Supporting Information for

Ammonia oxidizers in high-elevation rivers of the Qinghai-Tibetan Plateau display distinctive distribution patterns

Sibo Zhang[a], Xinghui Xia[a*], Siling Li[a], Liwei Zhang[a], Gongqin Wang[a], Meishui Li[a], Yinan Shi[a], Nengwang Chen[b]

[a] *School of Environment, Beijing Normal University / State Key Joint Laboratory of Environmental Simulation and Pollution Control, Beijing, 100875, China*

[b] Fujian Provincial Key Laboratory for Coastal Ecology and Environmental Studies, College of the Environment and Ecology, Xiamen University, Xiamen, *361102,* China

To whom correspondence should be addressed.
E-mail address: xiaxh@bnu.edu.cn;

Tel.: +86 10 58805314; fax: +86 10 58805314.

Table S1 Physicochemical properties of the overlying water samples collected from five rivers in the Qinghai-Tibetan Plateau (n = 28)

	Elevation (m.a.s.l.)	Latitude (° N)	Solar radiation (MJ m^{-2} d^{-1})	DO (mg L^{-1})	Temp. (℃)	ORP (mV)	pH	Conductivity (μS cm^{-1})	Suspended sediment concentration (g L^{-1})	NH_4-N concentration (mg L^{-1})	NO_x-N concentration (mg L^{-1})
MD	4221	34.89	21.1 / 22.6	6.12 / 7.89	16.5 / 11.3	193.0 / 124.8	8.85 / 8.63	678.7 / 1007.0	0.0069 / 0.0010	0.089 / 0.042	0.050 / 0.231
RQ	4223	34.94	21.2 / 22.5	5.96 / 7.21	13.3 / 7.2	214.7 / 127.4	8.83 / 8.49	442.0 / 490.0	0.0032 / 0.0042	0.080 / 0.041	0.253 / 0.352
DR	3918	33.77	22.1 / 22.5	5.76 / 7.25	15.8 / 9.7	160.3 / 142.6	8.44 / 8.48	465.3 / 388.0	0.0136 / 0.2513	0.050 / 0.043	0.472 / 1.134
MT	3642	33.77	22.9 / 22.0	5.97 / 7.03	15.7 / 12.5	198.0 / 132.0	8.54 / 8.60	418.0 / 159.0	0.0198 / 0.0506	0.069 / 0.036	0.555 / 0.818
JZ	3539	33.43	22.8 / 21.3	7.20 / 7.95	12.0 / 8.13	205.7 / 139.3	8.32 / 8.31	395.7 / 210.5	0.0141 / 0.0046	0.094 / 0.033	0.437 / 0.401
TK	3391	33.41	23.2 / 21.9	6.22 / 6.83	15.4 / 14.4	216.0 / 116.1	8.09 / 7.87	127.0 / 97.8	0.0644 / 0.0531	0.091 / 0.032	0.152 / 0.167
MQ	3423	33.96	23.5 / 22.6	6.09 / 7.05	17.3 / 12.8	172.7 / 294.0	8.48 / 8.41	315.5 / 125.3	0.0534 / 0.0509	0.099 / 0.038	0.481 / 0.570
JG	3100	34.68	24.2 / 23.6	7.43 / 7.97	19.9 / 14.0	127.0 / 88.7	8.46 / 8.60	328.0 / 302.0	0.1107 / 0.1112	0.105 / 0.059	0.498 / 0.590
BD	2726	35.32	25.0 / 25.5	6.41 / 7.57	19.3 / 12.6	168.0 / 101.7	8.51 / 8.54	358.0 / 338.0	0.1517 / 0.1480	0.104 / 0.044	0.691 / 0.778
TNH	2687	35.50	24.9 / 25.7	6.57 / 7.74	18.3 / 14.6	152.0 / 139.3	8.34 / 8.53	358.0 / 350.2	0.2198 / 0.4446	0.120 / 0.047	0.664 / 0.910
ZM03	3229	29.19	22.43	5.88	19.5	110.2	9.08	261.1	0.7106	0.039	0.360
ZM04	3957	29.18	24.27	6.13	17.0	88.4	7.99	210.3	0.4010	0.033	0.217
ZM05	3432	29.21	22.94	5.57	19.2	152.0	8.14	273.3	0.5217	0.036	0.140
YC	3412	29.25	22.89	5.65	18.6	98.0	8.15	275.9	0.5681	0.034	0.390
QML	4065	34.06	24.55	5.91	16.4	97.5	8.23	1271.0	0.2337	0.036	0.517
BM	3513	32.93	23.15	6.57	15.9	143.5	8.53	590.0	0.2666	0.037	0.213
XD	3690	32.31	23.6	6.38	17.1	130.3	7.68	721.0	0.0917	0.026	0.517
ZG	3774	29.67	23.81	6.51	13.9	161.6	8.40	201.6	0.0381	0.099	0.522

(I) The numbers in the left and right sides of the slash denote physicochemical factor values of water samples in the Yellow River source region during summer and spring, respectively.

