

Supplementary material

Seasonality and depth distribution of the abundance and activity of ammonia oxidizing microorganisms in marine coastal sediments

(North Sea)

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Table S1: Primer pairs described in the text, polymerase chain reaction (PCR) conditions and amplicon size used in this study.

Assay	Target	Primer pair	T _m	amplicon size [bp*]	Reference
qPCR	AOA 16S rRNA gene	MCGI-391F (5'-AAGGTTARTCCGAGTGRTTTC-3') MCGI-554R (5'-TGACCACTTGAGGTGCTG-3')	qPCR 61 °C	163	1
qPCR + PCR/cloning	AOA <i>amoA</i> gene	AmoA-ModF (5'-GGTCTGGYTWAGACGATG-3') AmoA-ModR (5'-GCCATCCATCTGTAWGTC-3')	qPCR 59.5 °C PCR 55 °C	626	2
qPCR + PCR/cloning	Anammox bacteria 16S rRNA gene	Brod541F (5'-GAGCACGTAGGTGGGTTTGT-3') Amx820R (5'-AAAACCCCTCTACTTAGTGCCC-3')	qPCR 59 °C PCR 58 °C	279	3
qPCR	Anammox bacteria <i>hzsA</i> gene	<i>hzsA</i> _1597F (5'-WTYGGKTATCARTATGTA-3') <i>hzsA</i> _1857R (5'-AAABGGYGAATCATARTGGC-3')	qPCR 51 °C	260	4
qPCR	AOB 16S rRNA gene	CTO189F_A (5'-GGAGRAAAGCAGGGGATCG-3') [†] CTO189F_C (5'-GGAGGAAAGTAGGGGATCG-3') [†] CTO654R (5'-CTAGCYTTGTAGTTTCAAACGC-3')	qPCR 60 °C	465	5
qPCR + PCR/cloning	AOB <i>amoA</i> gene	AOB-amoAF (5'-GGGGTTTCTACTGGTGGT-3') AOB-amoAR new (5'-CCCCTCBGSAAAVCCTTCTTC-3')	qPCR 61.6 °C PCR 58 °C	491	6, 7

PCR conditions: 95°C 5 min; 40 × [95°C 1 min, melting temperature (T_m) 40 s, 72°C 1 min]; 72°C 5 min; qPCR conditions: 95°C 4 min; 40 × [95°C 30 s, T_m 40 s, 72°C 30 s]; 80°C 25 s; *base pairs (bp); [†] The forward primer contains an equimolar mixture of the reported forward primers.

- References:
1. Coolen et al. (2007)
 2. Yakimov et al. (2011)
 3. Li et al. (2010)
 4. Harhangi et al. (2012)
 5. Kowalchuk et al. (1997)
 6. Rotthauwe et al. (1997)
 7. Hornek et al. (2006)

Table S2: Quantitative polymerase chain reaction (qPCR) efficiencies (E) and correlation coefficients (R^2).

qPCR assay	target molecule	February			May			August		
		T_m [°C]	E [%]	R^2	T_m [°C]	E [%]	R^2	T_m [°C]	E [%]	R^2
AOA 16S rRNA gene (MCGI-391F/MCGI-554R)	DNA	61.0	100.0	0.998	61.0	93.5	0.988	61.0	94.3	0.999
	cDNA	61.0	96.1	1.000	61.0	98.8	0.998	61.0	97.2	0.999
AOA <i>amoA</i> gene (AmoA_modF/AmoA_modR)	DNA	59.5	100.7	0.998	59.5	99.7	0.988	59.5	97.8	0.994
	cDNA	59.5	99.9	0.983	59.5	107.6	0.984	59.5	96.4	0.995
Anammox bacteria 16S rRNA gene (Brod541F/Amx820R)	DNA	59.0	99.0	0.998	59.0	96.4	0.995	59.0	98.9	0.993
	cDNA	59.0	98.4	0.999	59.0	93.7	0.996	59.0	106.8	0.989
Anammox bacteria <i>hzsA</i> gene (<i>hzsA</i> _1597F/ <i>hzsA</i> _1857R)	DNA	51.0	82.0	0.997	51.0	81.1	0.997	51.0	80.1	0.997
	cDNA	51.0	80.0	0.998	51.0	80.2	0.997	51.0	80.4	0.996
AOB 16S rRNA gene (CTO189F/CTO654R)	DNA	60.0	86.6	0.998	60.0	86.6	0.998	60.0	86.6	0.998
	cDNA	60.0	84.0	0.998	60.0	84.0	0.998	60.0	84.0	0.998
AOB <i>amoA</i> gene (AOB-amoAF/AOB-amoAR new)	DNA	61.6	85.4	0.994	61.6	85.4	0.994	61.6	85.4	0.994
	cDNA	61.6	91.0	0.993	61.6	91.0	0.993	61.6	91.0	0.993

