

Table S1. Abundances of the archaeal and β -proteobacterial *amoA* genes and *Nitrospira* and *Nitrospina* 16S rRNA genes in the PRE.

Station	Water Depth (m)	Sampling Depth (m)	Archaeal <i>amoA</i> (copies L ⁻¹)				β -proteobacterial <i>amoA</i> (copies L ⁻¹)				<i>Nitrospira</i> 16S rRNA (copies L ⁻¹)				<i>Nitrospina</i> 16S rRNA (copies L ⁻¹)			
			FL ^a	SD	PA ^b	SD	FL ^a	SD	PA ^b	SD	FL ^a	SD	PA ^b	SD	FL ^a	SD	PA ^b	SD
P1	8.9	1	0		1501	40	0		1338	799	1533	446	33025	525	909	747	4537	691
		7	0		1248	57	228	56	528	17	776	76	53287	1086	121	6	3902	179
P2	9.8	1			NS ^c				NS ^c				NS ^c				NS ^c	
		7	2768	27	4462	1243	526	6	7441	562	29374	5945	328697	15430	4851	447	45997	2792
P3	10.2	1			NS ^c				NS ^c				NS ^c				NS ^c	
		8	2556	251	11321	85	298	14	8239	482	25360	1478	573425	12573	5103	850	85027	9576
P4	21.5	1			NS ^c				NS ^c				NS ^c				NS ^c	
		18	657	22	12080	516	308	49	34158	2469	9175	1541	2024263	198739	3068	441	380537	14232
P5	22.5	1	4104	80	6535	30	961	58	5532	428	40070	3306	365741	18556	13556	1852	84860	3967
		19	3263	109	9162	327	672	70	12341	292	38076	5273	763345	93318	12186	1715	125427	10142
P6	18.8	1	3617	305	11219	241	488	71	4136	208	21516	2437	482519	13994	3763	250	56974	2244
		16			NS ^c					NS ^c				NS ^c				NS ^c
P7	12	1	40742	2180	108877	4425	2012	245	14259	3443	69806	5991	735150	42882	7820	652	95572	4870
		10	10212	2234	8267	677	221	11	974	171	11393	3342	46220	3334	950	103	7540	399
P8	5	1	2614	47	64350	3095	150	40	1024	72	16111	1427	70874	15050	3368	539	7479	625
		3.5	2904	272	49549	4515	201	117	1228	92	26179	3334	83656	10345	5913	668	11065	1838
P9	8	1	1018	55	23925	1716	70		553	127	3536	475	40278	3435	8309	1501	75342	10147
		6	501	22	454359	16727	0		1392	64	0		47889	10565	769	139	296757	20048
P10	12.9	1	1114	28	730	231	0		121		506	128	1663	946	1814	98	2033	214
		11	3683	160	9475	1631	298	71	165	29	63	23	973	292	2340	256	14241	2378
P11	14.2	1	0		0		0		172	118	43		495	135	412	151	51	

		12	4710	435	18304	1145	0	224	14	154	101	2542	750	3400	315	12913	2894
P12	16	1	714	52	0		0	0		68		0		6945	3360	362	
		14	21793	1731	33005	3976	0	1423	74	227	110	5294	807	6635	851	20858	530

1 ^a, Free-living; ^b, Particle-associated; ^c, No sample

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Table S2. Primer set sequences, PCR reaction mixtures and conditions for each gene.