(II) Data represents the mean value of three replicates.

Table S2 Pearson correlation analysis of physicochemical factors with ammonia oxidizer abundance as well as PNRs in the overlying water (n = 28)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Elevation	1.00																
2 Latitude	-.14	1.00															
3 Temp.	-.38*	-.34	1.00														
4 Solar radiation	.86**	-.03	-.32	1.00													
5 Conductivity	.45*	.27	-.01	.60**	1.00												
6 pH	.03	.28	-.07	.18	.08	1.00											
7 ORP	.09	.23	-.10	-.03	-.20	.15	1.00										
8 DO	-.23	.51**	-.63**	-.15	-.06	.10	-.15	1.00									
9 Suspended sediment concentration	-.57**	-.43*	.59**	-.51**	-.23	-.21	-.36	-.27	1.00								
10 NH_4-N	-.26	.32	.29	-.19	-.13	.24	.39*	-.08	-.14	1.00							
11 NO_x (NO_2^- + NO_3^-)-N	-.41*	.33	-.20	-.32	-.10	.13	-.09	.39*	.30	.03	1.00						
12 AOA	-.60**	-.41*	.74**	-.51**	-.36	-.04	-.18	-.42*	.86**	.22	.19	1.00					
13 AOB	-.50**	-.25	.21	-.45**	-.26	-.15	-.30	.02	.78**	-.13	.53**	.65**	1.00				
14 AOB/AOA ratio	.22	.12	-.64**	.05	.12	-.11	-.09	.45*	-.35	-.33	.09	-.64**	-.08	1.00			
15 Comammox	-.43*	-.25	-.06	-.41*	-.18	-.34	-.46*	.35	.56**	-.44*	.33	.32	.66**	.14	1.00		
16 Comammox/(AOA+AOB) ratio	.28	.14	-.29	.35	.41*	.09	-.14	.39*	-.48**	-.18	-.18	-.49**	-.38*	.20	.15	1.00	
17 Potential nitrification rates	-.72**	.45*	.12	-.62**	-.31	.22	.17	.34	.19	.44*	.65**	.31	.37	-.10	.17	-.13	1.00

$p \leqslant 0.01$

$0.01 < p \leqslant 0.05$

$p > 0.05$

*. Correlation is significant at the 0.05 level (two-tailed).

**. Correlation is significant at the 0.01 level (two-tailed).

Table S3 Results of the partial correlation analysis relating ammonia oxidizer abundance to environmental variables (n = 28)

Controlling for	Correlation with DO	Temperature	Solar radiation	Ammonium concentration	Suspended sediment concentration
AOA					
DO	—	0.68	-0.63	0.20	0.86
Temperature	0.10	—	-0.42	0.01	0.78
Solar radiation	-0.58	0.71	—	0.16	0.81
Ammonium	-0.41	0.73	-0.48	—	0.92
DO & Temperature & Solar radiation & Ammonium	—	—	—	—	0.83
Suspended sediment	-0.38	0.59	-0.16	0.68	—
AOB					
DO	—	0.28	-0.45	-0.13	0.82
Temperature	0.20	—	-0.41	-0.20	0.84
Solar radiation	-0.05	0.07	—	-0.25	0.71
Ammonium	0.01	0.26	-0.49	—	0.78
DO & Temperature & Solar radiation & Ammonium	—	—	—	—	0.80
Suspended sediment	0.38	-0.52	-0.10	-0.03	—
Comammox					
DO	—	0.22	-0.38	-0.44	0.72
Temperature	0.40	—	-0.45	-0.44	0.74
Solar radiation	0.32	-0.22	—	-0.61	0.40
Ammonium	0.35	0.07	-0.55	—	0.55
DO & Temperature & Solar radiation & Ammonium	—	—	—	—	0.59
Suspended sediment	0.62	-0.59	-0.18	-0.43	—