qPCR conditions: 95°C 4 min; 40 × [95°C 30 s, melting temperature (T_m) 40 s, 72°C 30 s]; 80°C 25 s

Table S3: Physical (CTD) and chemical (nutrient) properties of the bottom water (BW).

Parameter/ Sediment dbsf [cm]	Ammonium [μM]	Nitrite [μM]	Nitrate [μM]	Phosphate [μM]	Temperature [°C]	Salinity [psu]	water depth [m]
Feb-11 BW	3.0	0.6	6.8	1.0	5.0	34.4	43.0
May-11 BW	1.9	0.1	2.0	0.7	8.6	34.3	44.0
Aug-11 BW	1.6	0.1	0.5	0.5	15.4	34.7	45.0

Table S4: Total organic carbon (TOC) and total organic nitrogen (TON)

Parameter/ Sediment dbsf [cm]	TOC [%]	TON [%]
Feb-11		
0.5	0.22	0.02
1.5	0.22	bdl*
2.5	0.21	0.04
3.5	0.22	0.04
4.5	0.26	0.03
6.5	0.20	0.02
8.5	0.28	0.03
11.5	0.37	0.08
May-11		
0.5	0.20	0.02
1.5	0.30	0.05
2.5	0.25	0.04
3.5	0.27	0.03
4.5	0.38	0.08
6.5	0.25	0.05
8.5	0.15	0.03
11.5	0.21	0.02
Aug-11		
0.5	0.26	0.03
1.5	0.27	0.05
2.5	0.23	0.02
3.5	0.20	0.02
4.5	0.31	0.06
6.5	0.25	0.03
8.5	0.28	0.03
11.5	0.22	0.04

*bdl: below detection limit

Table S5: Spearman rank order correlation of all parameters observed in this study.

Parameter	NH ₄ ⁺ [μM]	NO ₃ ⁻ [μM]	PO ₄ ⁺ [μM]	TON [%]	AOA 16S rRNA gene (RNA:DNA ratio)	AOA <i>amoA</i> gene (DNA) [copies g ⁻¹]
Sediment depth [cm]	0.822***		0.801***			
NH ₄ ⁺ [μM]		0.734***				
NO ₃ ⁻ [μM]						
PO ₄ ⁺ [μM]						
AOA 16S rRNA gene (DNA) [copies g ⁻¹]						0.694***
AOA 16S rRNA gene (cDNA) [copies g ⁻¹]					0.846***	
AOA <i>amoA</i> gene (DNA) [copies g ⁻¹]						
AOA <i>amoA</i> gene (cDNA) [copies g ⁻¹]						
AOA <i>amoA</i> gene (RNA:DNA ratio)						
Crenarchaeol IPLs total [area g ⁻¹]						
HPH-crenarchaeol [area g ⁻¹]						
TOC [%]				0.657***		
Anammox bacteria 16S rRNA gene (DNA) [copies g ⁻¹]						
Anammox bacteria 16S rRNA gene (cDNA) [copies g ⁻¹]						
Anammox bacteria 16S rRNA gene (RNA:DNA ratio)						
Anammox bacteria <i>hzsA</i> gene (DNA) [copies g ⁻¹]						
Anammox bacteria <i>hzsA</i> gene (cDNA) [copies g ⁻¹]						
Anammox <i>hzsA</i> gene (RNA:DNA ratio)						
PC Monoether ladderane [ng g ⁻¹]						
AOB 16S rRNA gene (cDNA) [copies g ⁻¹]						
AOB <i>amoA</i> (DNA) gene [copies g ⁻¹]						
AOB <i>amoA</i> (cDNA) gene [copies g ⁻¹]						
AOB <i>amoA</i> gene (RNA:DNA ratio)						