Target gene	Primer	Sequence (5'-3')	PCR mixture	PCR conditions	References
<i>β</i> -proteobacterial <i>amoA</i>	amoA-34F	GCGGCRAAAATGCCGCCGGAAGCG	50 μL reaction mixture: Failsafe Premix F (Epicentre Biotechnologies, Madison, WI, U.S.A.) 25 μL, primers 0.5 μM, plantium Taq DNA polymerase (Invitrogen, Carlsbad, CA, U.S.A.) 1 U, DNA template 1 μL	95 °C for 2 min; hot start at 80 °C; and 25 x (95 °C for 30 s, 57 °C for 30 s, 73 °C for 3 min).	Kim et al., 2008; Hu et al., 2010
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
Archaeal <i>amoA</i>	Arch-amoAF	STAATGGTCTGGCTTAGACG	50 μL reaction mixture: Failsafe Premix F (Epicentre Biotechnologies) 25 μL, primers 0.5 μM, plantium Taq DNA polymerase (Invitrogen) 1 U, DNA template 1 μL	95 °C for 5 min; 30 x (94 °C for 45 s, 53 °C for 60 s, and 72 °C for 60 s); and 72 °C for 15 min.	Francis et al., 2005; Hu et al., 2010
	Arch-amoAR	GCGGCCATCCATCTGTATGT			
<i>Nitrobacter</i> and <i>Nitrococcus nxrB</i>	nxBF706	AAGACCTAYTTCAACTGGTC	50 μL reaction mixture: Ex Taq DNA polymerase 0.25 μL (TaKaRa), 10×Buffer 5 μL, dNTP 4 μL, Mg ²⁺ 4 μL, primers 1 μM, BSA (20 μg/μL) 0.125 μL, DNA template 2 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56 °C 30 s, 72 °C 30 s), 72 °C for 10 min.	Koch, 2009
	nxBR1431	CGCTCCATCGGYGGAACMAC			
<i>Nitrospira nxrB</i>	nxB169F	TACATGTGGTGGAACA	25 μL reaction mixture: Platinum Taq DNA polymerase 0.1 μL (Invitrogen), 10×Buffer 2.5 μL, dNTP 2 μL, Mg ²⁺ 4 μL, primers 1 μM, BSA (200 ng/μL) 2.5 μL, DNA template 1 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56.2 °C 40 s, 72 °C 90 s), 72 °C for 10 min.	Modified from Pester et al., 2013
	nxB638R	CGGTTCTGGTCRATCA			
<i>Nitrospina nxrB</i>	nxBNF	GGGCGACCAGATGGAAAC	25 μL reaction mixture: LA Taq DNA polymerase 0.25 μL (TaKaRa), 10×Buffer 2.5 μL, dNTP 1 μL, Mg ²⁺ 5 μL, primers 1 μM, BSA (200 ng/μL) 2.5 μL, DNA template 1 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56.2 °C 40 s, 72 °C 90 s), 72 °C for 10 min.	This study
	nxBNR	GGGCCGGACATAGAAAGG			

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Table S3. Primer pair sequences, qPCR mixtures and conditions for each gene.

Target gene	Primer	Sequence (5'-3')	PCR mixture	PCR conditions	Efficiency	References
<i>β</i> -proteobacterial <i>amoA</i>	amoA-1F	GGGGHTTYTACTGGTGGT	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa, Dalian, China) 12.5 μL, BSA 5 μg, primers 0.4 μM, DNA template 1 μL	94 °C for 30 s; 45 × (94 °C for 15 s, 60 °C for 60 s, and 72 °C for 90 s).	96-104 %	Rotthauwe et al., 1997; Hu et al., 2011; Mincer et al., 2007
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC				
Archaeal <i>amoA</i>	Arch-amoAF	STAATGGTCTGGCTTAGACG	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5 μL, BSA 5 μg, primers 0.4 μM, DNA template 1 μL	95 °C for 30 s; 40 × (95 °C for 30 s, 53 °C for 60 s, and 72 °C for 45 s).	91-98%	Francis et al., 2005; Hu et al., 2011
	Arch-amoAR	GCGGCCATCCATCTGTATGT				
<i>Nitrospira</i> 16S rRNA	Nspra-675f	GCGGTGAAATGCGTAGAKATCG	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5μL, BSA 15 μg, primers 0.2 μM, DNA template 1 μL	95 °C for 10 min; 45 × (94 °C for 30 s, 64 °C for 30 s, 72 °C for 60 s).	92-98 %	Graham et al., 2007; Attard et al., 2010
	Nspra-746r	TCAGCGTCAGRWAYGTTCCAGAG				
<i>Nitrospina</i> 16S rRNA	NitSSU_130F	GGGTGAGTAACACGTGAATAA	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5μL, BSA 1 μg, primers 0.4 μM, DNA template 1 μL	94 °C for 15 min; 45 × (94 °C for 15 s, 57.5 °C for 15 s, 72 °C for 30 s, 77 °C for 1 s).	90-100 %	Mincer et al., 2007
	NitSSU_282R	TCAGGCCGGCTAAMCA				

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1 **Table S4.** Diversity indices of AOA and β -AOB *amoA*, *Nitrospira*, *Nitrospina*, and
 2 *Nitrobacter nxrB* genes based on 5% nucleic acid sequences cutoff.