Grey: $p > 0.05$

Blue: $0.01 < p \leqslant 0.05$

Green: $p \leqslant 0.01$

Table S4 AOB community compositions in the overlying water samples

		Nitrosospira (%)				*Nitrosomonas* (%)		
Season	Site	C1	C3a	C10	C14	C6	C7	Nm143
Spring	TNH	57.69	7.69	7.69	0	23.08	0	3.85
	JG	59.38	12.50	6.25	0	21.88	0	0
	TK	50.00	14.71	2.94	0	32.35	0	0
	MT	63.64	0	6.06	0	30.30	0	0
	DR	16.67	23.33	6.67	0	53.33	0	0
Summer	TNH	19.35	9.68	6.45	0	61.29	3.23	0
	JG	70.00	6.67	10.00	0	13.33	0	0
	TK	6.06	6.06	54.55	0	27.27	6.06	0
	MT	48.48	6.06	18.18	0	27.27	0	0
	DR	22.86	2.86	42.86	0	31.43	0	0
	MD	3.70	18.52	22.22	0	55.56	0	0
	QML	10.34	3.45	44.83	3.45	37.93	0	0
	XD	57.58	27.27	3.03	0	12.12	0	0
	ZG	20.59	0	2.94	0	64.71	0	11.76
	ZM03	5.56	5.56	11.11	0	16.67	55.56	5.56
	ZM04	21.88	6.25	53.13	0	6.25	9.38	3.13
	ZM05	21.43	7.14	50.00	0	17.86	3.57	0

The shadow zone represents sampling sites in the Yellow River source region.

Table S5 Pearson's correlation analysis of spatial and environmental factors with AOA (AOB) community dissimilarity as determined by the Mantel test[a]

Variables	Pearson correlation coefficient (*r*)	*p*
Elevation	0.02 (0.10)	0.361 (0.762)
Latitude	0.03 (0.26)	0.326 (0.041)
Longitude	0.34 (0.21)	0.004 (0.068)
Temp.	-0.01 (0.08)	0.480 (0.243)
pH	0.08 (0.22)	0.237 (0.094)
Suspended sediment concentration	-0.04 (0.29)	0.601(0.06)
Conductivity	0.04 (0.05)	0.322 (0.245)
ORP	0.08 (0.05)	0.209 (0.335)
Ammonium	0.01 (-0.07)	0.422 (0.653)
DO	-0.04 (0.06)	0.641 (0.289)
All environmental factors [b]	0.05 (0.27)	0.296 (0.03)

[a] The Mantel test was conducted with 9999 permutations, and the distance matrixes for factors were calculated with the Euclidean method.

[b] All environmental factors included temperature, pH, suspended sediment concentration, conductivity, ORP, ammonium, and DO. Prior to the Mantel test, these environmental factor values were normalized using z-scores.

The numbers in the parentheses denote corresponding parameter values for AOB.

Table S6 Sources of reference sequences used in this study

	Source	Traits	References
AOA	the Dongjiang River	elevation is ~40 m, Guangdong province, China	(1)
	the Guyun River	elevation is ~10 m, Jiangsu province, China	KM881716.1-KM882056.1
	Qinghai lakes	in the Qinghai-Tibetan Plateau	GQ342628.1-GQ342681.1
	Arctic lakes	average annual air temperature of -20 °C	(2)
	Glacial circue lakes	in the central Spanish Pyrenees; elevation of 2,240 m	(3)
	Tibetan cold spring	in the Qinghai-Tibetan Plateau	(4)
AOB	the Yong River	elevation is ~20 m, Jiangsu province, China	(5)
	the Dongjiang River	elevation is ~40 m, Jiangsu province, China	(6)
	the Mississippi River	elevation is ~100 m	GQ906668.1-GQ906700.1
	the Yellow River estuary	elevation is ~5 m	KY130172.1-KY130403.1
	Tibetan cold spring	in the Qinghai-Tibetan Plateau	(4)

Elevation of reference rivers was estimated using the Google Earth based on their geographical location information

