

1 **Supplemental Material for**

2 Active ammonia oxidizers in an acid soil are phylogenetically closely related to
3 neutrophilic archaeon

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14 **Supplemental Table S1**

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18 Table S1. Pyrosequencing summary of the total 16S rRNA genes in the fractionated DNA from SIP microcosms after incubation for 56 days

DNA fractions	High Quality Read Number*			Proportion of nitrifier-like 16S rRNA genes to the total 16S rRNA gene reads in each DNA fractions, %								
				AOA†			AOB†			NOB†		
	¹³ CO ₂	¹² CO ₂	¹³ CO ₂ +C ₂ H ₂	¹³ CO ₂	¹² CO ₂	¹³ CO ₂ +C ₂ H ₂	¹³ CO ₂	¹² CO ₂	¹³ CO ₂ +C ₂ H ₂	¹³ CO ₂	¹² CO ₂	¹³ CO ₂ +C ₂ H ₂
14	648	2176	2722	0.80±0.22 ^b	15.35±4.97 ^a	0.51±0.25 ^b	- ^a	0.08±0.14 ^a	0.02±0.03 ^a	1.69±1.59 ^a	0.85±0.21 ^a	0.64±0.45 ^a
13	3130	6072	1939	3.04±0.97 ^b	11.93±1.08 ^a	0.06±1.03 ^c	- ^a	0.02±0.02 ^a	0.01±0.01 ^a	0.79±0.57 ^a	0.48±0.15 ^a	0.36±0.33 ^a
12	5254	8043	3768	2.73±1.85 ^b	13.53±0.39 ^a	0.54±0.94 ^b	0.04±0.05 ^b	0.28±0.11 ^a	0.05±0.03 ^b	0.99±0.45 ^b	2.48±0.22 ^a	0.54±0.51 ^b
11	7348	7879	6538	0.87±0.35 ^b	3.54±1.37 ^a	0.22±0.38 ^b	0.04±0.02 ^a	0.16±0.10 ^a	0.02±0.02 ^b	1.75±0.34 ^b	7.82±1.62 ^a	1.21±0.11 ^b
10	7417	7801	8046	0.11±0.08 ^a	0.19±0.03 ^a	---	0.03±0.01 ^a	0.01±0.02 ^a	0.01±0.02 ^a	0.95±0.17 ^b	2.44±0.27 ^a	0.39±0.09 ^c
9	8947	8059	7320	0.05±0.04 ^a	0.22±0.03 ^a	---	0.01±0.01 ^a	0.01±0.01 ^a	---	0.75±0.18 ^a	1.11±0.17 ^a	0.15±0.06 ^b
8	8205	7112	7554	0.17±0.10 ^a	0.31±0.04 ^a	0.01±0.02 ^b	0.01±0.01 ^a	0.01±0.01 ^a	---	6.07±1.00 ^a	0.89±0.21 ^b	0.20±0.09 ^c
7	5366	3790	3158	3.15±0.59 ^a	0.78±0.26 ^b	0.10±0.17 ^c	0.48±0.58 ^a	0.03±0.03 ^a	0.02±0.03 ^a	17.60±1.37 ^a	1.20±0.30 ^b	0.48±0.06 ^c
6	4243	11884	8919	22.64±3.28 ^a	1.06±0.71 ^b	---	1.63±0.36 ^a	0.03±0.02 ^b	0.01±0.01 ^b	35.31±7.17 ^a	0.85±0.28 ^b	0.51±0.45 ^b
5	6802	8149	14735	10.08±1.69 ^a	1.51±1.17 ^b	0.11±0.20 ^b	0.48±0.25 ^a	0.02±0.02 ^b	0.08±0.11 ^b	24.76±3.98 ^a	0.75±0.26 ^b	0.49±0.20 ^b
4	2309	7575	11944	1.05±0.99 ^a	0.82±0.21 ^a	0.22±0.38 ^a	0.12±0.15 ^a	0.09±0.12 ^a	0.03±0.04 ^a	3.23±3.81 ^a	0.73±0.20 ^a	0.60±0.06 ^a
3	1530	3398	8542	1.22±0.66 ^a	1.23±0.36 ^a	0.15±0.26 ^b	0.07±0.09 ^a	0.08±0.06 ^a	0.03±0.03 ^a	1.65±0.58 ^a	0.86±0.03 ^b	0.52±0.31 ^b
Total reads	684,972											

19 * denotes the mean of biological triplicate of 16S rRNA gene pyrosequencing was used.

20 --- denotes the sequence was not detectable.

21 † The same letter indicates no significant difference ($P > 0.05$) using analysis of variance.

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23 Table S2. Phylogenetic classification of ¹³C-labeled 16S rRNA genes of nitrifying communities

Microbe	Taxonomy	Reads Numbers in ¹³ C-DNA *
AOA	<i>Nitrososphaera</i>	1815 (100%)
	<i>Nitrosopumilis</i>	0
	<i>Nitrosotalea</i>	0
	<i>Nitrosocaldus</i>	0
AOB	<i>Nitrospira</i> cluster 3	127 (100%)
	<i>Nitrosomonas</i>	0
NOB	<i>Nitrospira</i>	4109 (99.6%)
	<i>Nitrobacter</i>	17 (0.4%)

24 * denotes the 16S rRNA gene reads of each phylotype that were pooled from heavy DNA-fraction-5,
 25 6 and 7 from labelled treatments. The number of 16S rRNA gene reads is followed by the values in
 26 bracket representing sequence proportion of each phylotype to the total AOA, AOB or NOB 16S
 27 rRNA genes.