Genes	n	No. of OTUs	C (%)	H'	1/D	Chao1
AOA <i>amoA</i> (SCS)	392	60	0.94	3.04	10.64	49.46
AOA <i>amoA</i> (PRE)	127	23	0.90	2.13	4.87	42.5
β -AOB <i>amoA</i> (PRE)	26	3	0.96	0.43	1.28	3
<i>Nitrospira nxrB</i>	345	29	0.96	1.79	3.57	42
<i>Nitrospina nxrB</i>	185	12	0.98	1.79	4.83	12.75
<i>Nitrobacter nxrB</i>	48	3	0.98	0.78	2.13	3

3 n, number of sequences; OTU, operational taxonomic unit; C, coverage; H',
 4 Shannon-Wiener Index; 1/D, Simpson's diversity Index; SCS, South China Sea; PRE,
 5 Pearl River estuary

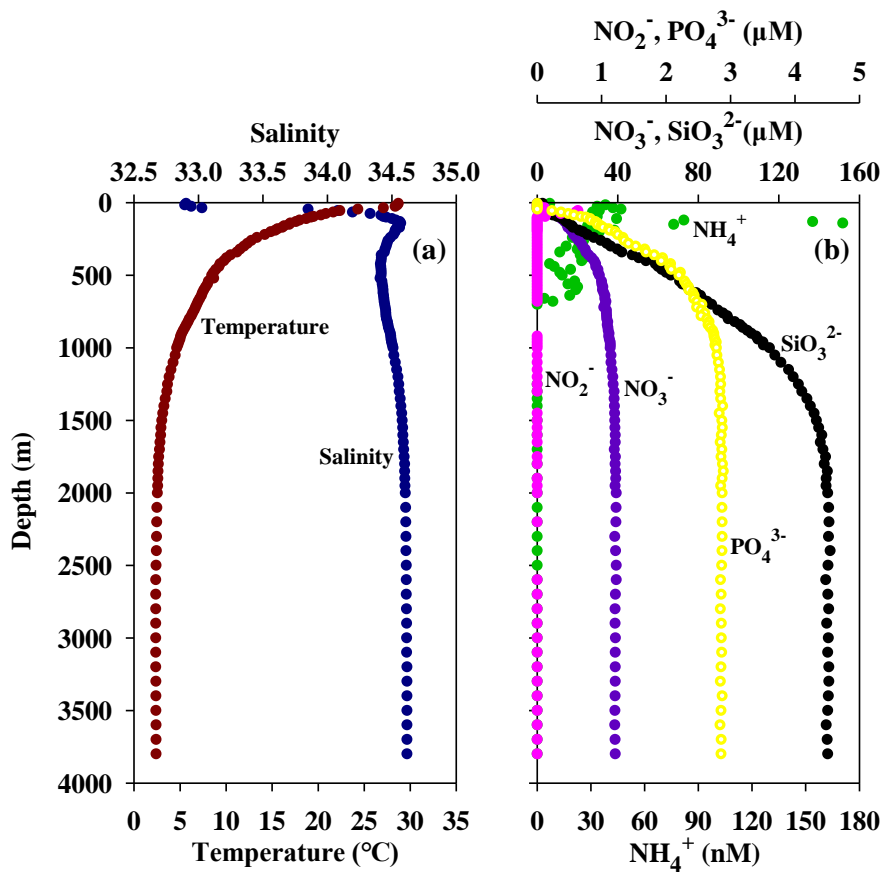
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Table S5. *r* values for the relationship between gene abundances of nitrifiers and environmental parameters in the PRE.

