Ammonia-oxidizing Bacteria of the *Nitrosospira* cluster 1 dominate over ammonia-oxidizing Archaea in oligotrophic surface sediments near the South Atlantic Gyre

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Summary

Sediments across the Namibian continental margin feature a strong microbial activity gradient at their surface. This is reflected in ammonium concentrations of <10 μM in oligotrophic abyssal plain sediments near the South Atlantic Gyre compared with ammonium concentrations of >700 μM in upwelling areas near the coast. Here we address changes in apparent abundance and structure of ammonia-oxidizing archaeal and bacterial communities (AOA and AOB) along a transect of seven sediment stations across the Namibian shelf by analysing their respective ammonia monooxygenase genes (*amoA*). The relative abundance of archaeal and bacterial *amoA* (g−1 DNA) decreased with increasing ammonium concentrations, and bacterial *amoA* frequently outnum-bered archaeal *amoA* at the sediment–water interface [0–1 cm below seafloor (cmbsf)]. In contrast, AOA were apparently as abundant as AOB or dominated in several deeper (>10 cmbsf), anoxic sediment layers. Phylogenetic analyses showed a change within the AOA community along the transect, from two clusters without cultured representatives at the gyre to *Nitrososphaera* and *Nitrosopumilus* clusters in the upwelling region. AOB almost exclusively belonged to the *Nitrosospira* cluster 1. Our results suggest that this predominantly marine AOB lineage without cultured representatives can thrive at low ammonium concentrations and is active in the marine nitrogen cycle.

Introduction

The Benguela upwelling system (BUS) along the southwest African continental margin is one of the most productive upwelling regions in the world oceans (Carr, 2001). Decreasing water depth and increasing primary productivity result in (i) a >100-fold increase in sediment organic matter content from oligotrophic surface sediments near the South Atlantic Gyre (SAG) to sediments underlying the hypoxic and seasonally anoxic water column of the BUS (Inthorn et al., 2006; Lavik et al., 2009; Lin et al., 2012), and (ii) a strong decrease in bottom water dissolved oxygen concentrations from lower shelf regions to the BUS (Lavik et al., 2009). The decrease in bottom water dissolved oxygen concentrations is reflected in a two order of magnitude decrease in benthic macrofaunal abundance (Sanders, 1969).

Ammonia oxidation is carried out by chemolithotrophic ammonia-oxidizing Archaea and Bacteria (AOA and AOB). As the first step towards the removal of fixed nitrogen via denitrification or anaerobic ammonium oxidation, it is a key process in the marine environment. As AOA and AOB presumably take up the same ecological role in the environment, niche specialization and subsequently niche differentiation between AOA and AOB have been suggested (Erguder et al., 2009; Schleper, 2010; Pester et al., 2011; Prosser and Nicol, 2012). Salinity (Mosier and Francis, 2008; Santoro et al., 2008), pH (Nicol et al., 2008; Zhang et al., 2011) and oxygen (Bouskill et al., 2012; Pett-Ridge et al., 2013) have been identified as important environmental variables for such niche differentiation. Additionally, studies of terrestrial (Di et al., 2009; Jia and Conrad, 2009; Baolan et al., 2012; Prosser and Nicol, 2012) and marine environments (Wuchter et al.,

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2006), and of AOA in culture (Martens-Habbena et al., 2009), suggest that AOA are better adapted to low ammonium concentrations, while AOB outcompete AOA at high ammonium concentrations, and that therefore ammonium concentrations may be the driving force for niche partitioning between AOA and AOB.

In this study, we utilized the strong gradient in microbial activity and sediment organic matter content in the surface sediments along a 550 km transect from the rim of the SAG into the BUS on the inner continental shelf off Namibia. Sediments from seven stations were collected during the RV METEOR cruise M76-1 in April 2008. The sediments ranged from highly oxidized, oligotrophic deep sea red clays in the SAG to organic-rich sites with oxygen-rich bottom water on the slope and outer shelf, to extremely organic-rich sediments underlying permanently hypoxic, seasonally anoxic bottom water on the inner shelf (Fig. 1, Table S1 for site characteristics; Lavik et al., 2009). We hypothesized that the drastic increase of ammonium concentrations from SAG to BUS surface sediments would drive a transition from AOA-dominated to AOB-dominated ammonia oxidizer communities along the transect. To this end, we analysed ammonium pore water concentrations as well as diversity and apparent abundance of benthic AOA and betaproteobacterial AOB across the gradient.