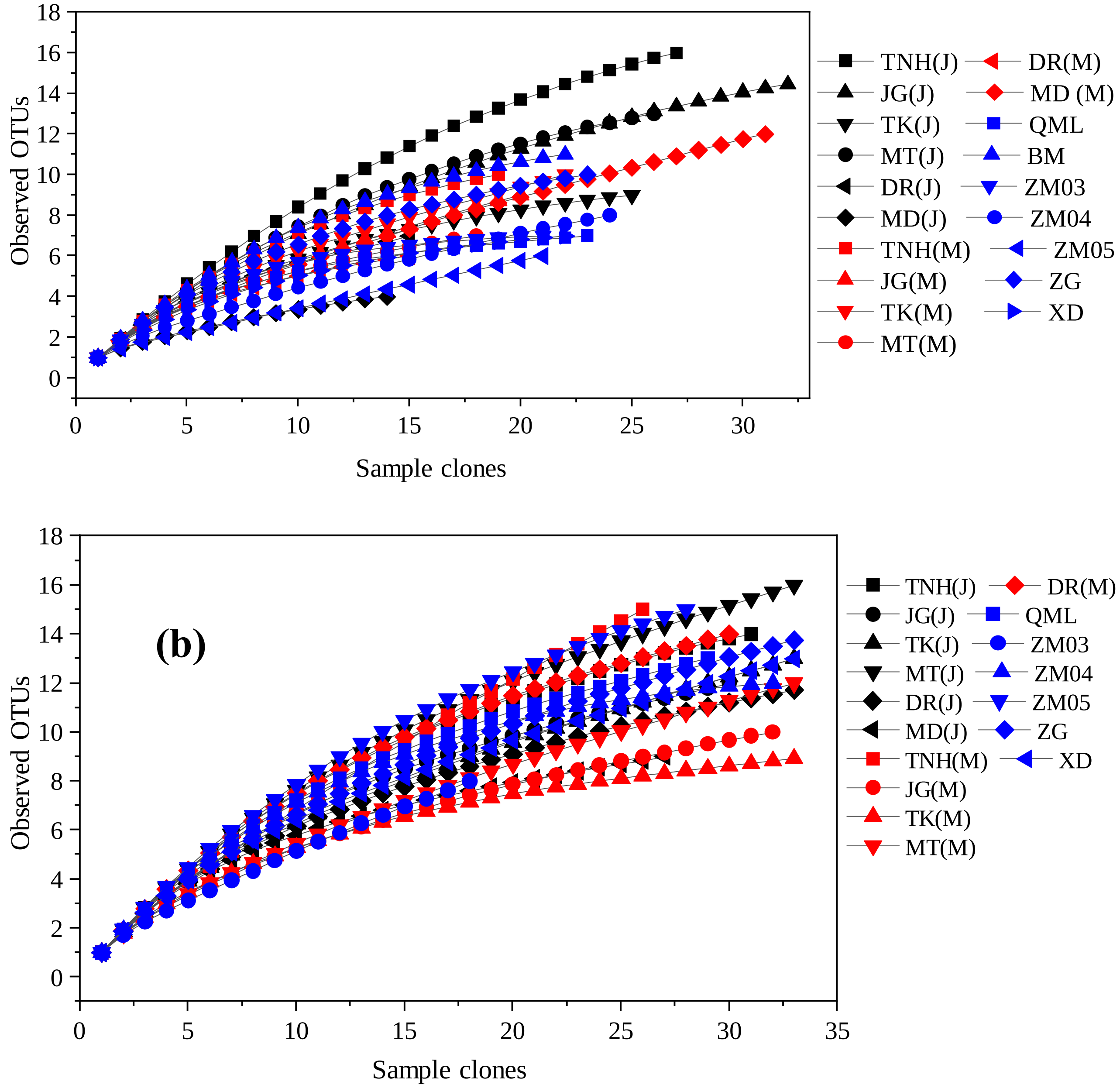

Fig. S1 Rarefaction curve analysis for archaeal (a) and bacterial (b) *amo*A gene clone libraries with 5% distance cutoff. M and J in the legend respectively represent May (spring) and July (summer).

