Supplementary Information

Functional Characterization and Diversity of Ammonia-Oxidizing Microorganisms in Streams South of the Dabie Mountains, China

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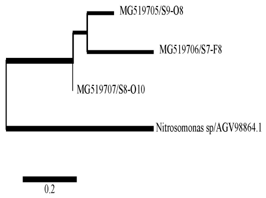

Figure S1. Phylogenetic tree the neighbor-joining showing the phylogenetic relationships of ammonia oxidizing bacteria (AOB) gene sequences. Bootstrap support values (1000 replicates) are shown. The scale bar represents 0.2; evolutionary analyses were implemented in MEGA 7.

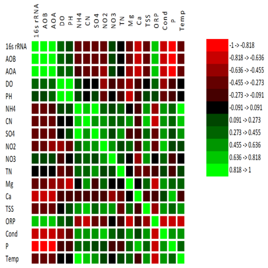

Figure S2. Spearman correlation matrix representative significant relationships between investigation parameters, for correlations among log-transformed qPCR data, gene copies (g.soil^{-1}), and physicochemical data of environments samples. The colors of the scale bar indicate the nature of the correlation with 1 denoting perfect positive correlation (green), and -1 denoting perfect negative correlation (red) were tested at $p<0.01$ and $p<0.05$. The used geo-physicochemical data of the samples applied to Xlstat (www.xlstat.com).

Table S1. The PCR primer pairs and thermal programs

Target gene	Primer	Thermal program	bp	References
16S rRNA	27F 5′-AGAGTTTGATCCTGGCTCAG-3′ 1492R 5′-(S)*ACGGCTACCTTGTTACGACT-3′	95°C, 5 min, 1 cycle 95°C for 1 min, 58°C for 45 s, 72°C for 1 min, 30 cycles 72°C for 8 min, 1 cycle	1500	[1]
Bacterial 16S rRNA gene-qPCR	27F 5′-AGAGTTTGATCCTGGCTCAG-3′ 16S-rtR 5′-GCTGCCTCCCGTAGGAGT-3′	95°C for 10 min, 1 cycle 95°C for 60 s, 60°C for 30 s, 72°C for 30 s, 40 cycles, 72°C for 8min , 1 cycle	310	[2]
Archaeal 16S rRNA gene-qPCR	ARC-rtF 5′-ACGGGGYGCAGCAGGCGCGA-3′ ARC-rtR 5′-GWATTACCGCGGCKGCTG-3′	94°C for 8 min, 1 cycle 94°C for 60 s, 56°C for 60 s, 72°C for 60 s, 45 cycles, 72°C for 6min , 1 cycle	190	[3]
AOA	Arch amoA F: 5′-TAATGGTCTGGCTTAGACG-3′ Arch amoA R: 5′-GCGGCCATCCATCTGTATGT-3′	5 min at 95 °C, 35 cycles of 45 s at 94 °C, 60 s at 53 °C, and 60 s at 72 °C, followed by 15 min at 72 °C.	635	[4]
AOB	amoA-1F: 5′-GGGGTTTCTACTGGTGGT-3′ amoA-2R KS: 5′-CCCCTCTGGAAAGCCTTCTTC-3′	5 min at 95 °C, 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C, and a final cycle consisting of 60 s at 60 °C and 10 min at 72 °C.	491	[5]

*(S= G, C), Abbreviations: AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; PCR, polymerase chain reaction

Table S2. Gene copy numbers of 16S rRNA bacteria and archaea (stream and sediment), ammonia oxidizing archaea (AOA), and ammonia oxidizing bacteria (AOB) in sampling sites. Values and standard deviations were estimated from triplicate an analysis within a single q-PCR.

	May			**August**			**October**		
Sampling Sites	**16S rRNA (copies/μg of DNA extract)**	**AOA (copies/μg of DNA extract)**	**BOA (copies/μg of DNA extract)**	**16S rRNA (copies/μg of DNA extract)**	**AOA (copies/μg of DNA extract)**	**BOA (copies/μg of DNA extract)**	**16S rRNA (copies/μg of DNA extract)**	**AOA (copies/μg of DNA extract)**	**BOA (copies/μg of DNA extract)**
S1	1.56E+09	5.23E+08	4.64E+08	1.19E+09	4.79E+08	2.03E+08	1.98E+09	3.85E+08	4.63E+08
S2	6.79E+08	5.03E+08	2.55E+08	6.79E+08	5.23E+08	3.24E+08	6.09E+08	5.21E+08	3.83E+08
S3	6.17E+08	3.22E+08	3.36E+08	6.89E+08	2.23E+08	3.45E+08	6.04E+08	3.69E+08	3.25E+08
S4	3.83E+08	3.19E+08	3.30E+07	3.87E+08	2.83E+08	3.60E+08	3.08E+08	3.92E+08	2.66E+08
S5	7.42E+08	4.41E+08	5.45E+08	7.62E+08	4.25E+08	5.05E+08	7.40E+08	4.08E+08	4.97E+08
S6	1.01E+09	5.27E+08	1.23E+08	1.25E+09	5.13E+08	1.08E+08	1.31E+09	5.22E+08	1.25E+08
S7	6.51E+08	5.28E+08	4.79E+08	6.68E+08	5.02E+08	4.53E+08	6.07E+08	5.24E+08	4.49E+08
S8	7.17E+08	4.00E+08	5.09E+08	7.79E+08	4.43E+08	5.05E+08	7.17E+08	4.68E+08	5.11E+08
S9	5.85E+08	3.78E+08	4.56E+08	4.91E+08	3.82E+08	4.60E+08	5.85E+08	4.74E+08	4.58E+08

Table S3. Comparison of AOA and AOB diversities as well as coverage estimates in sampling sites base on 95% nucleotide sequences similarity.

	AOA					AOB				
Sampling sites	**No. of clone sequenced**	**No. of OTUs**[*]	**Coverage**[**] **(C %)**	**Simpson index D**	**Shannon-Weiner**	**No. of clone sequenced**	**No. of OTUs**[*]	**Coverage**[**] **(C %)**	**Simpson index D**	**Shannon-Weiner**
S1	4	4	0	2.33	0.69	8	-	100	-	1
S2	5	4	20	2.25	0.68	6	-	100	-	1
S3	5	1	80	1.5	0.45	5	-	100	-	1
S4	5	1	80	1.5	0.45	4	-	100	-	1
S5	6	4	33.3	2.14	0.67	9	-	100	-	1
S6	5	1	80	1.5	0.4	5	-	100	-	1
S7	5	4	20	2.25	0.68	7	1	85.7	0.25	1.33
S8	14	12	14.28	2.07	0.69	9	1	88.88	0.2	1.25
S9	5	3	40	2.15	0.66	8	1	87.5	0.22	1.29
Total	54	34	37.04	1.92	0.66	61	3	95.08	0.09	1.09

–, undetected

[*] Unique OTUs of the *amoA* sequences were determined using the DOTUR program. The coverage (C), Shannon-Weiner (H), and Simpson (D) estimators were calculated based on the OUT data.

[**] Coverage % = (1 − no. of singletons)/no. of clones sequenced × 100.

References:

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