**Results and discussion**

**Chemical gradients**

At the abyssal plain (GeoB12815) and the lower rise (GeoB12808) stations, ammonium was below the detection limit (10 μmol l⁻¹) throughout the upper 30 cm below seafloor (cmbsf) (Fig. 2A). The lower rise station GeoB12811 had an ammonium profile similar to the shelf break and slope stations, but here the ammonium flux (as predicted from transport-reaction models) from below did not reach the sediment surface. At the shelf break and upper slope stations (GeoB12802, 12803 and...
ammonium concentrations were higher (10–50 μmol l⁻¹), and the predicted ammonium flux from the subsurface reached the sediment–water interface. Station GeoB12806, the only station located in the intense upwelling zone, where the seabed consisted of an extremely organic-rich diatom ooze (Borchers et al., 2005), featured ammonium concentrations at least one order of magnitude higher (572–1914 μmol l⁻¹) than all the other stations. The oxygen penetration depth predicted from in situ data from the region (Wenzhöfer and Glud, 2002) decreased from 112 mm at the most oligotrophic station GeoB12815 to 15 mm at GeoB12803, and was below 10 mm at the upper slope, shelf break and shelf stations (Fig. 2A, Table S1, Appendix S1). Ammonium production in the oxic surface sediment, as estimated from carbon oxidation rates via the oxygen penetration depths (for details, see Appendix S1, Table S1), exceeded the ammonium diffusing into the oxic zone from below at all stations, most strongly at the offshore sites. Since there was no accumulation of ammonium in the oxic zone, this locally produced ammonium must have been concurrently oxidized, and it constitutes the most significant source of ammonium for the AOA and AOB. Thus, the volumetric ammonium oxidation rate increased >500-fold from the SAG to the BUS. Bioturbation was apparent at stations GeoB12802, GeoB12803, GeoB12811 and GeoB12815, but not GeoB12807 and GeoB12806 (Zabel et al., 2008), indicating that actual sediment oxygenation may occasionally reach deeper than modelled (up to 100 mm) towards the SAG but not at the BUS. Overall, the data documented that the organic matter gradient from the SAG rim sediments towards the BUS is reflected by an increase of ammonium concentration and a decrease of oxygen availability.

Quantification of archaeal and bacterial amoA genes

Archaeal and bacterial 16S rRNA gene copy numbers were determined by quantitative real-time PCR (qPCR) for several depths at the seven sampling stations (25 samples; Fig. 2B). Total 16S rRNA gene abundance within the uppermost sampled sediment layer (0–1 cmbsf) increased towards the coast, with abundance increasing from 1.15 × 10⁶ copies g⁻¹ sediment at station GeoB12815 closest to the SAG to 6.96 × 10⁸ copies g⁻¹ sediment at station GeoB12806 beneath the upwelling cell. Assuming average 16S rRNA gene copy numbers of 1.64 for Archaea and 3.98 for Bacteria (averaged based on 165 archaeal and 2649 bacterial finished genomes available on 20 March 2014, on the Integrated Microbial Genomes (IMG) webpage; Markowitz et al., 2006), the corresponding apparent cell numbers increased from 4.82 × 10⁷ g⁻¹ sediment (GeoB12815) to 2.05 × 10⁸ g⁻¹ sediment (GeoB12806) (Table S2).

This trend was even more pronounced in deeper sediments layers (>10 cmbsf) where total 16S rRNA gene abundance increased from 5.06 × 10⁴ copies g⁻¹ sediment off shelf (2.34 × 10⁴ cells g⁻¹ sediment; 14 cmbsf, GeoB12815) to 2.01 × 10⁶ copies g⁻¹ sediment near shore (6.49 × 10⁴ cells g⁻¹ sediment; 12.5 cmbsf, GeoB12806). The observed trend was probably caused by the low sedimentation rate in the gyre, and generally speaking older sediment with more recalcitrant organic matter below the surface at offshore sites compared with sites located closer to shore (Inthorn et al., 2006; Mollenhauer et al., 2007).