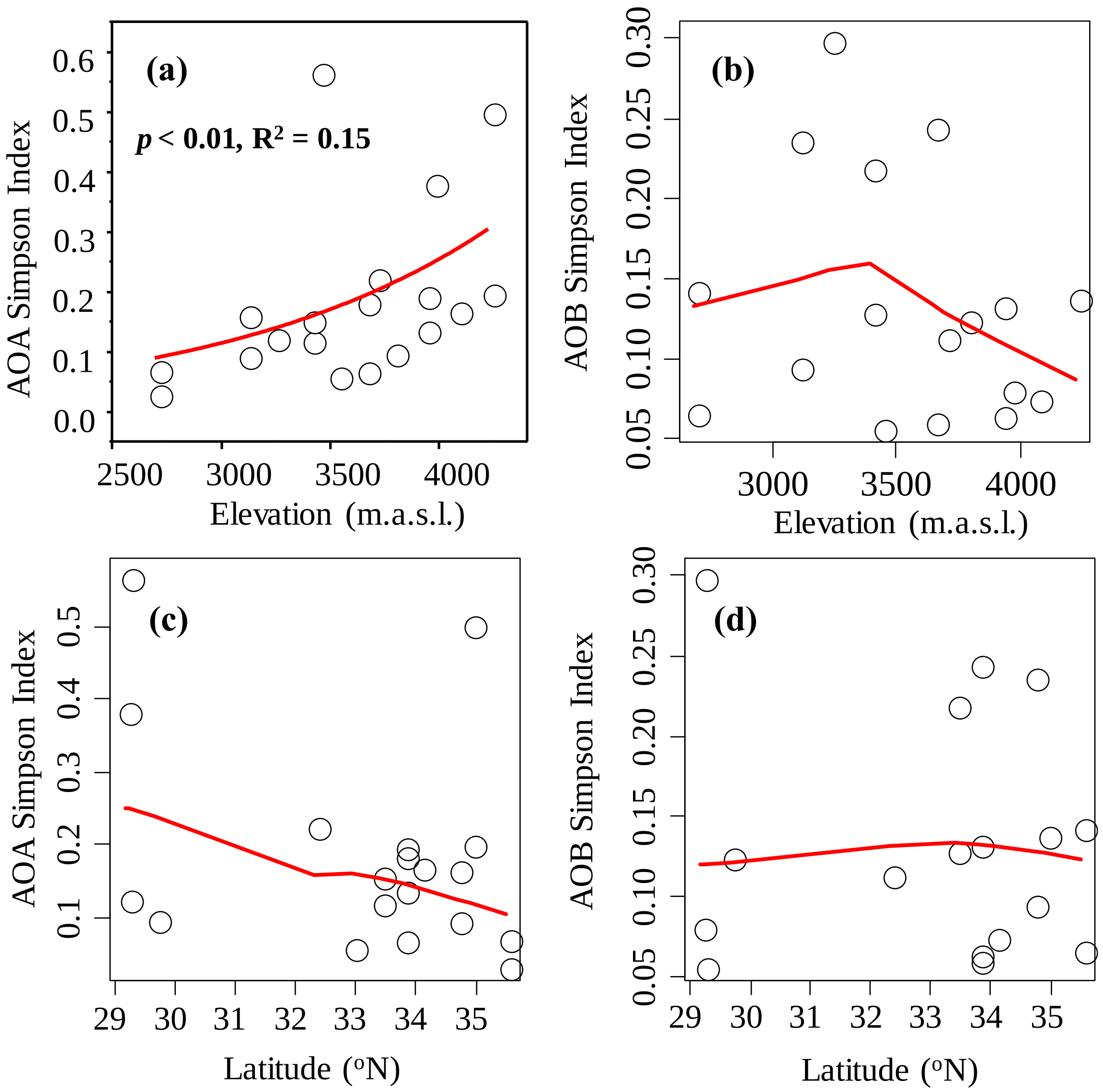

Fig. S2 Variations of AOA (a and c) and AOB (b and d) Simpson diversity along the elevation and latitude gradients in five rivers of the Qinghai-Tibetan Plateau. The trends along the elevation or latitude gradient in the b, c and d were indicated by solid lines with locally Weighted Smooth Regression.