Correlation coefficients $r_s \geq 0.6$ and $r_s \leq -0.6$ with P -values ≤ 0.05 were reported. The degree of significance was indicated by using a single asterisk for P -values ≤ 0.05 and ≥ 0.01 , two asterisks for P -values ≤ 0.01 and three asterisks for P -values ≤ 0.005 .

Table S5: Spearman rank order correlation of all parameters observed in this study.

Parameter	AOA <i>amoA</i> gene (cDNA) [copies g ⁻¹]	AOA <i>amoA</i> gene (RNA:DNA ratio)	Anammox 16S rRNA gene (DNA) [copies g ⁻¹]	Anammox 16S rRNA gene (cDNA) [copies g ⁻¹]
Sediment depth [cm]				
NH ₄ ⁺ [μM]				
NO ₃ ⁻ [μM]			-0.647***	-0.743***
PO ₄ ⁺ [μM]	-0.745***	-0.739***		
AOA 16S rRNA gene (DNA) [copies g ⁻¹]				
AOA 16S rRNA gene (cDNA) [copies g ⁻¹]				
AOA <i>amoA</i> gene (DNA) [copies g ⁻¹]			0.725***	
AOA <i>amoA</i> gene (cDNA) [copies g ⁻¹]		0.994***		0.629***
AOA <i>amoA</i> gene (RNA:DNA ratio)				
Crenarchaeol IPLs total [area g ⁻¹]				
HPH-crenarchaeol [area g ⁻¹]				
TOC [%]				
Anammox bacteria 16S rRNA gene (DNA) [copies g ⁻¹]				0.797***
Anammox bacteria 16S rRNA gene (cDNA) [copies g ⁻¹]				
Anammox bacteria 16S rRNA gene (RNA:DNA ratio)		0.717***		
Anammox bacteria <i>hzsA</i> gene (DNA) [copies g ⁻¹]				
Anammox bacteria <i>hzsA</i> gene (cDNA) [copies g ⁻¹]				
Anammox <i>hzsA</i> gene (RNA:DNA ratio)				
PC Monoether ladderane [ng g ⁻¹]				
AOB 16S rRNA gene (cDNA) [copies g ⁻¹]				
AOB <i>amoA</i> (DNA) gene [copies g ⁻¹]				
AOB <i>amoA</i> (cDNA) gene [copies g ⁻¹]				
AOB <i>amoA</i> gene (RNA:DNA ratio)				

Correlation coefficients $r_s \geq 0.6$ and $r_s \leq -0.6$ with P -values ≤ 0.05 were reported. The degree of significance was indicated by using a single asterisk for P -values ≤ 0.05 and ≥ 0.01 , two asterisks for P -values ≤ 0.01 and three asterisks for P -values ≤ 0.005 .

Table S5: Spearman rank order correlation of all parameters observed in this study.

