

Supplementary Material

Table S1. Physical and chemical properties of different treated soil (0–20 cm) before sampling

Soil properties		CK	U	UI	NI
Total C (g kg ⁻¹)		13.5±0.09 ^c	15.2±0.09 ^b	15.7±0.02 ^b	16.6±0.22 ^a
Total N (g kg ⁻¹)		1.29±0.03 ^c	1.63±0.04 ^a	1.42±0.03 ^b	1.55±0.03 ^b
TOC (g kg ⁻¹)		12.4±0.40 ^c	13.9±0.26 ^b	14.9±0.62 ^b	15.7±0.24 ^a
CO ₃ ²⁻ (mg g ⁻¹)		34.2±0.17 ^a	34.2±0.34 ^a	34.5±0.3 ^a	33.6±0.15 ^a
Available-P (mg kg ⁻¹)		21.7±0.68 ^a	16.2±0.79 ^b	10.7±0.58 ^d	13.3±0.34 ^c
Available-K (mg kg ⁻¹)		162±0.64 ^a	108±7.67 ^b	114±1.45 ^b	116±1.45 ^b
NH ₄ ⁺ -N (mg kg ⁻¹)		0.92±0.11 ^b	1.66±0.28 ^a	1.02±0.17 ^b	1.17±0.11 ^{ab}
NO ₃ ⁻ -N (mg kg ⁻¹)		21.2±0.75 ^c	26.9±1.07 ^b	33.2±1.68 ^a	30.2±1.68 ^{ab}
pH		7.5±0.06 ^a	7.51±0.18 ^a	7.76±0.13 ^a	7.74±0.03 ^a
Particle size	Clay (< 0.002 mm)	27.9±0.63			
	Silt (0.002-0.02 mm)	29.62±1.41			
	Sand (0.02-2 mm)	42.48±0.79			

Values represent means ± standard errors (n = 3). Lower case letters in column indicate significant differences between treatment means (*p* < 0.05). CK, no nitrogen fertilizer application; U, urea; UI, urea with NBPT; NI, urea with DMPP.

Table S2. The primer sets used for analyzing the populationwith archaeal *amoA*, bacterial *amoA*, *nxrA*, *nxB*, *narG* and *nosZ* genes

Primers	Sequence (5'-3')	References
archaeal <i>amoA</i>		
Arch- <i>amoA</i> F	STAATGGTCTGGCTTAGACG	[82]
Arch- <i>amoA</i> R	GCGGCCATCCATCTGTATGT	
bacterial <i>amoA</i>		
<i>bamoA</i> 1F	GGGGTTTCTACTGGTGGT	[83]
<i>bamoA</i> 2R	CCCCTCKGSAAAGCCTTCTTC	
<i>nxrA</i>		
F1 <i>norA</i>	CAGACCGACGTGTGCGAAAG	[84]
F2843 R2 <i>nxrA</i>	TCCACAAGGAACGGAAGGTC	
<i>nxrB</i>		
<i>nxrB</i> 169f	TACATGTGGTGGAACA	[85]
<i>nxrB</i> 638r	CGGTTCTGGTCRATCA	
<i>narG</i>		
<i>narGG</i> -F	TCGCCSATYCCGGCSATGTC	[86]
<i>narGG</i> -R	GAGTTGTACCAGTCRGCSGAYTCSG	
<i>nosZ</i>		
<i>nosZ</i> -F	CG(C/T) TGT TC(A/C) TCG ACA GCC AG	[87]
<i>nosZ</i> 1622R	CGC (G/A)A(C/G) GGC AA(G/C) AAG GT(G/C) CG	

Table S3. Pearson's correlation coefficients (r) between soil PNR and cumulative N₂O emissions, and microbial population diversity (Shannon index).

	N ₂ O emission			Shannon index			
	kg N ha ⁻¹	AOA	AOB	<i>nxrA</i> - bearing bacteria	<i>nxrB</i> - bearing bacteria	<i>narG</i> - bearing bacteria	<i>nosZ</i> - bearing bacteria
PNR	0.687*	-0.609*	0.631*	-0.225	0.434	-0.01	0.372

* Indicate correlations are significant at 5% level; PNR: potential nitrification rate.