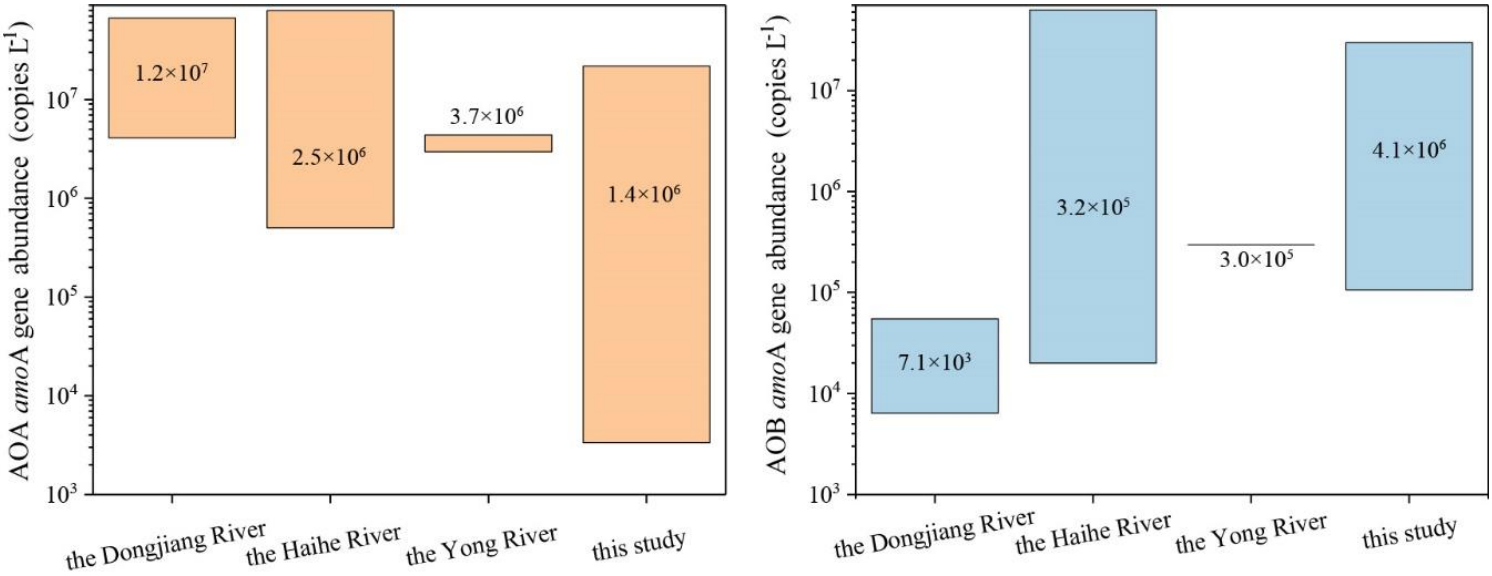

Fig. S3 Brief summary of AOA and AOB *amo*A gene abundance ranges in the overlying water of river systems. Values are extracted from text, tables, and figures of literatures studying the Dongjiang River (1), the Haihe River (7), and the Yong River (5). The values in these figures denote average *amo*A gene abundance.

