

**Table B1.** Ammonia oxidation rates measured via  $^{15}\text{NH}_4^+$ -incubation experiments at various controlled oxygen levels in the central-NE Arabian Sea OMZ. Rates are determined as the slope ( $\pm$  standard error) in a linear regression between  $^{15}\text{NO}_2^-$  production and incubation time.

Sample	Oxygen ( $\mu\text{M}$ )	Ammonia oxidation rates ( $\text{nM N d}^{-1}$ )
<b>St. 949 (150 m)</b>	2	0
	5	0
	8	$1.2 \pm 0.2$
	12	$3.6 \pm 0.04$
<b>St. 953 (125 m)</b>	0	0
	2.5	0
	5	0
	10	$3.5 \pm 0.3$

**Table B2.** Primers used in the detection of various N-cycling functional genes and their expression in this study. Unless otherwise stated, the same PCR protocols are used for real-time PCR with the following modifications: (1) the initial denaturing step was 10 minutes at 95°C, (2) no final extension, (3) 50 cycles were performed followed by melting analyses<sup>1</sup>. Primers designed in this study are marked by asterisks. For real-time PCR, the SYBR Green chemistry was used in most cases, except when a probe was listed alongside the primers, in which case the TaqMan chemistry was applied. ‘F’ indicates false positive PCR products of targeted size identified after cloning and sequencing.

Functional Group	Target Gene	Primers	Sequence (5'-3')	PCR conditions	Ref.	(RT)-PCR/ sequencing	Real-time PCR
Anammox (Scalindua)	<i>nirS</i>	Scnir372F	TGT AGC CAG CAT TGT AGC GT	95 °C for 2 min, 30x (95°C for 45s, 60°C for 1 min, 72°C for 1 min), 72°C for 15 min	2	+	+
	<i>nirS</i>	Scnir845R	TCA AGC CAG ACC CAT TTG CT		2	+	+
Denitrifier	<i>nirS</i>	cd3aF	GTS AAC GTS AAG GAR ACS GG	94 °C for 2 min, 35 x (94°C for 30s, 51°C for 1 min, 72°C for 1 min), 72 °C for 10 min	3	+	+
	<i>nirS</i>	R3cd	GAS TTC GGR TGS GTC TTG A		4	+	+
Denitrifier	<i>nirS</i>	nirS1F	CCT AYT GGC CGC CRC ART	<u>Touchdown PCR:</u> 95°C for 5 min, 10 x [95°C for 30 s, 55°C (-0.5°C per cycle) for 30 s, 72°C for 1 min], 25x [95°C for 30 s, 49°C for 30 s, 72°C for 1 min]; 72°C for 7 min	5	+	
	<i>nirS</i>	nirS6R	CGT TGA ACT TRC CGG T		5	+	
Nitrate reducers	<i>narG</i>	narG1960f	TAY GTS GGS CAR GAR AA	<u>Touchdown PCR:</u> 95°C for 5 min, 10 x [94°C for 30 s, 60°C (-0.5°C per cycle) for 30 s, 72°C for 6 min], 30x [95°C for 30 s, 49°C for 30 s, 72°C for 1 min]; 72°C for 7 min <u>Real-time PCR:</u> 95 °C for 10 min, 40x (95°C for 45s, 51°C for 1 min, 72°C for 3 min), 72°C for 15 min	6	+	
	<i>narG</i>	narG2650r	TYT CRT ACC ABG TBG C		6	+	
DNRA	<i>nrfA</i>	nrfA F1	GCN TGY TGG WSN TGY AA	<u>Touchdown PCR:</u> 94 °C for 5 min; 30 x [94 °C for 1 min, 60 °C (-0.5°C per cycle) for 1 min, 72 °C for 90 s] 30 x [94 °C for 30 s; 45 °C for 30 s, 72 °C for 1 min]; 72 °C for 10 min.	7	+	
	<i>nrfA</i>	nrfA R1	TWN GGC ATR TGR CAR TC		7	+	
DNRA	<i>nrfA</i>	nrfA6F	GAY TGC CAY ATG CCR AAA GT	<u>Real-time PCR:</u> 95°C for 10 min, 40 x [94°C for 20 s, 54.5°C for 30s, 72°C for 1 min]	8	+	
	<i>nrfA</i>	nrfA6R	GCB KCT TTY GCT TCR AAG TG		8	+	
$\beta$ -proteobacterial ammonia- oxidizers	<i>amoA</i>	amoA1F'	GGG GTT TCT ACT GGT GG	94°C for 3 min, 30 x (94°C for 20s, 55°C for 1 min, 72°C for 1 min), 72°C for 10 min	9	+	+
	<i>amoA</i>	amoA2R	CCT CKG SAA AGC CTT CTT C		9	+	+
$\gamma$ -proteobacterial ammonia oxidizers	<i>amoA</i>	amoA3F	GGT GAG TGG GYT AAC MG	94°C for 3 min, 30 x (94°C for 20s, 48°C for 1 min, 72°C for 1 min), 72°C for 10 min	10	+	+
	<i>amoA</i>	amoB4R	GCT AGC CAC TTT CTG G		10	+	+