Table S4. Information of AOB operational Units (OTUs) in different trearments

Group	OTU	CK		U		NI		UI	
<i>Nitrosospira</i> Cluster 3a	OTU52	3012±90	a	2265±395	ab	1146±116	b	3319±293	a
	OTU95	143±11	ab	524±75	a	16±2	b	528±71	a
	OTU222	0	b	12±5	b	145±34	a	7±3	b
	OTU84	0	b	84±28	a	0	b	29±2	b
	OTU183	0	b	111±63	a	8±3	b	31±3	ab
	OTU28	23±7	a	132±26	a	62±11	a	83±6	a
	OTU214	150±95	a	54±10	a	338±73	a	159±41	a
	OTU88	53±12	b	764±136	a	55±34	b	157±52	b
	OTU13	46±10	ab	28±3	ab	17±6	b	56±15	a
	OTU21	169±29	a	78±27	ab	48±12	b	69±14	b
<i>Nitrosospira</i> Cluster 3b	OTU86	3514±203	ab	969±167	b	4636±707	a	1874±249	b
	total	7110±239	a	5021±208	b	6471±44	a	6312±152	a
	OTU178	8±1	b	215±54	a	109±28	ab	47±9	ab
	OTU19	124±42	b	323±31	a	0	b	186±53	ab
	OTU11	52±14	b	335±59	a	11±4	b	119±7	b
	OTU70	27±9	c	433±57	a	0	c	211±45	b
	OTU89	39±14	b	142±15	a	0	c	50±4	b
	OTU202	261±34	bc	749±38	a	128±6	c	476±52	b
	OTU75	215±25	b	532±13	a	79±7	b	303±25	ab
	OTU10	52±20	b	88±19	b	1012±215	a	165±32	ab
	total	778±158	b	2817±167	a	1339±437	b	1558±212	b

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<i>Nitrospira</i>									
	OTU198	0	a	45±15	a	0	a	118±30	a
Cluster 4									
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Note: Values represent means ± standard errors (n = 3). Lower case letters in column indicate significant differences between treatment means (*p* < 0.05). CK: no nitrogen fertilizer application, U: urea, UI: urea with NBPT; NI: urea with DMPP.