Quantification of archaeal and bacterial amoA genes

A subset of 19 samples used for bacterial and archaeal 16S rRNA gene quantification, as well as four additional samples (GeoB12811: 10 cm; GeoB12807: 24 cmbsf and 29 cmbsf; GeoB12806: 31 cmbsf), were screened for the presence of archaeal and bacterial amoA, which were detected across all stations and depths (Fig. 2C). Average archaeal amoA copy numbers ranged from 5.2 × 10² genes g⁻¹ sediment (14 cmbsf, GeoB12811) to 5.2 × 10⁵ genes g⁻¹ sediment (14 cmbsf, GeoB12815). Bacterial amoA copy numbers ranged from 1.45 × 10² genes g⁻¹ sediment (14 cmbsf, GeoB12808) to 6.6 × 10⁶ genes g⁻¹ sediment (19 cmbsf, GeoB12803).

These numbers are comparable to previous amoA surveys in marine sediments (e.g. Dang et al., 2010; Cao et al., 2012; Zheng et al., 2013). Similar to marine sediments off the coast of California (Beman et al., 2012), log-transformed amoA copy numbers of AOA show a significant positive correlation with amoA copy numbers of AOB across the analysed samples (Pearson correlation; r = 0.84, P = 3.2 × 10⁻⁶). This suggests that AOA and AOB respond similarly to environmental parameters at the sampling sites. Correlations were calculated between qPCR data and all available geochemical data along the transect, but none of them was significant (for details, see Tables S5 and S6). AmoA copy numbers of AOA and, to a lesser degree, also of AOB showed a decreasing trend from the oligotrophic sediments near the SAG towards the eutrophic upper slope and shelf sediments (Fig. 3). This decrease coincided with a decreasing predicted oxygen penetration depth (Fig. 2A). The biggest drop-off in relative amoA abundance (Fig. 3) was observed at the two shallow shelf stations GeoB12807 and GeoB12806, an area for which hypoxic and seasonally anoxic bottom water has been reported (Brüchert et al., 2009).

Most samples showed a dominance of amoA copy numbers of AOB over those of AOA, with a trimean ratio
of 7.4 (range: 0.2–102; Fig. 2C). A survey of published genomic information of AOA and AOB (Norton et al., 1996; Norton et al., 2008; Walker et al., 2010; Blainey et al., 2011; and retrieved from IMG website: Markowitz et al., 2006) showed that betaproteobacterial AOB usually feature multiple amoA gene copies per genome compared with only one copy in AOA. Specifically, AOB of the genus Nitrosospira, which formed the majority of AOB detected in the present study (see below), contain three amoA copies per genome. Taking this into account, apparent AOA and AOB cell numbers were comparable (AOB/AOA ratios of 0.9 and 1) in 5 of the 23 analysed samples (Table S3). Higher AOB/AAO ratios (3.8–34) that cannot readily be explained by varying copy numbers in the genome were detected in the upper, mostly oxic, surface sediment samples (0–1 cmbsf) of the five intermediate transect sites (all but stations GeoB12815 and GeoB12806), where ammonium concentrations were ≤ 21 μmol l⁻¹ and active ammonium oxidation took place. This observation falsified our starting hypothesis of a shift from AOA to AOB dominance from the ammonium-poor gyre towards the ammonium-rich BUS sediments, and suggests a more complex niche differentiation of ammonia oxidizers in this system. AmoA gene copy numbers of AOA and AOB generally increased by an order of magnitude from the surface layer (0–1 cmbsf) to the next deeper layer (≥ 5 cmbsf) (Table S3), which indicates growth of AOA and AOB in the top sediment layers.