Archaeal ammonia-oxidizers	<i>amoA</i>	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	95°C for 5 min, 30 x (94°C for 45s, 53°C for 1 min, 72°C for 1 min), 72°C for 15 min	11	+	+
	<i>amoA</i>	Arch-amoAR	GCG GCC ATC CAT CTG TAT GT		11	+	+
Nitric oxide reducers (cytochrome bc containing <i>norB</i> )	<i>cnorB</i>	norB1F	CGN GAR TTY CTS GAR CAR CC	94 °C for 4 min, 30 x [94 °C for 1 min, 50 °C for 1.5 min, 72 °C for 2 min], 72°C for 5 min (with norB1F): 94 °C for 4 min, 30 x [94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min], 72°C for 7 min	12		
	<i>cnorB</i>	norB3R	CCY TCV ACC CAG ASA TGC AC		12	+/F	
	<i>cnorB</i>	norB8R	CRT ADG CVC CRW AGA AVG C	Touchdown PCR: 95°C for 5 min, 10 x [95°C for 30 s, 57°C (-0.5°C per cycle) for 40 s, 72°C for 1 min], 30x [95°C for 30 s, 55°C for 40 s, 72°C for 1 min]; 72°C for 7 min	12	+/F	
	<i>cnorB</i>	cnorB1F	GAR TTY CTN GAR CAR CC		13	+/F	
	<i>cnorB</i>	cnorB2F	GAC AAG NNN TAC TGG TGG T		13	+/F	
Arabian Sea cluster 2	<i>cnorB</i>	cnorB6R	GAA NCC CCA NAC NCC NGC	<u>Real-time PCR</u> : 50°C for 2 min, 95 °C for 10 min, 50x [95°C for 15s, 54°C for 30s, 72°C for 1 min, 76°C for 10s (data recording)]	13		
	<i>cnorB</i>	ASc2-1f	GAR TTY CTN GAG CAG CC		*		+
	<i>cnorB</i>	ASc2-307r	TTA CYT CRC GGT CAA CAC C		*		+
Arabian Sea cluster 3	<i>cnorB</i>	ASc3-179f	ACC TTG CGG TGG ACA AGA TGT ACT	<u>Real-time PCR</u> : 50°C for 2 min, 95°C for 10 min, 50x [95°C for 15s, 58°C for 1 min]	*		-
	<i>cnorB</i>	ASc3-239	GGG AAC TGA TCA TGG CGT CGA TCC TGG		*		-
	<i>cnorB</i>	probe	CGT		*		-
Arabian Sea cluster 4	<i>cnorB</i>	ASc3-378r	ATA GTG ATG GCC GGT ACC CAG AAT	<u>Real-time PCR</u> : 50°C for 2 min, 95°C for 10 min, 50x [95°C for 15s, 58°C for 1 min]	*		-
	<i>cnorB</i>	ASc4-34f	GGC GTA TGG GAA TTG ATC ATG GCT		*		+
	<i>cnorB</i>	ASc4-86	TGA CCG GTG TGG ATC GCG AAG TGA TCG		*		+
Nitric oxide reducers (quinol-containing <i>norB</i> )	<i>cnorB</i>	probe	AAA	<u>Real-time PCR</u> : 50°C for 2 min, 95°C for 10 min, 50x [95°C for 15s, 60°C for 1 min]	*		+
	<i>cnorB</i>	ASc4-153r	GGA GAA CAG TGC CAA CCC AAC AAT		*		+
	<i>qnorB</i>	qnorB2F	GGN CAY CAR GGN TAY GA		13		
Arabian Sea cluster	<i>qnorB</i>	qnorB7R	GGN GGR TTD ATC ADG AAN CC	<u>Touchdown PCR</u> : 95°C for 5 min, 10 x [95°C for 30 s, 57°C (-0.5°C per cycle) for 40 s, 72°C for 1 min], 30x [95°C for 30 s, 55°C for 40 s, 72°C for 1 min]; 72°C for 7 min	13	+/F	
	<i>qnorB</i>	qnorB5R	ACC CAN AGR TGN ACN ACC CAC CA		13	-	
	<i>qnorB</i>	ASqnor270f	ATT CTT CGA GGT CTT TGC CAC G		*		+
Denitrifiers (nitrous oxide reducers)	<i>qnorB</i>	ASqnor458	TGT TCA GTG CTC TGG AAG TGG TGC CGC T	<u>Real-time PCR</u> : 50°C for 2 min, 95°C for 10 min, 50x [95°C for 15s, 60°C for 2 min]	*		+
	<i>qnorB</i>	probe	ATC GGC CAT TTG TAA CGC TGG A		*		+
	<i>nosZ</i>	nosZ-F	CGY TGT TCM TCG ACA GCC AG		14		
	<i>nosZ</i>	nosZ-R	CAT GTG CAG NGC RTG GCA GAA	94 °C for 2 min, 35 x (94°C for 30s, 50°C for 1 min, 72°C for 1 min), 72 °C for 10 min (with nosZ-F): 94 °C for 2 min, 35 x (94°C for 30s, 55°C for 1 min, 72°C for 1 min), 72 °C for 10 min	14	-	
	<i>nosZ</i>	Nos1773R	AAC GAV CAG YTG ATC GAY AT		15	-	