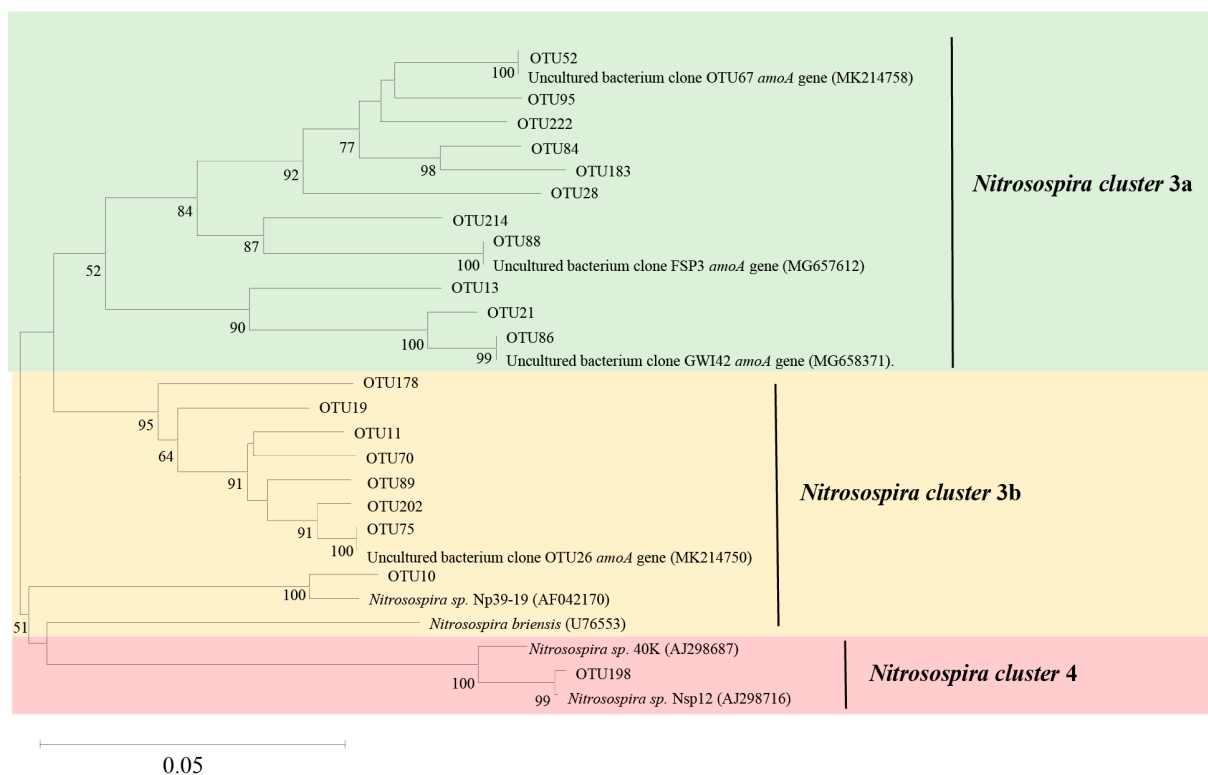


Figure S1. Phylogenetic trees (NJ) of the partial AOB *amoA* gene sequences. The most abundant OTUs ($n = 20$, more than 97% numbers of total OTU) and reference sequences available downloaded from NCBI were used to construct the phylogenetic tree. Bootstrap values of $> 50\%$ based on 1000 replicates are shown next to the branches. Bars indicate 0.05 substitutions per position.

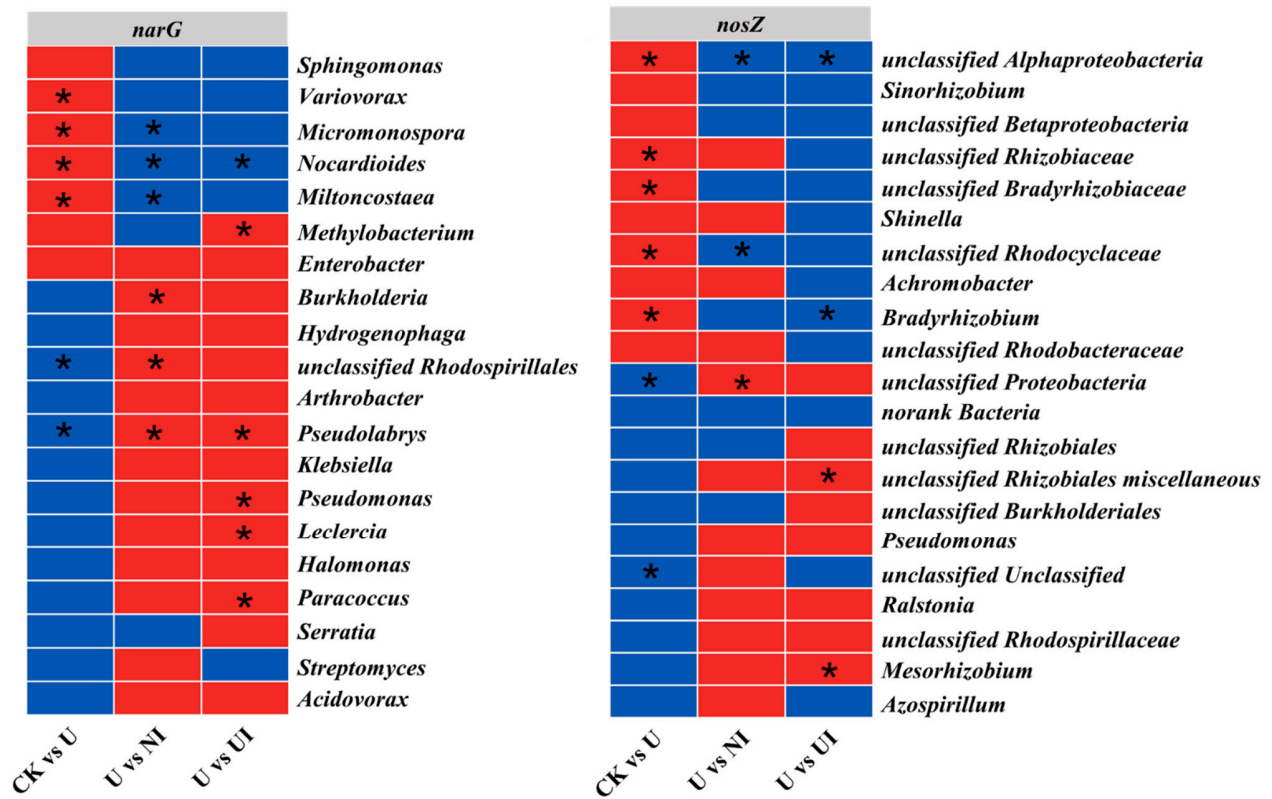


Figure S2. Heatmap depicting significant differentially abundant genera (relative abundance > 0.01%) between pairs of treatments in *narG*-bearing and *nosZ*-bearing bacterial populations. Red and blue signifies greater and reduced prevalence respectively in the first treatment of the treatment comparison. * Indicate significant differences for genera abundance between pairs of treatments at $p < 0.05$.

Reference

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