Parameter	Anammox 16S rRNA (RNA:DNA ratio)	Anammox <i>hzsA</i> (DNA) [copies g ⁻¹]	Anammox <i>hzsA</i> (cDNA) [copies g ⁻¹]	Anammox <i>hzsA</i> (RNA:DNA ratio)	PC-ME ladderane [ng g ⁻¹]
Sediment depth [cm]					-0.662***
NH ₄ ⁺ [μM]					
NO ₃ ⁻ [μM]					
PO ₄ ⁺ [μM]					
AOA 16S rRNA gene (DNA) [copies g ⁻¹]					
AOA 16S rRNA gene (cDNA) [copies g ⁻¹]					
AOA <i>amoA</i> gene (DNA) [copies g ⁻¹]		0.662***			
AOA <i>amoA</i> gene (cDNA) [copies g ⁻¹]	0.708***				
AOA <i>amoA</i> gene (RNA:DNA ratio)					
Crenarchaeol IPLs total [area g ⁻¹]				0.797***	
HPH-crenarchaeol [area g ⁻¹]				0.760***	
TOC [%]					
Anammox bacteria 16S rRNA gene (DNA) [copies g ⁻¹]		0.848***		-0.751***	
Anammox bacteria 16S rRNA gene (cDNA) [copies g ⁻¹]	0.748***	0.729***			
Anammox bacteria 16S rRNA gene (RNA:DNA ratio)					
Anammox bacteria <i>hzsA</i> gene (DNA) [copies g ⁻¹]					
Anammox bacteria <i>hzsA</i> gene (cDNA) [copies g ⁻¹]					
Anammox <i>hzsA</i> gene (RNA:DNA ratio)					
PC Monoether ladderane [ng g ⁻¹]					
AOB 16S rRNA gene (cDNA) [copies g ⁻¹]					
AOB <i>amoA</i> (DNA) gene [copies g ⁻¹]					
AOB <i>amoA</i> (cDNA) gene [copies g ⁻¹]			0.713***		
AOB <i>amoA</i> gene (RNA:DNA ratio)					

Correlation coefficients $r_s \geq 0.6$ and $r_s \leq -0.6$ with P -values ≤ 0.05 were reported. The degree of significance was indicated by using a single asterisk for P -values ≤ 0.05 and ≥ 0.01 , two asterisks for P -values ≤ 0.01 and three asterisks for P -values ≤ 0.005 .

Table S5: Spearman rank order correlation of all parameters observed in this study.

Parameter	AOB 16S rRNA (DNA) [copies g ⁻¹]	AOB 16S rRNA (cDNA) [copies g ⁻¹]	AOB 16S rRNA (RNA:DNA ratio)	AOB <i>amoA</i> (cDNA) [copies g ⁻¹]	AOB <i>amoA</i> (RNA:DNA ratio)
Sediment depth [cm]					-0.646***
NH ₄ ⁺ [μM]					
NO ₃ ⁻ [μM]					
PO ₄ ⁺ [μM]					
AOA 16S rRNA gene (DNA) [copies g ⁻¹]					
AOA 16S rRNA gene (cDNA) [copies g ⁻¹]					
AOA <i>amoA</i> gene (DNA) [copies g ⁻¹]					
AOA <i>amoA</i> gene (cDNA) [copies g ⁻¹]					
AOA <i>amoA</i> gene (RNA:DNA ratio)					
Crenarchaeol IPLs total [area g ⁻¹]					
HPH-crenarchaeol [area g ⁻¹]					
TOC [%]					
Anammox bacteria 16S rRNA gene (DNA) [copies g ⁻¹]					
Anammox bacteria 16S rRNA gene (cDNA) [copies g ⁻¹]					
Anammox bacteria 16S rRNA gene (RNA:DNA ratio)					
Anammox bacteria <i>hzsA</i> gene (DNA) [copies g ⁻¹]					
Anammox bacteria <i>hzsA</i> gene (cDNA) [copies g ⁻¹]		0.656***			
Anammox <i>hzsA</i> gene (RNA:DNA ratio)					
PC Monoether ladderane [ng g ⁻¹]					
AOB 16S rRNA gene (cDNA) [copies g ⁻¹]			0.620***		
AOB <i>amoA</i> (DNA) gene [copies g ⁻¹]	0.617***				
AOB <i>amoA</i> (cDNA) gene [copies g ⁻¹]		0.702***			
AOB <i>amoA</i> gene (RNA:DNA ratio)				0.887***	

Correlation coefficients $r_s \geq 0.6$ and $r_s \leq -0.6$ with P -values ≤ 0.05 were reported. The degree of significance was indicated by using a single asterisk for P -values ≤ 0.05 and ≥ 0.01 , two asterisks for P -values ≤ 0.01 and three asterisks for P -values ≤ 0.005 .

