Supplementary Material for

Soil pH and phosphorus drive the canonical nitrifiers and comammox *Nitrospira* communities in citrus orchards with different cultivating years

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1. Supplementary Figures

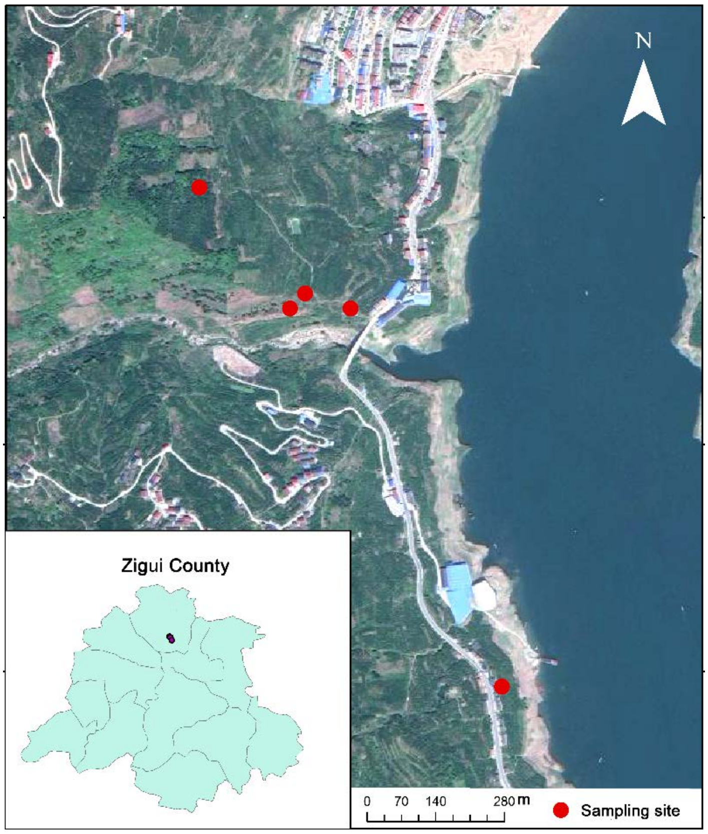


Fig. S1 The sampling sites of this study in Zigui County. Detailed information about the sampling sites is listed in Supplementary Table S1.