Gene	Community	Water mass parameters			Substrate parameters			Parameters influencing substrate availability		
		Temperature (n = 20)	Salinity (n = 20)	SiO ₃ ²⁻ (n = 20)	NH ₄ ⁺ (n = 15)	NO ₂ ⁻ (n = 20)	NO ₃ ⁻ (n = 20)	TSM (n = 19)	DO (n = 20)	pH (n = 20)
AOB <i>amoA</i>	FL ^a	0.302	-0.441	0.439	-0.108	0.527*	0.759**	-0.053	-0.425	-0.512*
	PA ^b	0.332	-0.474*	0.475*	-0.048	0.706**	0.464*	0.520*	-0.525*	-0.496*
	FL+PA	0.341	-0.471*	0.487*	-0.053	0.718**	0.491*	0.504*	-0.536*	-0.513*
AOA <i>amoA</i>	FL ^a	-0.158	0.016	-0.051	-0.208	0.005	0.013	-0.125	0.024	-0.050
	PA ^b	-0.213	0.239	-0.223	-0.341	-0.186	-0.144	0.562*	0.209	0.191
	FL+PA	-0.226	0.238	-0.225	-0.321	-0.184	-0.142	0.543*	0.208	0.184
<i>Nitrospira</i> 16S rRNA	FL ^a	0.426	-0.580**	0.537*	-0.205	0.643**	0.772**	-0.099	-0.464*	-0.625**
	PA ^b	0.356	-0.474*	0.491*	-0.073	0.730**	0.518*	0.504*	-0.541*	-0.524*
	FL+PA	0.367	-0.475*	0.503*	-0.080	0.743**	0.539*	0.493*	-0.550*	-0.540*
<i>Nitrospina</i> 16S rRNA	FL ^a	0.097	-0.167	0.158	-0.268	0.436	0.253	-0.315	-0.190	-0.230
	PA ^b	0.108	-0.134	0.162	-0.105	0.453*	0.173	0.822**	-0.276	-0.221
	FL+PA	0.111	-0.140	0.167	-0.115	0.468*	0.182	0.811**	-0.282	-0.229

2 ^a, Free-living; ^b, Particle-associated; *, *P* < 0.05; **, *P* < 0.01; TSM, Total suspended material; DO, Dissolved oxygen

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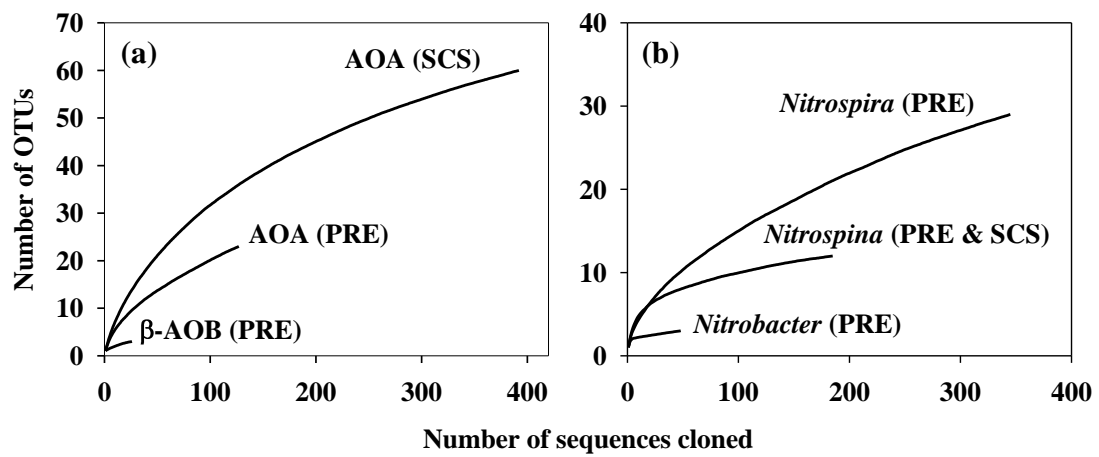


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Figure S1. Depth profiles of biogeochemical parameters at SEATS.