The proportion of archaeal relative to bacterial amoA increased with sediment depth at all sites except site GeoB12803 (Fig. 2C, Table S3), especially below the oxic zone, where ammonia oxidizers presumably are inactive. Similar trends were previously observed in soil (Leininger et al., 2006) and marine sediments (Beman et al., 2012), and may be explained by the Archaea’s suggested ability to better persist with little or no energy sources due to their lower basal power requirement (Valentine, 2007; Hoehler and Jørgensen, 2013).

**Phylogenetic diversity of AOA and AOB**

Archaeal and bacterial amoA diversities were analysed in surface sediments (0–1 cmbsf) of the abyssal plain site closest to the SAG (GeoB12815), the upper rise site (GeoB12811) and the shelf break site (GeoB12807). With 13 operational taxonomic units (OTUs) of archaeal amoA (Fig. S2) and 6 OTUs of bacterial amoA (Fig. S3), overall diversity was low, and neither diversity indices nor richness estimates showed a significant trend across the transect (Table 1). Archaeal amoA sequences affiliated with the Nitrosopumilus cluster (NPUM), the Nitrososphaera subcluster 1.1 (NSP) and five distinct sequence clusters (A–E) without cultured representatives (NPUM and NSP nomenclature based on Pester et al., 2012) (for details, see Table 1, Fig. S2). Clusters NPUM and NSP were rare at stations GeoB12815 and GeoB12811, but dominated the clone library at station GeoB12807. In contrast, clusters A and D dominated the clone libraries at stations GeoB12815 and GeoB12811, but were rare (cluster D) or absent (cluster A) at station GeoB12807. The remaining sequence clusters showed low abundance in clone libraries from all three stations. Assuming that the observed amoA clone abundance represents AOA cell abundance, this distribution may indicate different adaptations of AOA in all clusters to ammonium, oxygen and organic carbon availability, with NPUM and NSP occupying the most eutrophic, oxygen-limited niche, and clusters A and D occupying more oligotrophic, oxygen-rich niches.

All six bacterial amoA OTUs clustered with other marine amoA sequences in a sister group to the Nitrosospira lineage (Fig. S3). 16S rRNA gene analysis confirmed this affiliation, as all but one sequence affiliated with Nitrososira cluster 1 (Stephen et al., 1996; Fig. S4). The difference in the exact tree topologies can be explained by the different resolution of the amoA and 16S rRNA genes (Koops et al., 2006).

Nitrososira cluster 1 contains currently no cultured representative and is the only Nitrososira cluster frequently found in marine environments (Stephen et al., 1996; McCaig et al., 1999; Koops et al., 2006). As all cultured Nitrososira species have low salt tolerance (Koops et al., 2006), Nitrososira cluster 1 has been proposed as solely allochthonous inhabitants in marine settings without significant physiological activity (Koops et al., 2006). On the other hand, their widespread distribution and even predominance in marine ammonia-oxidizing communities (Bano and Hollibaugh, 2000; Freitag and Prosser, 2003; Freitag et al., 2006) suggest a more active role in the marine nitrogen cycle. Our qPCR data showed (i) an increase of Nitrososira cluster 1 off coast towards the gyre, while one would expect the opposite trend if they were allochthonous input from land; and (ii) an increase of apparent AOB cell numbers from the top sediment layer (0–1 cmbsf) to the next deeper layer (see above), indicating active growth of Nitrososira cluster 1 at sampling sites GeoB12815, GeoB12803, GeoB12802 and GeoB1207. Finally, at least at station GeoB12807 active ammonia oxidation by Nitrososira cluster 1 is implied, as AOA alone are unlikely to account for the estimated ammonia oxidation rate: published cell-specific rates for AOA are 0.2–0.5 fmol NH₃ cell⁻¹ h⁻¹ (Martens-Habbema et al., 2009; Tourna et al., 2011), while rates of > 2 fmol cell⁻¹ h⁻¹ would be needed if ammonia oxidation was solely performed by AOA (see Appendix S1 for details of
A \( \text{NH}_4^+ \) concentration

B 16S rRNA gene copy numbers

C \textit{amoA} gene copy numbers

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calculation). These combined observations indicate that *Nitrosospiro* cluster 1 actively grow and oxidize ammonia in these marine sediments.