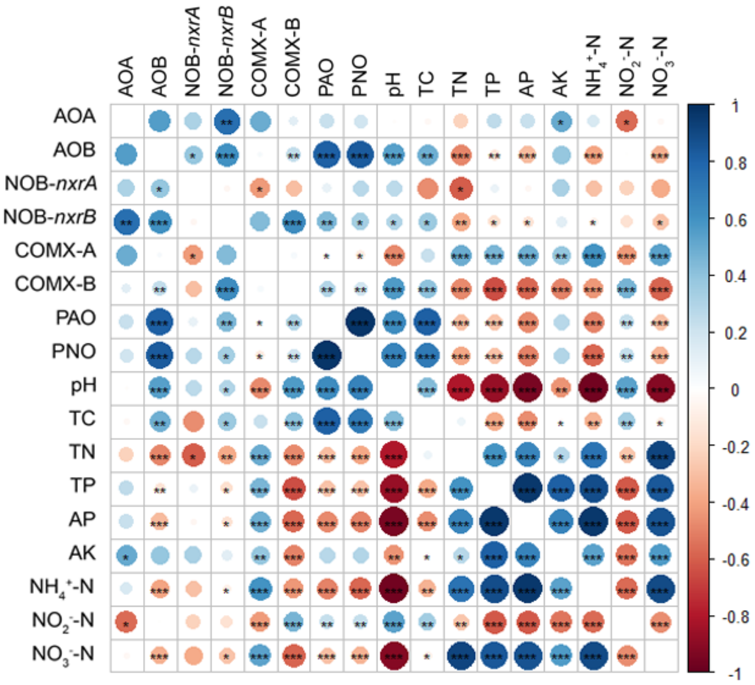
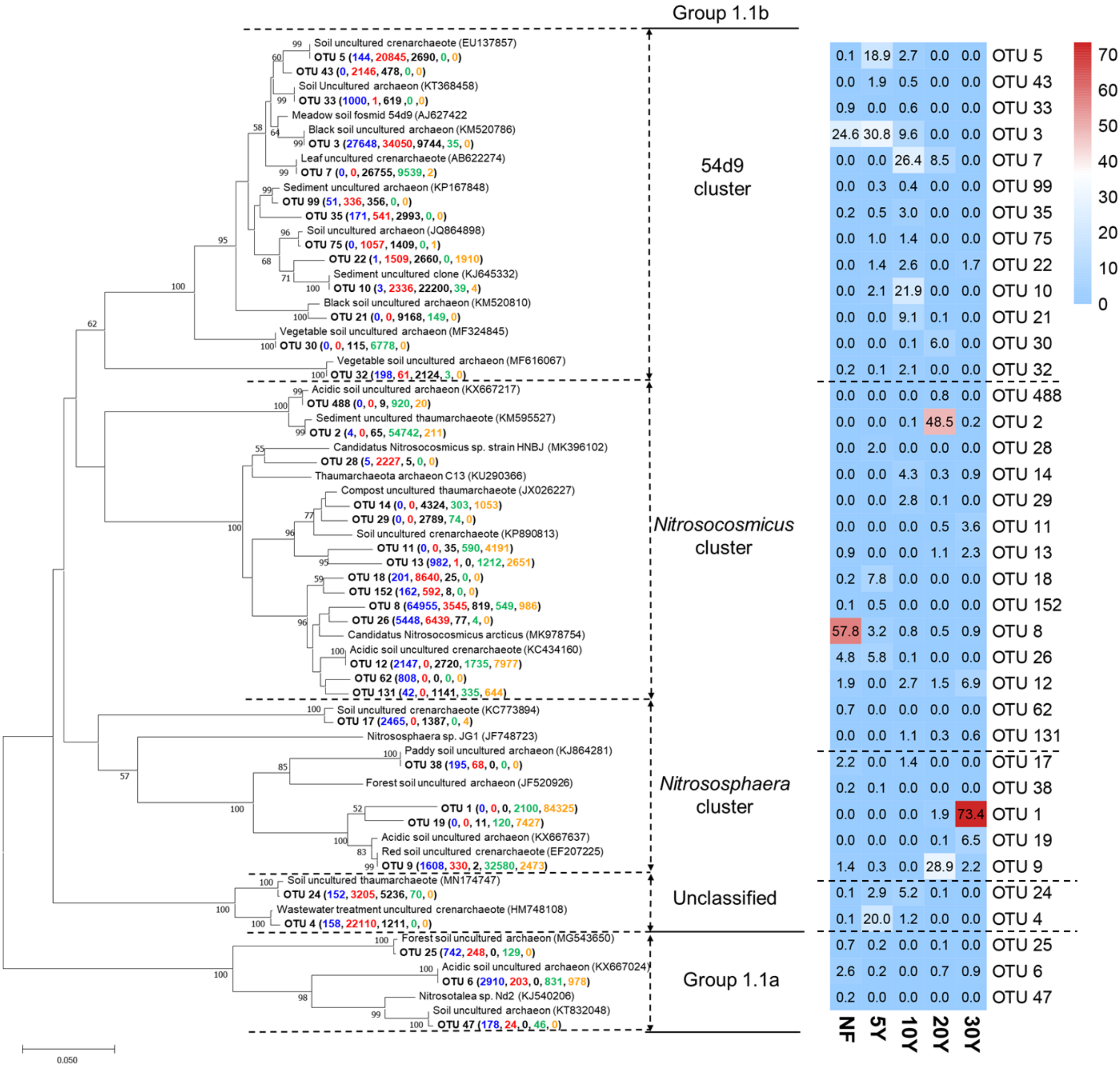