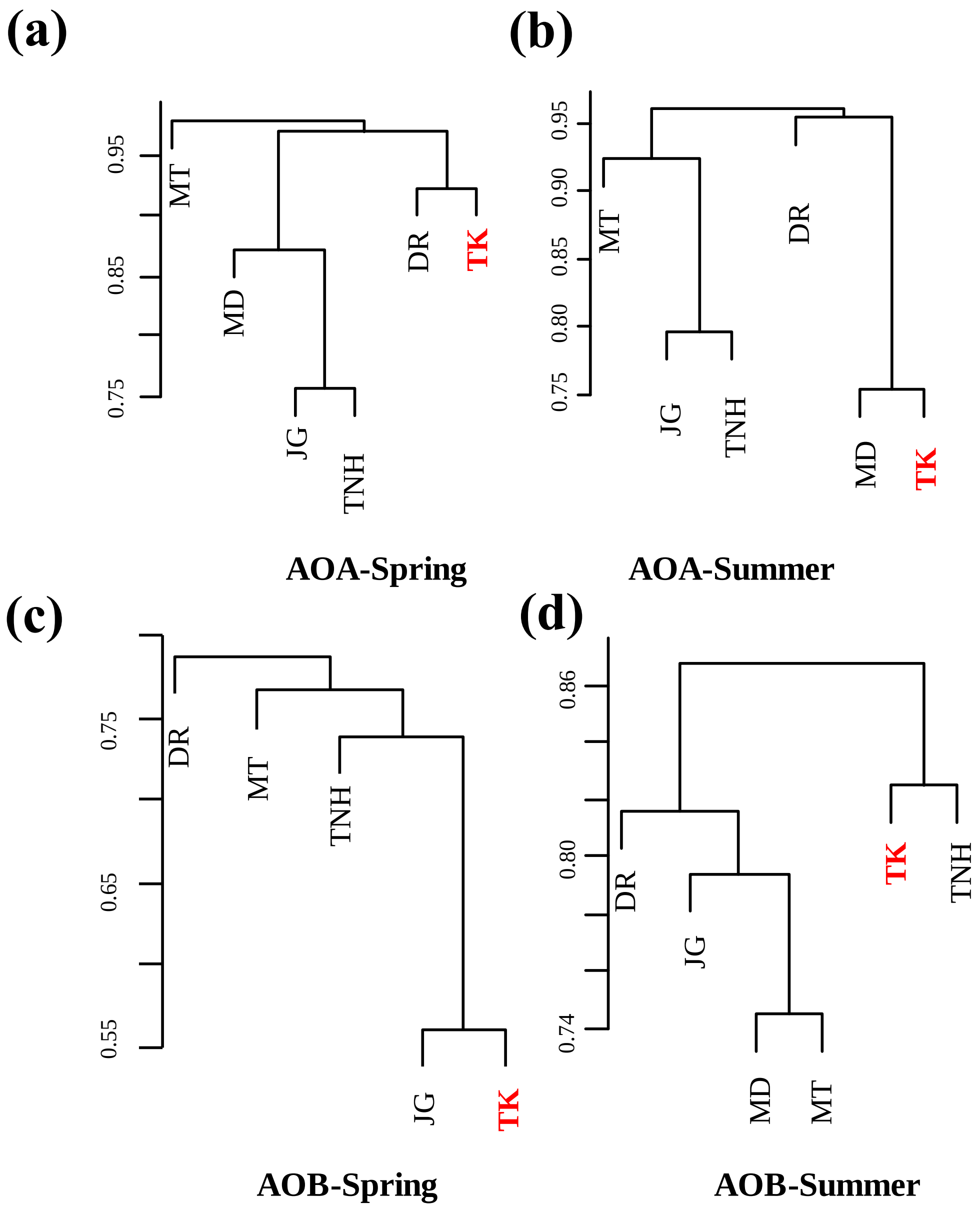

Fig. S4 Unweighted Unifrac UPGMA (unweighted pair group method with mathematical averages) cluster analysis for AOA (a and b) and AOB (c and d) communities of the Yellow River source region in the spring and in the summer. The numbers in the figures indicate the unweighted Unifrac dissimilarities. TK was sampled in one tributary, while other sampling sites were collected in the main channel (overlying water in MD station successively flows to DR, MT, JG, and TNH).

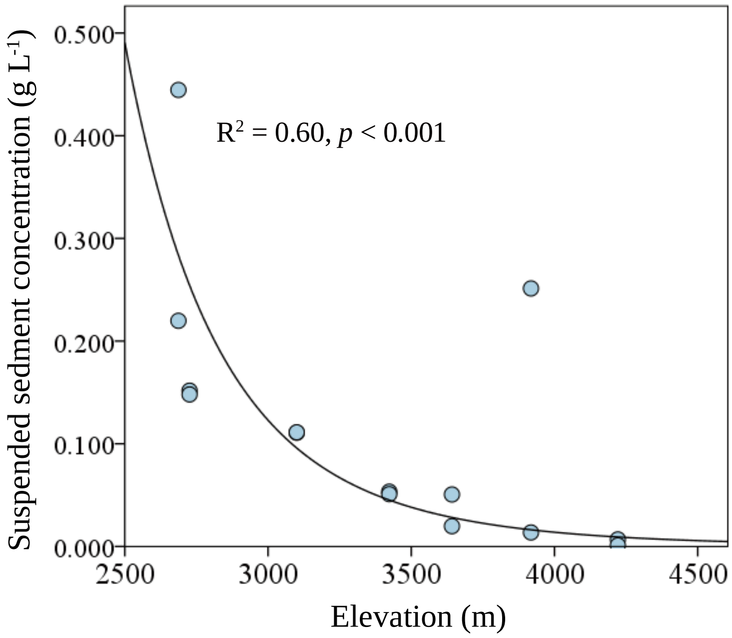

Fig. S5 Variations of suspended sediment concentration along the elevation gradient in the main channel of the Yellow river source region.

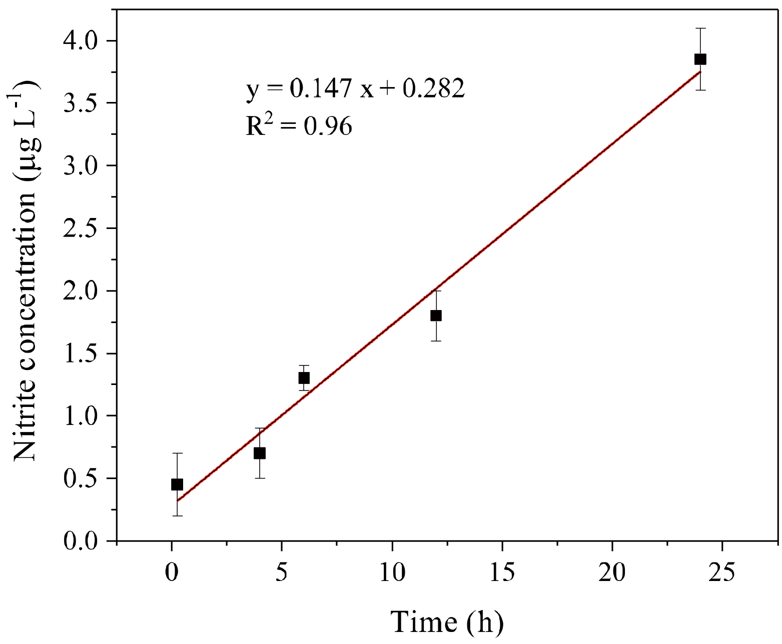

Fig. S6 Production of nitrite vs time in the Tangke station (may). The nitrite concentration is the net value obtained by $\text{Nitrite}_{(\text{non-sterile})}$ - $\text{Nitrite}_{(\text{sterile})}$.