Half saturation constants ($K_m$) for betaproteobacterial ammonia oxidation range from 10 to 3300 μM ($NH_4^+ + NH_3$) (Stehr *et al.*, 1995; Pommerening-Röser *et al.*, 1996; Jiang and Bakken, 1999; Schramm *et al.*, 1999; Bollmann *et al.*, 2005). The presence and growth of *Nitrosospiro* cluster 1 in sediments with ammonium concentrations < 10 μM thus likely position them among the AOB with the highest ammonia affinities; adaptation of *Nitrosospiro* cluster 1 to low ammonium was also previously indicated by their increased relative abundance in oligotrophic compared with eutrophic mangrove sediment (Laanbroek *et al.*, 2012). Whether *Nitrosospiro* cluster 1 is able to compete for ammonium with high-affinity AOA, whose $K_m$ is in the nM range, cannot be resolved on the basis of our data.

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Table 1. Diversity, phylogenetic affiliation and relative abundance of AOA and AOB clones from three sites along the BUS transect.

| Diversity measures | Ammonia-oxidizing Archaea (amoA<sup>A</sup>) | Ammonia-oxidizing Bacteria (amoA<sup>A</sup>) | Ammonia-oxidizing Bacteria (16S rRNA<sup>B</sup>) |
|-------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Simpson's index of diversity | 0.75 | 0.22 | 0.55 |
| Shannon-Wiener index | 1.6 | 0.68 | 0.89 |
| Richness | 9 | 3 | 4 |
| Chao1 | 13 | 3 | 4 |
| Phylogeny | | | |
| Nitrospumilus cluster | 5% | 100% | 100% |
| Nitrosospheara cluster | 16% | 100% | 100% |
| Cluster A | 29% | 0% | 0.16 |
| Cluster B | 0% | 0% | 0.44 |
| Cluster C | 0% | 0% | 7 |
| Cluster D | 44% | 0% | 37 |
| Cluster E | 4% | 0% | 3 |
| Total number of clones (n) | 87 | 88 | 91 |

Diversity measures were calculated based on OTU clustering using a 13% sequence divergence cut-off for archaeal amoA (Pester et al., 2012), an 8% cut-off for bacterial amoA (this study; Fig. S1) and a 1% cut-off for 16S rRNA genes (Ebers and Stackebrandt, 2006). Chao1 analysis was performed using *ESTIMATES* 9.1.0 (Colwell, 2013). Phylogenetic affiliation of amoA and 16S rRNA gene clones is based on consensus phylogeny of maximum likelihood (RAxML version 7.4.2; Stamatakis et al., 2008), maximum parsimony, and neighbour joining (both PHYLIP 3.69; Felsenstein, 2005) analyses (Figs S2–S4). Sequences are available under European Nucleotide Archive Accession Numbers LK055903-LK056644 and LK392634-LK392675.

References

Bano, N., and Hollibaugh, J.T. (2000) Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the β subdivision of the class Proteobacteria in the Arctic Ocean. *Appl Environ Microbiol* 66: 1960–1969.

Baolan, H., Shuai, L., Lidong, S., Ping, Z., Xiangyang, X., and Liping, L. (2012) Effect of different ammonia concentrations on community succession of ammonia-oxidizing microorganisms in a simulated paddle soil column. *PLoS ONE* 7: e44122.

Beman, J.M., Bertics, V.J., Braunschweiler, T., and Wilson, J.M. (2012) Quantification of ammonia oxidation rates and the distribution of ammonia-oxidizing Archaea and Bacteria in marine sediment depth profiles from Catalina Island, California. *Front Microbiol* 3: 263.

Berg, P., Risgaard-Petersen, N., and Rysgaard, S. (1998) Interpretation of measured concentration profiles in sediment pore water. *Limnol Oceanogr* 43: 1500–1510.

Blainey, P.C., Mosier, A.C., Potanina, A., Francis, C.A., and Quake, S.R. (2011) Genome of a low-salinity ammonia-oxidizing archaeon determined by single-cell and metagenomic analysis. *PLoS ONE* 6: e16626.