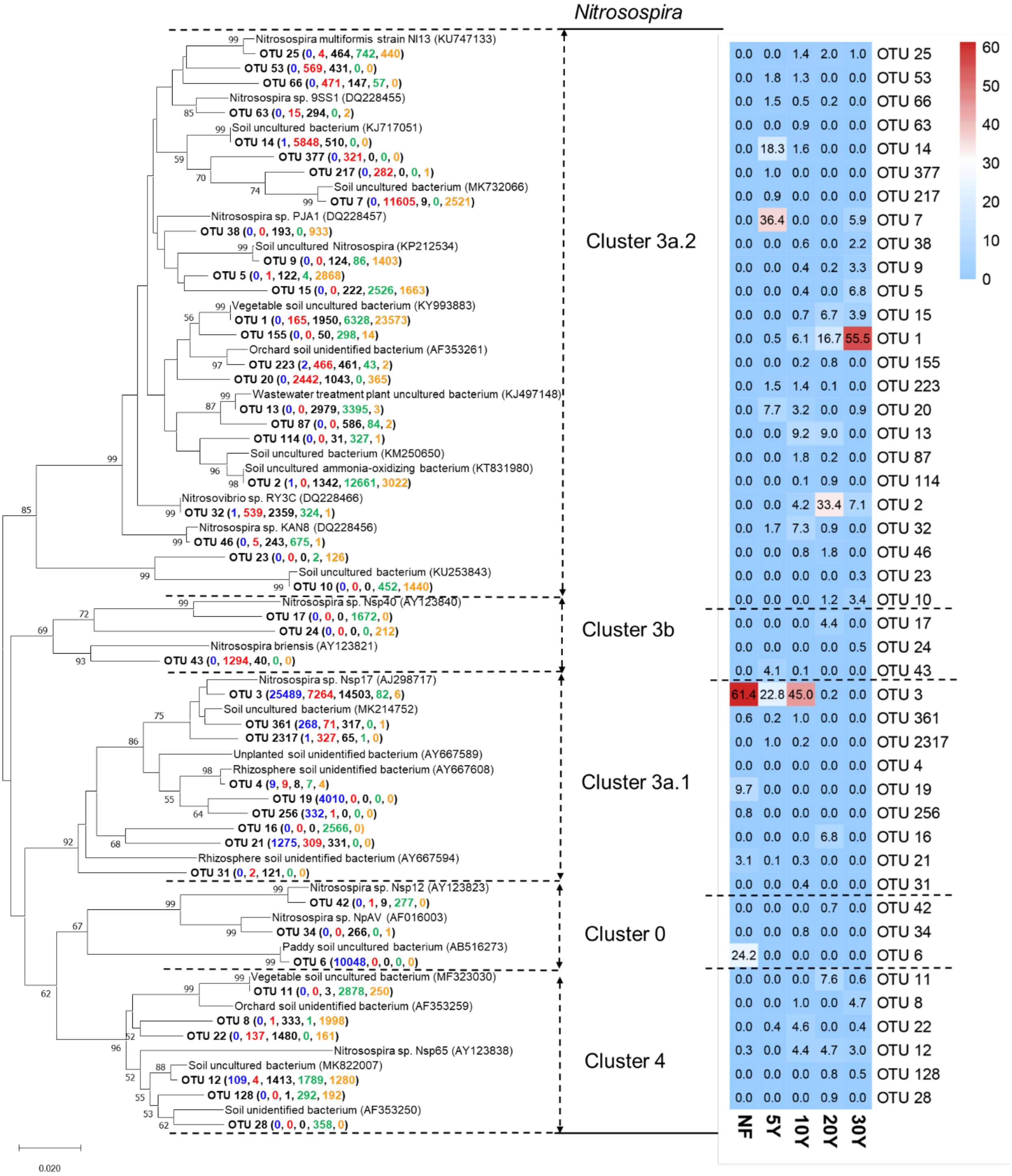