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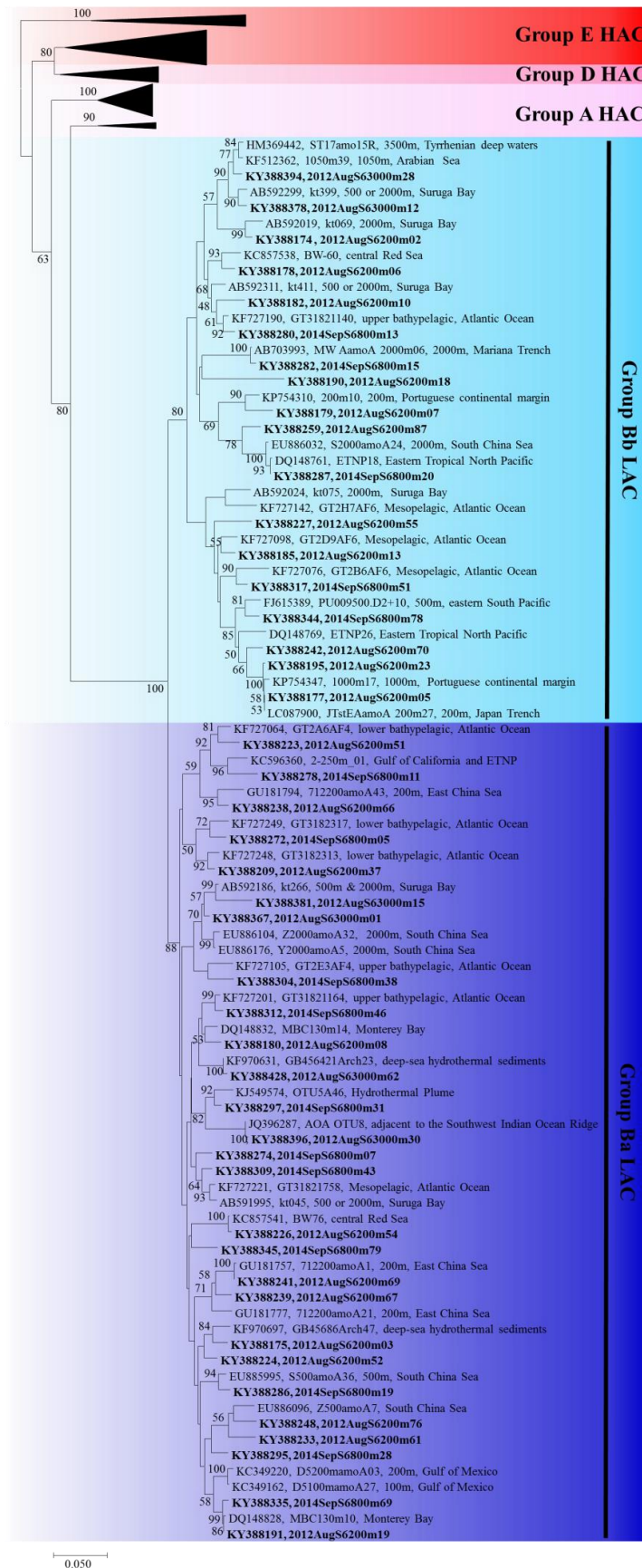
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2 **Figure S2.** Rarefaction curves of (a) AOA and β -AOB *amoA* gene sequences and (b)

3 *Nitrospira*, *Nitrospina*, and *Nitrobacter nxrB* gene sequences. The curves were

4 generated at 95% DNA sequence identity.

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2 **Figure S3.** Unrooted neighbor-joining (NJ) phylogenetic tree of the archaeal *amoA*

1 gene sequences (expanded view for group Ba and Bb (LAC)). Clone sequences from
2 this study are shown in bold and sequences sharing 95% DNA identity are grouped.
3 Phylogenetic relationships were bootstrapped 1000 times, and bootstrap values greater
4 than 50% are shown. The scale bar indicates 5% estimated sequence divergence.
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