Figure S1

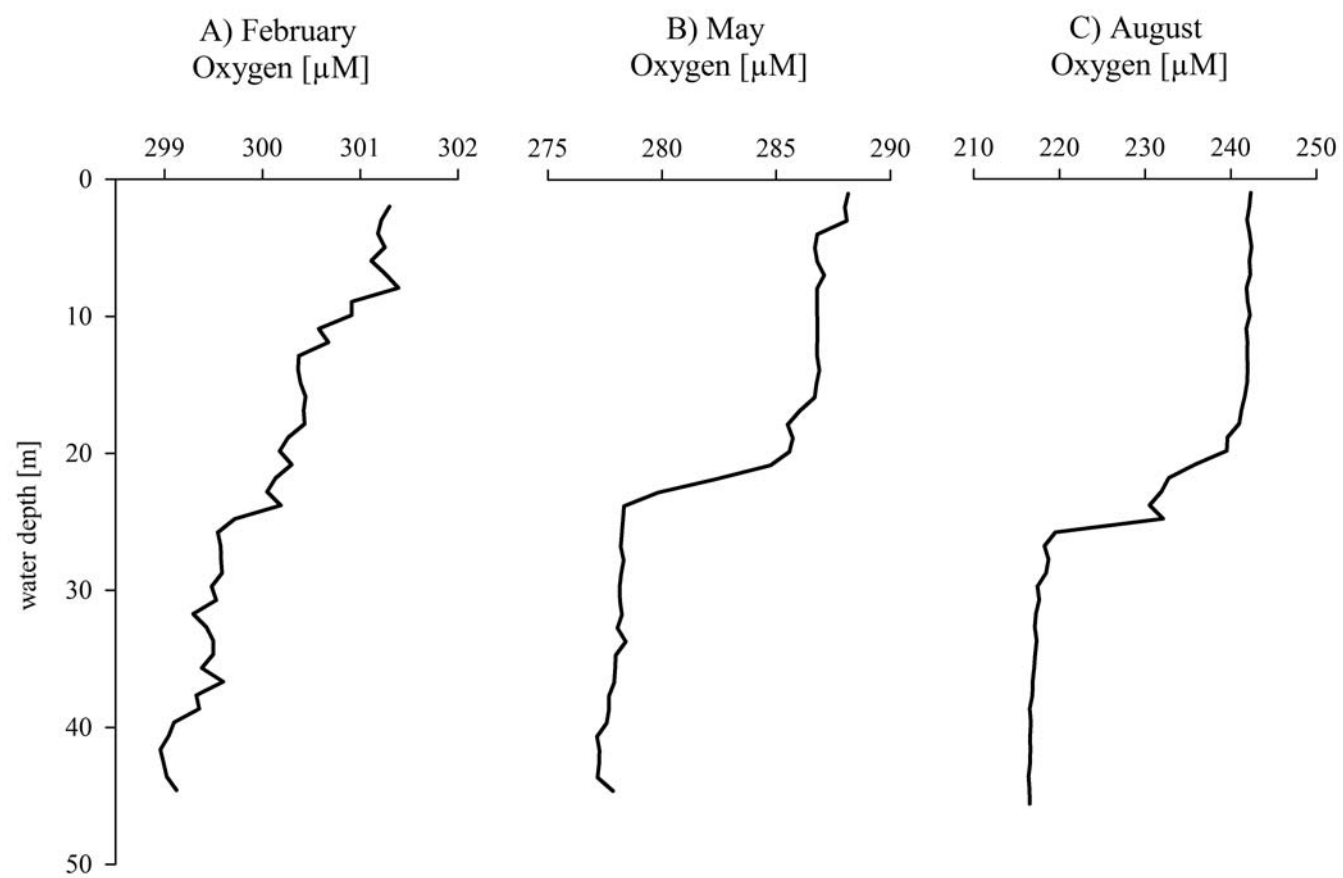