Fig. S2 Pairwise comparisons between physicochemical variables, potential nitrification activity and nitrifying abundance. The color gradient and circle denote correlation coefficients. Single asterisk, double asterisk and three asterisks represent significance at $P < 0.05$, 0.01 and 0.001, respectively.



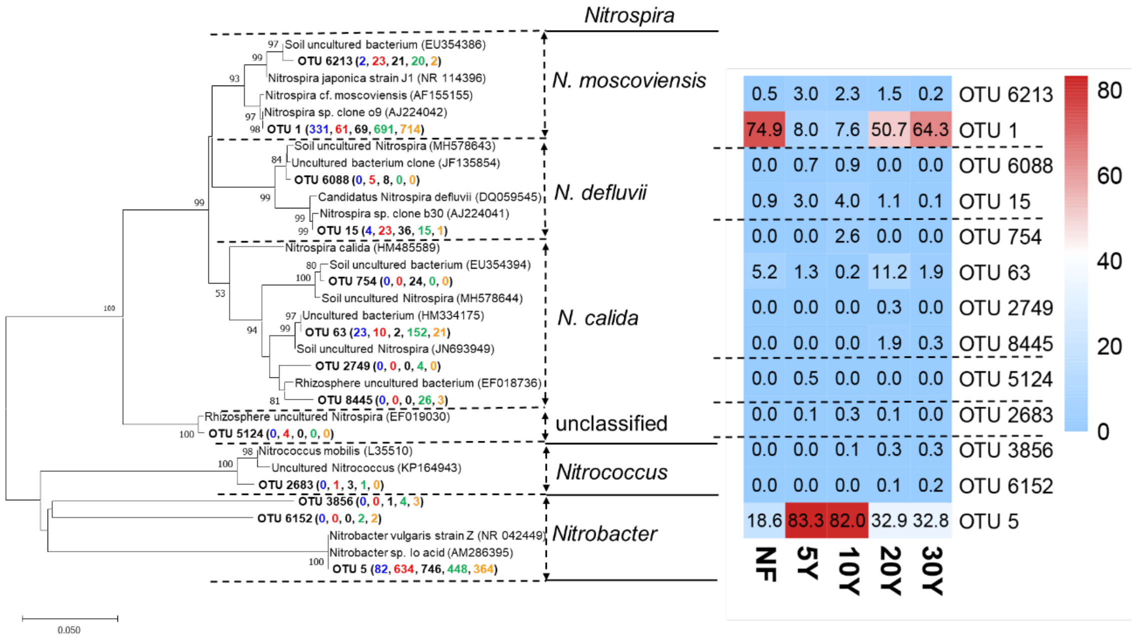
NF	5Y	10Y	20Y	30Y	
0.1	18.9	2.7	0.0	0.0	OTU 5
0.0	1.9	0.5	0.0	0.0	OTU 43
0.9	0.0	0.6	0.0	0.0	OTU 33
24.6	30.8	9.6	0.0	0.0	OTU 3
0.0	0.0	26.4	8.5	0.0	OTU 7
0.0	0.3	0.4	0.0	0.0	OTU 99
0.2	0.5	3.0	0.0	0.0	OTU 35
0.0	1.0	1.4	0.0	0.0	OTU 75
0.0	1.4	2.6	0.0	1.7	OTU 22
0.0	2.1	21.9	0.0	0.0	OTU 10
0.0	0.0	9.1	0.1	0.0	OTU 21
0.0	0.0	0.1	6.0	0.0	OTU 30
0.2	0.1	2.1	0.0	0.0	OTU 32
0.0	0.0	0.0	0.8	0.0	OTU 488
0.0	0.0	0.1	48.5	0.2	OTU 2
0.0	2.0	0.0	0.0	0.0	OTU 28
0.0	0.0	4.3	0.3	0.9	OTU 14
0.0	0.0	2.8	0.1	0.0	OTU 29
0.0	0.0	0.0	0.5	3.6	OTU 11
0.9	0.0	0.0	1.1	2.3	OTU 13
0.2	7.8	0.0	0.0	0.0	OTU 18
0.1	0.5	0.0	0.0	0.0	OTU 152
57.8	3.2	0.8	0.5	0.9	OTU 8
4.8	5.8	0.1	0.0	0.0	OTU 26
1.9	0.0	2.7	1.5	6.9	OTU 12
0.7	0.0	0.0	0.0	0.0	OTU 62
0.0	0.0	1.1	0.3	0.6	OTU 131
2.2	0.0	1.4	0.0	0.0	OTU 17
0.2	0.1	0.0	0.0	0.0	OTU 38
0.0	0.0	0.0	1.9	73.4	OTU 1
0.0	0.0	0.0	0.1	6.5	OTU 19
1.4	0.3	0.0	28.9	2.2	OTU 9
0.1	2.9	5.2	0.1	0.0	OTU 24
0.1	20.0	1.2	0.0	0.0	OTU 4
0.7	0.2	0.0	0.1	0.0	OTU 25
2.6	0.2	0.0	0.7	0.9	OTU 6
0.2	0.0	0.0	0.0	0.0	OTU 47