				(with nosZ-F): 94 °C for 2 min, 35 x (94°C for 30s, 53°C for 1 min, 72°C for 1 min), 72 °C for 10 min	4	F
Nitrogen fixers	<i>nosZ</i>	<i>nosZ1622R</i>	CGC RAS GGC AAS AAG GTS CG			
	<i>nifH</i>	nifH3	ATR TTR TTN GCN GCR TA	95°C for 5 min, 30 x [95°C for 1 min, 55°C for 1 min, 72°C for 1 min], 72°C for 5 min	16	-
	<i>nifH</i>	nifH4	TTY TAY GGN AAR GGN GG		16	-
	<i>nifH</i>	nifH1	TGY GAY CCN AAR GCN GA	<u>Nested PCR from nifH3-nifH4:</u> 95°C for 5 min, 30 x [95°C for 1 min, 55°C for 1 min, 72°C for 1 min], 72°C for 5 min	16	F
Crenarchaea	<i>16S rRNA</i>	Cren334F	AGA TGG GTA CTG AGA CAC GGA C		1	+
	<i>16S rRNA</i>	Cren554R Cren519	CTG TAG GCC CAA TAA TCA TCC T	<u>Real-time PCR:</u> 50°C for 2 min, 95°C for 10 min, 40x [95°C for 15s, 60°C for 1 min]	1	+
	<i>16S rRNA</i>	probe	TTA CCG CGG CGG CTG ACA C		1	+