Figure S2

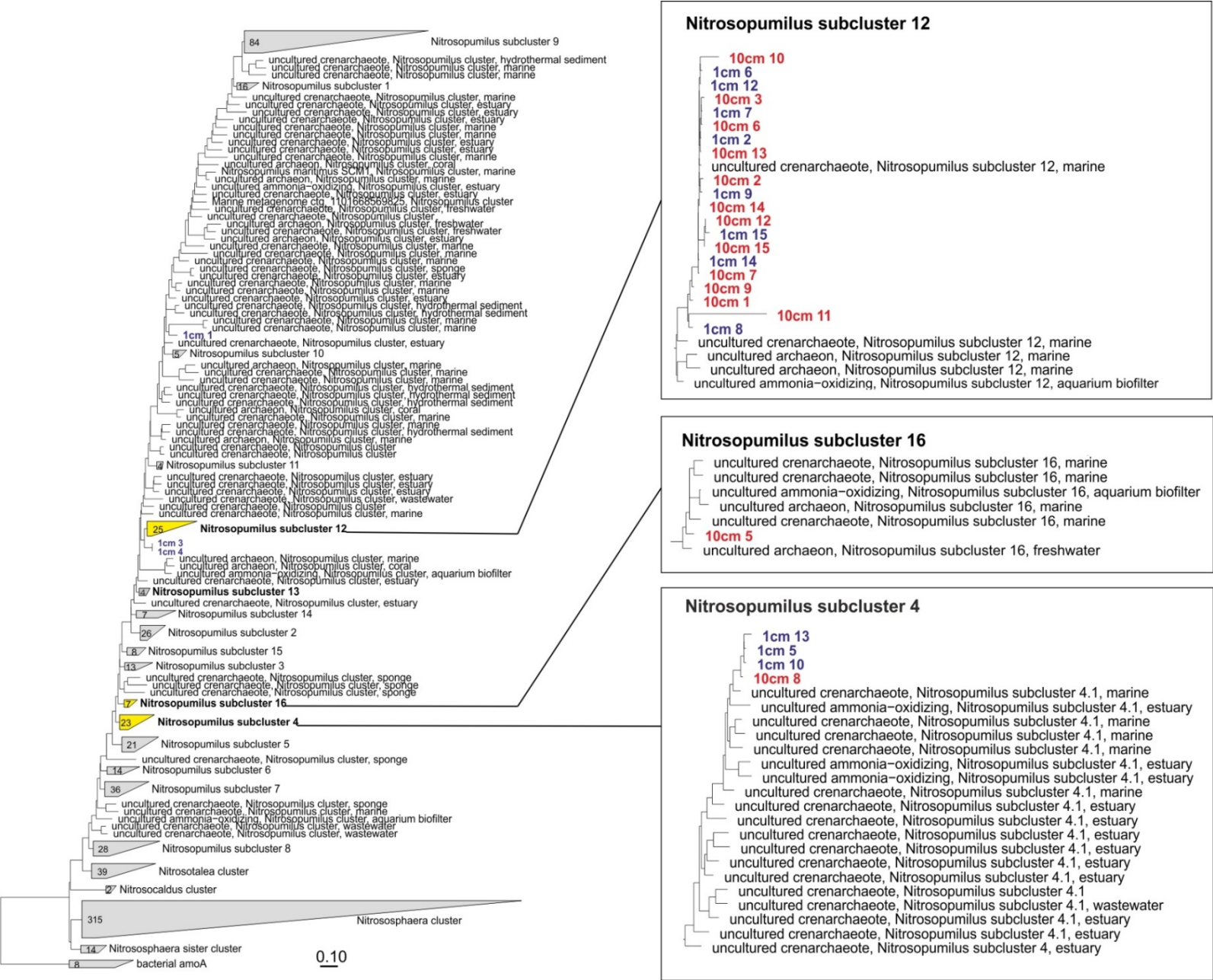


Figure S3