Fig. S3. Phylogenetic analysis of the AOA *amoA* gene in different soils, and heatmap displaying the proportion of each OTU to total OTUs in each soil. Numbers with blue, red, black, green and yellow colors represent sequencing numbers in NF, 5Y, 10Y, 20Y and 30Y soils, respectively. Bootstrap values higher than 50% are indicated at the branch nodes. The scale bars represent 5% nucleic acid sequence divergence.



NF	5Y	10Y	20Y	30Y	
0.0	0.0	1.4	2.0	1.0	OTU 25
0.0	1.8	1.3	0.0	0.0	OTU 53
0.0	1.5	0.5	0.2	0.0	OTU 66
0.0	0.0	0.9	0.0	0.0	OTU 63
0.0	18.3	1.6	0.0	0.0	OTU 14
0.0	1.0	0.0	0.0	0.0	OTU 377
0.0	0.9	0.0	0.0	0.0	OTU 217
0.0	36.4	0.0	0.0	5.9	OTU 7
0.0	0.0	0.6	0.0	2.2	OTU 38
0.0	0.0	0.4	0.2	3.3	OTU 9
0.0	0.0	0.4	0.0	6.8	OTU 5
0.0	0.0	0.7	6.7	3.9	OTU 15
0.0	0.5	6.1	16.7	55.5	OTU 1
0.0	0.0	0.2	0.8	0.0	OTU 155
0.0	1.5	1.4	0.1	0.0	OTU 223
0.0	7.7	3.2	0.0	0.9	OTU 20
0.0	0.0	9.2	9.0	0.0	OTU 13
0.0	0.0	1.8	0.2	0.0	OTU 87
0.0	0.0	0.1	0.9	0.0	OTU 114
0.0	0.0	4.2	33.4	7.1	OTU 2
0.0	1.7	7.3	0.9	0.0	OTU 32
0.0	0.0	0.8	1.8	0.0	OTU 46
0.0	0.0	0.0	0.0	0.3	OTU 23
0.0	0.0	0.0	1.2	3.4	OTU 10
0.0	0.0	0.0	4.4	0.0	OTU 17
0.0	0.0	0.0	0.0	0.5	OTU 24
0.0	4.1	0.1	0.0	0.0	OTU 43
61.4	22.8	45.0	0.2	0.0	OTU 3
0.6	0.2	1.0	0.0	0.0	OTU 361
0.0	1.0	0.2	0.0	0.0	OTU 2317
0.0	0.0	0.0	0.0	0.0	OTU 4
9.7	0.0	0.0	0.0	0.0	OTU 19
0.8	0.0	0.0	0.0	0.0	OTU 256
0.0	0.0	0.0	6.8	0.0	OTU 16
3.1	0.1	0.3	0.0	0.0	OTU 21
0.0	0.0	0.4	0.0	0.0	OTU 31
0.0	0.0	0.0	0.7	0.0	OTU 42
0.0	0.0	0.8	0.0	0.0	OTU 34
24.2	0.0	0.0	0.0	0.0	OTU 6
0.0	0.0	0.0	7.6	0.6	OTU 11
0.0	0.0	1.0	0.0	4.7	OTU 8
0.0	0.4	4.6	0.0	0.4	OTU 22
0.3	0.0	4.4	4.7	3.0	OTU 12
0.0	0.0	0.0	0.8	0.5	OTU 128
0.0	0.0	0.0	0.9	0.0	OTU 28

Fig. S4. Phylogenetic analysis of the AOB *amoA* gene in different soils, and heatmap displaying the proportion of each OTU to total OTUs in each soil.



