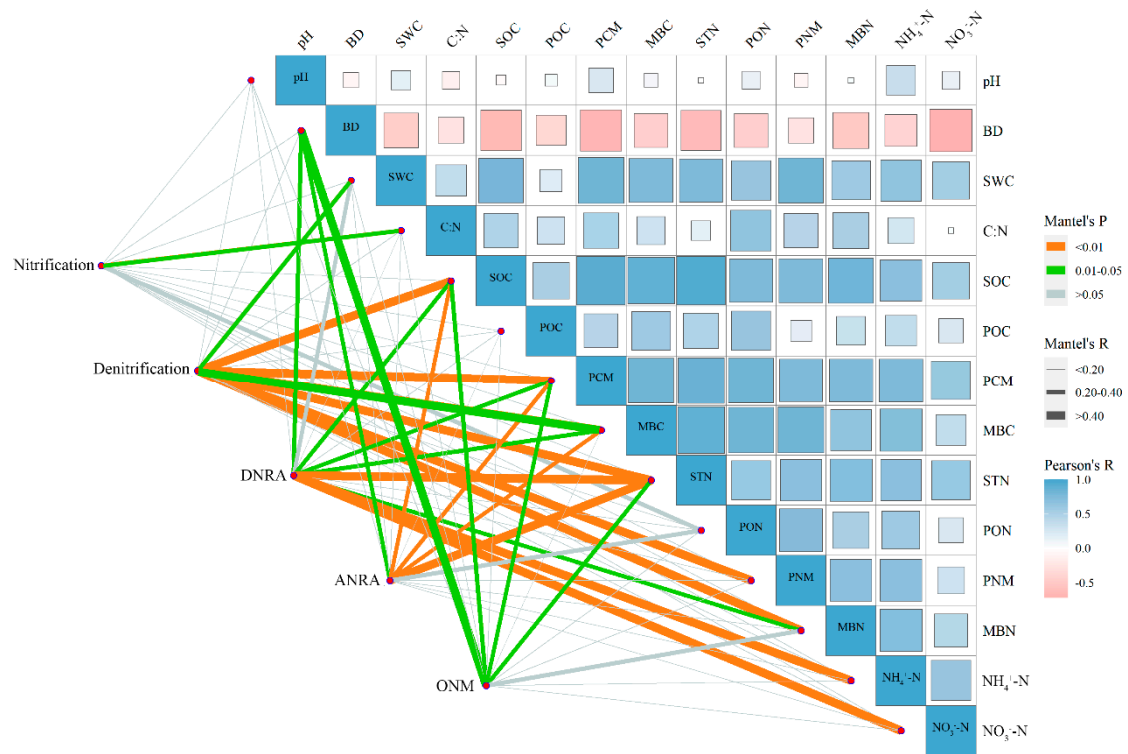
# Supplementary Figures



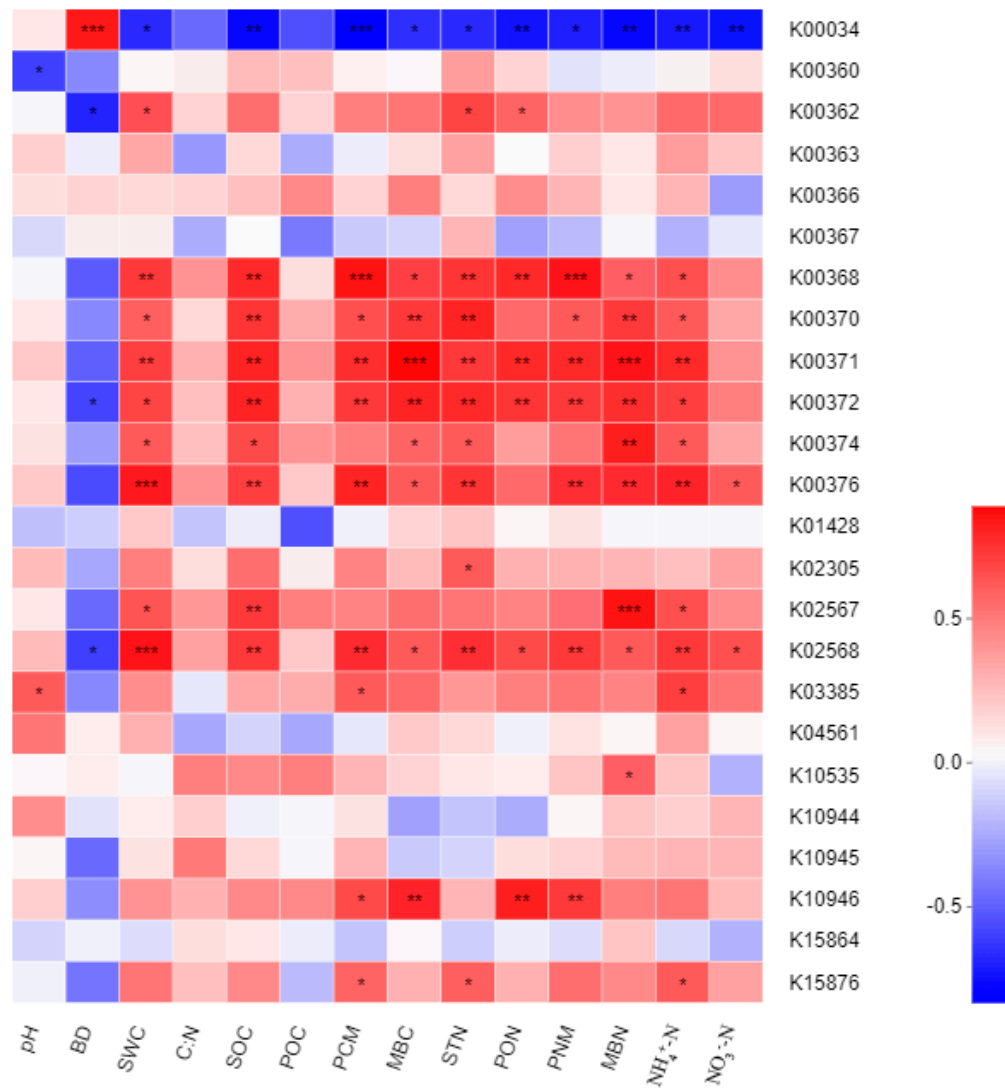
**Figure S1.** The relative abundances of genes involved in different N metabolism pathways under different fertilization treatments. Treatments were CK, control; NF, chemical N fertilization; OM, organic manure application; and NM, combination of chemical N fertilizer and organic manure application.



**Figure S2.** The abundances of genes involved in different N metabolism pathways under different fertilization treatments. Treatments were CK, control; NF, chemical N fertilization; OM, organic manure application; and NM, combination of chemical N fertilizer and organic manure application. Error bars represent standard error of the means (n =3). Different lowercase letters indicate significant difference ( $p < 0.05$ ) among treatments.



**Figure S3.** Relationships between the functional gene composition involved in different N metabolism pathways and soil properties.



**Figure S4.** Spearman correlations between the abundances of N-cycling genes and soil properties. Red means positive relationship, blue means negative relationship. Significance is represented by \* -  $p < 0.05$ , \*\* -  $p < 0.01$ , \*\*\* -  $p < 0.001$ .