## References

1. Lam, P. et al. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proc. Natl. Acad. Sci. USA* **104**, 7104-7109 (2007).
2. Lam, P. et al. Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc. Natl. Acad. Sci. USA* **106**, 4752-4757 (2009).
3. Michotey, V., Mejean, V. & Bonin, P. Comparison of Methods for Quantification of Cytochrome cd1-Denitrifying Bacteria in Environmental Marine Samples. *Appl. Environ. Microbiol.* **66**, 1564-1571 (2000).
4. Throback, I.N., Enwall, K., Jarvis, A. & Hallin, S. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.* **49**, 401-417 (2004).
5. Braker, G., Fesefeldt, A. & Witzel, K.-P. Development of PCR Primer Systems for Amplification of Nitrite Reductase Genes (nirK and nirS) To Detect Denitrifying Bacteria in Environmental Samples. *Appl. Environ. Microbiol.* **64**, 3769-3775 (1998).
6. Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S. & Germon, J.C. Molecular Analysis of the Nitrate-Reducing Community from Unplanted and Maize-Planted Soils. *Appl. Environ. Microbiol.* **68**, 6121-6128 (2002).
7. Mohan, S.B., Schmid, M., Jetten, M. & Cole, J. Detection and widespread distribution of the nrfA gene encoding nitrite reduction to ammonia, a short circuit in the biological nitrogen cycle that competes with denitrification. *FEMS Microbiol. Ecol.* **49**, 433-443 (2004).
8. Takeuchi, J. Habitat Segregation of a Functional Gene Encoding Nitrate Ammonification in Estuarine Sediments. *Geomicrobiol. Journal* **23**, 75-87 (2006).
9. Rotthauwe, J.-H., Witzel, K.-P. & Liesack, W. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **63**, 4704-4712 (1997).
10. Purkhold, U. et al. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. *Appl. Environ. Microbiol.* **66**, 5368-5382 (2000).
11. Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E. & Oakley, B.B. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl. Acad. Sci. USA* **102**, 14683-14688 (2005).
12. Casciotti, K.L. & Ward, B.B. Phylogenetic analysis of nitric oxide reductase gene homologues from aerobic ammonia-oxidizing bacteria. *FEMS Microbiol. Ecol.* **52**, 197-205 (2005).

13. Braker, G. & Tiedje, J.M. Nitric Oxide Reductase (norB) Genes from Pure Cultures and Environmental Samples. *Appl. Environ. Microbiol.* **69**, 3476-3483 (2003).
14. Kloos, K., Mergel, A., sch, C. & Bothe, H. Denitrification within the genus *Azospirillum* and other associative bacteria. *Functional Plant Biology* **28**, 991-998 (2001).
15. Scala, D.J. & Kerkhof, L.J. Nitrous oxide reductase (nosZ) gene-specific PCR primers for detection of denitrifiers and three nosZ genes from marine sediments. *FEMS Microbiol. Lett.* **162**, 61-68 (1998).
16. Zani, S., Mellon, M.T., Collier, J.L. & Zehr, J.P. Expression of nifH Genes in Natural Microbial Assemblages in Lake George, New York, Detected by Reverse Transcriptase PCR. *Appl. Environ. Microbiol.* **66**, 3119-3124 (2000).

**Table B3.** The measured variables and the respective abbreviations used in the non-metric multidimensional scaling analysis based on Spearman rank correlation in this study. Five final dimensions were chosen with a final stress of 0.0633. Analyses were conducted with the Statistics Toolbox of Matlab (R2008b, The Mathworks, Inc.).