NF	5Y	10Y	20Y	30Y	
0.5	3.0	2.3	1.5	0.2	OTU 6213
74.9	8.0	7.6	50.7	64.3	OTU 1
0.0	0.7	0.9	0.0	0.0	OTU 6088
0.9	3.0	4.0	1.1	0.1	OTU 15
0.0	0.0	2.6	0.0	0.0	OTU 754
5.2	1.3	0.2	11.2	1.9	OTU 63
0.0	0.0	0.0	0.3	0.0	OTU 2749
0.0	0.0	0.0	1.9	0.3	OTU 8445
0.0	0.5	0.0	0.0	0.0	OTU 5124
0.0	0.1	0.3	0.1	0.0	OTU 2683
0.0	0.0	0.1	0.3	0.3	OTU 3856
0.0	0.0	0.0	0.1	0.2	OTU 6152
18.6	83.3	82.0	32.9	32.8	OTU 5

Fig. S5. Phylogenetic analysis of the NOB 16S rRNA gene in different soils, and heatmap displaying the proportion of each OTU to total OTUs in each soil.

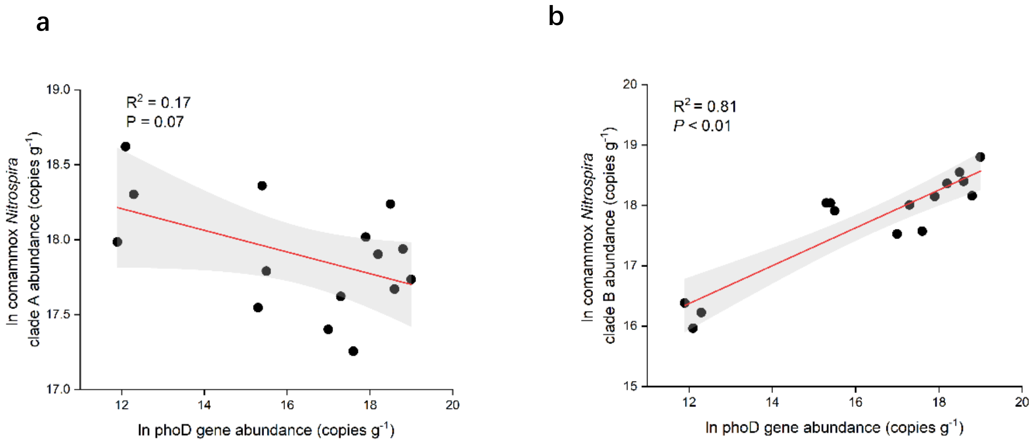


Fig. S6. The associations between *phoD* gene abundance and cmoammox *Nitrospira* clade A and clade B *amoA* gene abundances.

2. Supplementary Tables

Table S1 Geographic location of sampling sites and morphological characteristics of trees

	NF	5Y	10Y	20Y	30Y
Longitude	110°40′25″	110°40′31″	110°40′35″	110°40′32"	110°40′45″
Latitude	31°3′47″	31°3′39″	31°3′39″	31°3′40″	31°3′16″
Altitude (m)	290	220	200	230	190
Tree height (cm)	-	145	153	186	242
Tree diameter (cm)	-	14	43	32	60
Tree crown (cm)	-	84	148	124	195

Table S2 Physicochemical properties of each soil used in this study

	NF	5Y	10Y	20Y	30Y
pH	7.59±0.03b	8.48±0.01a	7.38±0.02c	4.96±0.01d	4.29±0.01e
TC (g kg^{-1})	17.60±0.26b	30.80±0.05a	7.76±0.03e	16.63±0.03c	12.93±0.06d
TN (g kg^{-1})	1.30±0.00c	1.30±0.00c	1.00±0.00d	1.90±0.00a	1.66±0.03b
TP (g kg^{-1})	0.21±0.01e	0.61±0.01d	0.85±0.01c	1.15±0.00b	1.43±0.05a
AP (mg kg^{-1})	5.00±0.26e	24.5±4.0d	72.0±2.1c	131.8±0.5b	188.2±0.2a
AK (mg kg^{-1})	10.6±0.2d	36.3±0.4b	34.5±0.1c	35.2±0.2c	40.0±0.1a
NH_4^+-N (mg kg^{-1})	4.13±0.28d	4.12±0.29d	6.47±0.69c	21.95±0.36b	35.48±0.92a
NO_3^--N (mg kg^{-1})	4.54±0.21c	8.20±0.54b	4.79±0.11c	20.07±0.27a	19.19±0.82a
NO_2^--N (mg kg^{-1})	1.16±0.03a	1.09±0.02ab	1.00±0.04ab	1.00±0.01ab	0.91±0.02b