28 Table S3. The putative contribution of AOA and AOB to the nitrification activity in the acidic soil tested.

Treatment*	Microbes	Copy number of genes in total DNA (g ⁻¹ d.w.s)†	Ratio of gene copy number in heavy DNA fractions to total gene copy number across the entire DNA fractions‡	Copy number of genes in 'heavy' DNA fractions (g ⁻¹ d.w.s)	Number of labeled cells (g ⁻¹ d.w.s)§	NO ₃ ⁻ -N after 56-day incubation (µg g ⁻¹ d.w.s)	Putative cell-specific ammonia oxidation rate (fmol cell ⁻¹ h ⁻¹)
¹³ CO ₂	AOA (<i>amoA</i> gene)	3.12×10 ⁸	84.6%	2.64×10 ⁸	2.64×10 ⁸	178.2	0.036 (0.058)
	AOB (<i>amoA</i> gene)	3.20×10 ⁷	2.10%	6.72×10 ⁵	2.69×10 ⁵		35.2 (5.3~7.1)

29 * represents the labeled treatments incubated with ¹³CO₂ in the absence of acetylene with three true replicates.

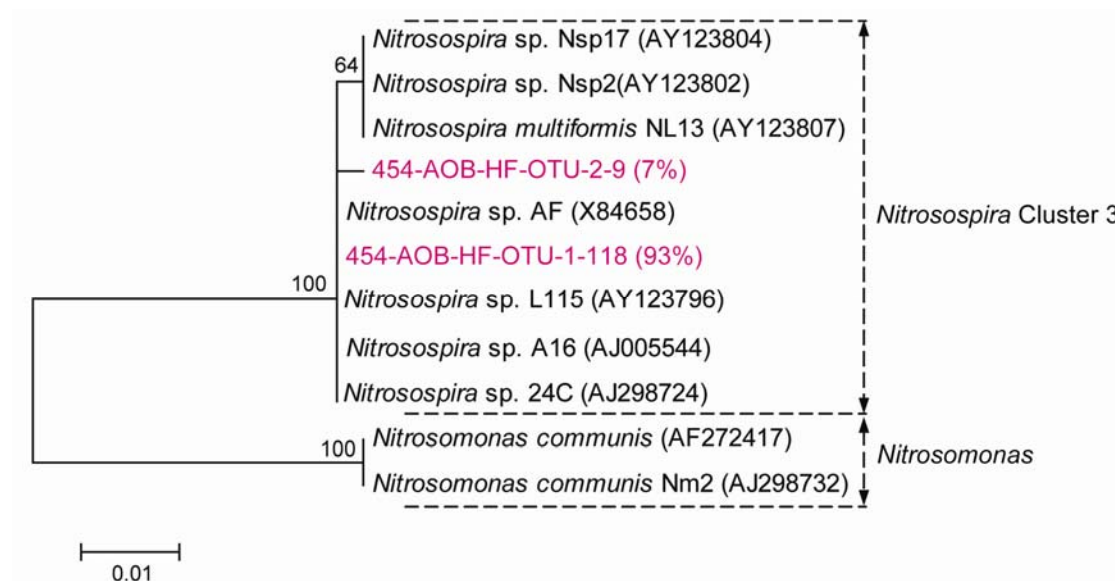
30 † represents the copy numbers of archaeal or bacterial *amoA* genes in the total DNA in g⁻¹ d.w.s.

31 ‡ represents the ratio of archaeal or bacterial *amoA* gene copy numbers in the heavy DNA fraction-5, 6 and 7 to the sum of archaeal or bacterial *amoA* gene copies in all DNA gradient fractions.

33 § represents cell numbers of ¹³C-labeled AOA and AOB assuming each AOB and AOA cell contains 2.5 and 1.0 of *amoA* gene copy respectively.

34 || represents the putative cell-specific ammonia oxidation rate of AOA or AOB, and it was calculated by dividing the production rate of soil nitrate by the ¹³C-labeled AOA or AOB cell numbers in the ¹³C-labeled DNA fractions from the labelled treatment spanning 8-week incubation, assuming the soil nitrate production resulted solely from either ¹³C-labeled AOA or AOB. The number in bracket of 0.058 represents the cell-specific rate of ammonia oxidation of strain JG1 (1), and the numbers of 5.3 and 7.1 represent the cell-specific rates of ammonia oxidation of strains L115 and AF (2), respectively.

38 **Supplementary Figure S1.** Phylogenetic relationship of the 16S rRNA genes of AOB
 39 in the ¹³C-DNA from the labeled microcosms after incubation for 56 days. The 16S
 40 rRNA reads of AOB in the ‘heavy’ DNA fraction-5, 6 and 7 were pooled for analysis
 41 with a total number of 127. The designation 454-AOB-HF-OTU-1-118-(93%) indicates
 42 that OTU-1 contains 118 sequences with >97% identity, accounting for 93% of the total
 43 16S rRNA sequences of AOB in the ¹³C-DNA. The scale bar represents nucleotide acid
 44 substitution percentage.



46 **Reference**

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