Calculations of a specific volume of headspace air which should be extracted from serum vials to simulate *in situ* air pressure

To simulate *in situ* conditions when determining potential nitrification rates (PNRs), a specific volume of headspace air should be drawn out from serum vials to allow their air pressures approximately same as those in the field. The specific volumes were calculated as follows. We presume that air pressures in serum vial and syringe are P_1 and P_2 (Fig. S7), respectively, and in the field is P_3, then according to the ideal gas law:

$$P_1 = n_1RT/V_1 \text{ (in serum vials)} \qquad (1\text{-}1)$$

$$P_2 = n_2RT/V_2 \text{ (in syringes)} \qquad (1\text{-}2)$$

where, n is the amount of substance of gas (in moles); R is the ideal gas constant; T is the absolute temperature of the gas, and V is the volume of the gas. To achieve our goal, we used syringes to draw out some volume of air from serum vials to make $P_1 = P_2 = P_3$. During this process, the sum of gas substance amount in vials (n_1) and syringes (n_2) should be equal to that in original vials (n). The total volume of serum vials used to determine PNR was 300 mL with an addition of 100-mL overlying water, therefore, headspace volume was 200 mL. The original amount of gas in our serum vials is PV/RT according to ideal gas law, that is, 101.3 kpa*200 mL/RT. Based on equation (1-1), $n_1 = P_1$*200 mL/RT, thus, n_2 could be described to be (101.3 - P_1) kpa*200 mL/RT. Then, equation (1-2) could be rearranged as $P_2 = (101.3-P_1) * 200/V_2$, and $P_1 = P_2 = P_3$ (*in situ* air pressure), therefore we could know how much gas should be extracted from the vials.

To assure $P_1 = P_2 = P_3$, the syringe was held for 5 min when we drew out gas from incubation vials.

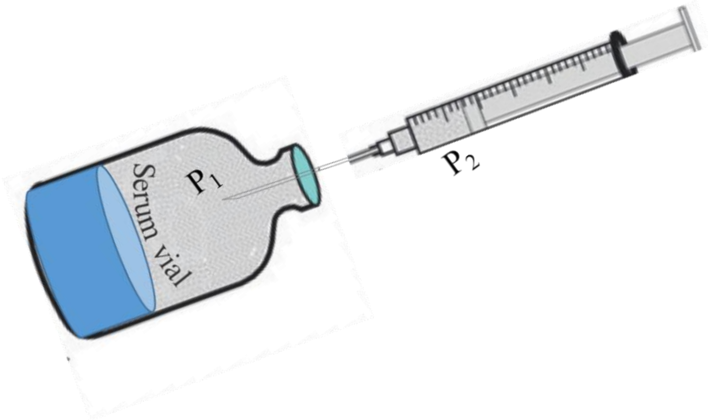

Fig. S7 Schematic diagram of air pressure adjustment for PNR determination.

References

1. Liu Z, Huang S, Sun G, Xu Z, Xu M. 2011. Diversity and abundance of ammonia-oxidizing archaea in the Dongjiang River, China. Microbiological research 166:337-345.
2. Pouliot J, Galand PE, Lovejoy C, Vincent WF. 2009. Vertical structure of archaeal communities and the distribution of ammonia monooxygenase A gene variants in two meromictic High Arctic lakes. Environmental Microbiology 11:687-699.
3. Auguet J-C, Triadó-Margarit X, Nomokonova N, Camarero L, Casamayor EO. 2012. Vertical segregation and phylogenetic characterization of ammonia-oxidizing Archaea in a deep oligotrophic lake. The Isme Journal 6:1786.
4. Peng C, Jiang H, Huang L, Hou W, Yang J, Wang S, Huang Q, Deng S, Dong H. 2013. Abundance and Diversity of Ammonia-Oxidizing Bacteria and Archaea in Cold Springs on the Qinghai-Tibet Plateau. Geomicrobiology Journal 30:530-539.
5. Zhang Q, Tang F, Zhou Y, Xu J, Chen H, Wang M, Laanbroek HJ. 2015. Shifts in the pelagic ammonia-oxidizing microbial communities along the eutrophic estuary of Yong River in Ningbo City, China. Frontiers in Microbiology 6.
6. Sun W, Xia C, Xu M, Guo J, Sun G, Wang A. 2014. Community structure and distribution of planktonic ammonia-oxidizing archaea and bacteria in the Dongjiang River, China. Research in Microbiology 165:657-670.
7. Wang C, Shan B, Zhang H, Zhao Y. 2014. Limitation of spatial distribution of ammonia-oxidizing microorganisms in the Haihe River, China, by heavy metals. Journal of Environmental Sciences 26:502-511.