Table S3 Primers and conditions used for qPCR and MiSeq sequencing

Primer Name	Primer sequence (5′-3′)	Target gene	Thermal Profile	Molecular analysis	Reference
Arch-*amo*AF Arch-*amo*AR	STA ATG GTC TGG CTT AGA CG GCG GCC ATC CAT CTG TAT GT	Archaeal *amoA*	94°C, 1min; 40 × (94°C, 10s; 55°C, 30s; 72°C, 60s with plate read)	qPCR	(Francis et al., 2005)
*amo*A-1F *amo*A-2R	GGG GTT TCT ACT GGT GGT CCC CTC KGS AAA GCC TTC TTC	Bacterial *amoA*	95°C, 1min; 40 × (95°C, 10s; 57°C, 30s; 72°C, 60s with plate read)	qPCR and MiSeq sequencing	(Rotthauwe et al., 1997)
comaA-244F comaA-659R	TAYAAYTGGGTSAAYTA ARATCATSGTGCTRTG	Comammox clade A *amoA*	95°C, 10min; 40 × (94°C, 30s; 52°C, 35s; 72°C, 35s with plate read)	qPCR	(Pjevac et al., 2017)
comaB-244F comaB-659R	TAYTTCTGGACRTTYTA ARATCCARACDGTGTG	Comammox clade B *amoA*	95°C, 10min; 40 × (94°C, 30s; 52°C, 35s; 72°C, 35s with plate read)	qPCR	(Pjevac et al., 2017)
F1norA R2norA	CAGACCGACGTGTGCGAAAG TCCACAAGGAACGGAAGGTC	*Nitrobacter nxrA*	95°C, 5min; 40 × (95°C, 15s; 55°C, 15s; 72°C, 35s with plate read)	qPCR	(Attard et al., 2010)
*nxr*B169f *nxr*B 638r	TACATGTGGTGGAACA CGGTTCTGGTCRATCA	*Nitrospira nxrB*	96°C, 5min; 35 × (95°C, 40s; 56°C, 40s; 72°C, 90s with plate read)	qPCR	(Pester et al., 2014)
Arch-amoA26F Arch-amoA417R	GACTACATMTTCTAYACWGAYTGGGC GGKGTCATRTATGGWGGYAAYGTTGG	Archaeal *amoA*	95°C, 5min; 35 × (95°C, 30s; 60°C, 30s; 72°C, 45s); 72°C, 7min	MiSeq sequencing	(Park et al., 2008)

Continued Table S3

Primer Name	Primer sequence (5′-3′)	Target gene	Thermal Profile	Molecular analysis	Reference
A189Y C576r (the first step)	GGNGACTGGGAYTTYTGG GAAGCCCATRTARTCNGCC	Comammox *Nitrospira amoA*	94°C, 5min; 20 × (94°C, 1 min; 52°C, 50s; 72°C, 50 s); 72°C, 7min	MiSeq sequencing	(Xia al., 2018)
CA209f C576r-barcode (the second step)	GAYTGGAARGAYCGNCA GAAGCCCATRTARTCNGCC		94°C, 5min; 35 × (94°C, 1 min; 50°C, 50s; 72°C, 50s); 72°C, 10min		
515F 907R	GTG CCA GCM GCC GCG G CCG TCA ATT CMT TTR AGT TT	Universal 16S rRNA	95°C, 3min; 35 × (95°C, 45s; 56°C, 45s; 72°C, 60s); 72°C, 7min	MiSeq sequencing	(Stubner, 2002)

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