Abbreviations	Measured Variables
ndens	Neutral density
O2	Dissolved oxygen
NH4	Ammonium concentration
NO2	Nitrite concentration
NO3	Nitrate concentration
N*	Nitrogen deficit expressed as N*=[Total measured inorganic N]-16[PO <sub>4</sub> <sup>3-</sup> ]+2.9×density
PO4	Phosphate concentration
N2O	Nitrous oxide concentration
PN	Surface particulate nitrogen
POC	Surface particulate organic carbon
CN	Particulate carbon to nitrogen ratios
AO	Ammonia oxidation rates
NR	Nitrate reduction rates
AmxNH4	Anammox rates measured via <sup>15</sup> NH <sub>4</sub> <sup>+</sup> incubations
AmxNO2	Anammox rates measured via <sup>15</sup> NO <sub>2</sub> incubations
pAmxNO2	Potential anammox rates measured via <sup>15</sup> NO <sub>2</sub> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup> incubations
pAmxNH4	Potential anammox rates measured via <sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> incubations
DNRA	Dissimilatory nitrate/nitrite reduction rates
BS820	Anammox bacterial cell abundance enumerated via CARD-FISH with anammox-specific probe BS820c
RNA	Total RNA concentration
DNA	Total DNA concentration
RNA:DNA	Total RNA:DNA ratios
FCM	Total microbial abundance measured via flow cytometry
Cren16S	Crenarchaeal abundance measured by 16S rDNA targeted real-time PCR
AamoAm	Archaeal ammonia monooxygenase subunit A ( <i>amoA</i> ) gene expression levels as mRNA
AamoAd	Archaeal <i>amoA</i> gene abundance
RAmoA	Gene expression ratios (mRNA: DNA) of archaeal <i>amoA</i> genes
AamoA:16S	Ratios of archaeal <i>amoA</i> gene copies to crenarchaeal 16S rRNA gene copies
bamoAm	β-Proteobacterial <i>amoA</i> gene expression levels as mRNA
bamoAd	β-Proteobacterial <i>amoA</i> gene abundance
RbamoA	Gene expression ratios (mRNA: DNA) of β-proteobacterial <i>amoA</i> genes
gamoAm	γ-Proteobacterial <i>amoA</i> gene expression levels as mRNA
gamoAd	γ-Proteobacterial <i>amoA</i> gene abundance
RgamoA	Gene expression ratios (mRNA: DNA) of γ-proteobacterial <i>amoA</i> genes
Scnirm	Anammox (Scalindua)-specific <i>cd</i> <sub>1</sub> -nitrite reductase gene ( <i>nirS</i> ) expression levels as mRNA
Scnird	Anammox (Scalindua)- specific <i>nirS</i> gene abundance
RScnir	Gene expression ratios (mRNA: DNA) of Scalindua-specific <i>nirS</i> genes
nirSm	Denitrifier <i>nirS</i> gene expression levels as mRNA
nirSd	Denitrifier <i>nirS</i> gene abundance
RnirS	Gene expression ratios (mRNA: DNA) of denitrifier <i>nirS</i> genes
narGm	Membrane-bound nitrate reductase gene large subunit ( <i>narG</i> ) expression levels as mRNA
narGd	<i>narG</i> gene abundance
RnarG	Gene expression ratios (mRNA: DNA) of <i>narG</i>
nrfAm	Cytochrome <i>c</i> nitrite reductase subunit A ( <i>nrfA</i> ) gene expression levels as mRNA
nrfAd	<i>nrfA</i> gene abundance
RnrfA	Gene expression ratios (mRNA: DNA) of <i>nrfA</i> genes
cnorBc2m	Expression levels of Arabian Sea cluster 2 cytochrome <i>bc</i> containing nitric oxide reductase subunit B ( <i>cnotB</i> ) genes as mRNA
cnotBc4m	Arabian Sea cluster 4 <i>cnotB</i> gene expression as mRNA
TnorBm	Total Arabian Sea type quinol- and cytochrome- <i>bc</i> - containing <i>norB</i> gene expressions as mRNA