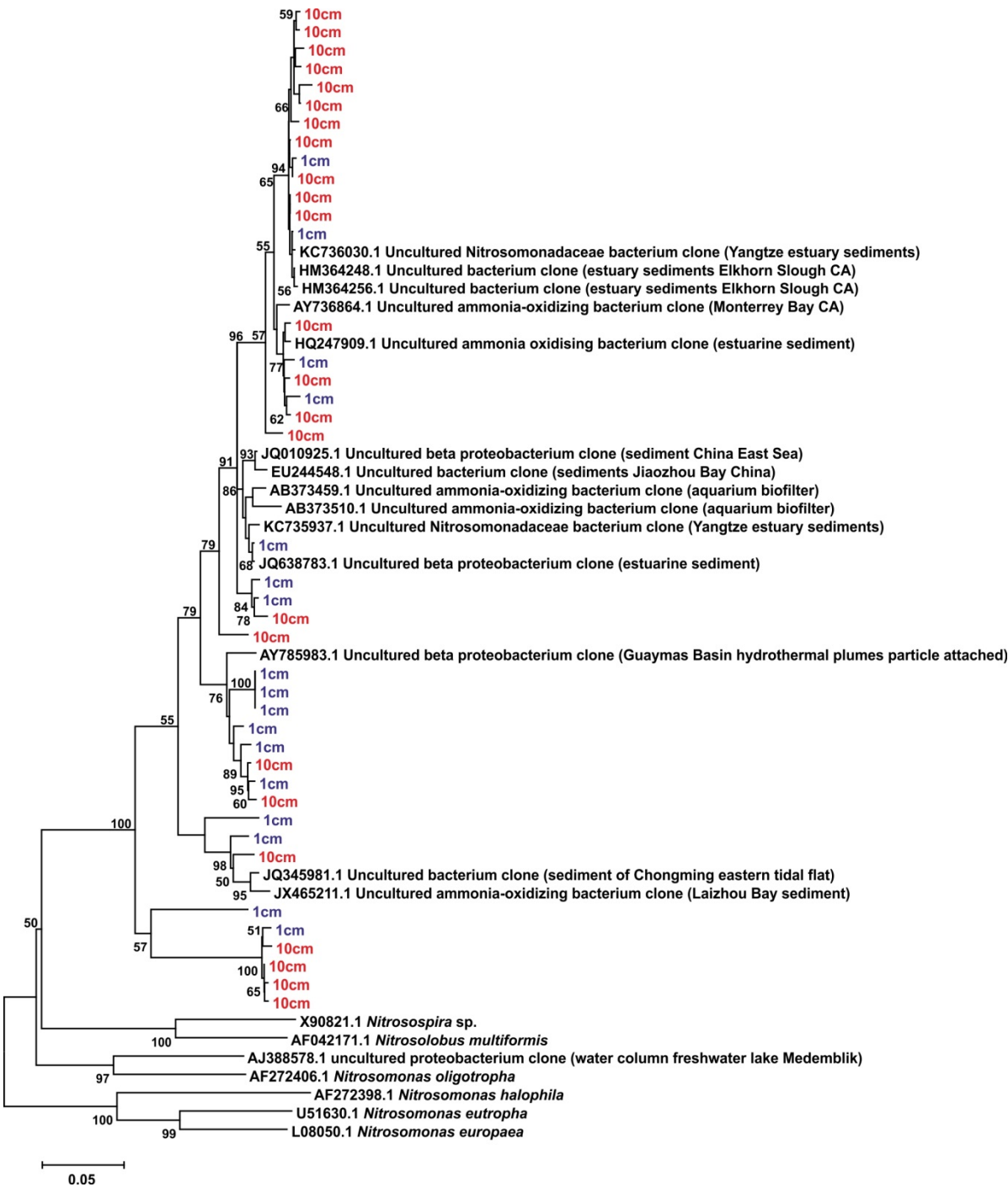


Figure S4