Bollmann, A., Schmidt, I., Saunders, A.M., and Nicolaisen, M.H. (2005) Influence of starvation on potential ammonia-oxidizing activity and amoA mRNA levels of *Nitrosospira briensis*. *Appl Environ Microbiol* 71: 1276–1282.

Borchers, S.L., Schnetger, B., Böning, F., and Brumsack, H.J. (2005) Geochemical signatures of the Namibian diatom belt: perennial upwelling and intermittent anoxia. *Geochem Geophys Geosyst* 6: Q06006.

Bouskill, N.J., Eveillard, D., Chien, D., Jayakumar, A., and Ward, B.B. (2012) Environmental factors determining ammonia-oxidizing organism distribution and diversity in marine environments. *Environ Microbiol* 14: 714–729.

Brüchert, V., Currie, B., and Peard, K.R. (2009) Hydrogen sulphide and methane emissions on the central Namibian shelf. *Prog Oceanogr* 83: 169–179.

Cao, H., Hong, Y., Li, M., and Gu, J.-D. (2012) Lower abundance of ammonia-oxidizing archaea than ammonia-oxidizing bacteria detected in the subsurface sediments of the Northern South China Sea. *Geomicrobiol J* 29: 332–339.

Carr, M.-E. (2001) Estimation of potential productivity in eastern boundary currents using remote sensing. *Deep Sea Res Part 2 Top Stud Oceanogr* 49: 59–80.

Colwell, R.K. (2013) Estimates: statistical estimation of species richness and shared species from samples [WWW document]. URL http://viceroy.eeb.uconn.edu/estimates.
Alex, B., (2008) Shifts in the relative abundance of ammonia-oxidizing bacteria. Appl Environ Microbiol 74: 4704–4712.

Embley, T.M. (1996) Molecular diversity of soil and marine bacteria and archaea? Trends Microbiol 4: 302–309.

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Pester, M., Schleper, C., and Wagner, M. (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr Opin Microbiol 14: 300–306.

Pester, M., Rattei, T., Flechl, S., Gröngröft, A., Richter, A., Overmann, J., et al. (2012) amoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. Environ Microbiol 14: 525–539.

Pett-Ridge, J., Petersen, D.G., Nuccio, E., and Firestone, M.K. (2010) Influence of oxic/anoxic fluctuations on ammonia oxidizers and nitrification potential in a wet tropical soil. FEMS Microbiol Ecol 85: 179–194.

Pommerening-Röser, A., Rath, G., and Koops, H.-P. (1996) Phylogenetic diversity within the genus Nitrosomonas. Syst Appl Microbiol 19: 344–351.

Prosser, J.I., and Nicol, G.W. (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol 20: 523–531.

Reyesenbach, A.L., Wickham, G.S., and Pace, N.R. (1994) Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. Appl Environ Microbiol 60: 2113–2119.

Rothaue, J.-H., Witzel, K.-P., and Liesack, W. (1997) 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.

Sanders, H.L. (1969) Benthic marine diversity and the stability time hypothesis. Brookhaven Symp Biol 22: 71–81.

Santoro, A.E., Francis, C.A., De Sieyes, N.R., and Boehm, A.B. (2008) Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environ Microbiol 10: 1068–1079.

Schleper, C. (2010) Ammonia oxidation: different niches for bacteria and archaea? ISME J 4: 1092–1094.

Schramm, A., de Beer, D., van den Heuvel, J.C., Ottengraf, S., and Amann, R. (1999) Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Appl Environ Microbiol 65: 3690–3696.

Stamatakis, A., Hoover, P., and Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. Syst Biol 57: 758–771.

Stehr, G., Böttcher, B., Dittberner, P., Rath, G., and Koops, H.-P. (1995) The ammonia-oxidizing nitrifying population of the River Elbe estuary. FEMS Microbiol Ecol 17: 177–186.

Stephen, J.R., McCaig, A.E., Smith, Z., Prosser, J.I., and Embley, T.M. (1996) Molecular diversity of soil and marine 16S rRNA gene sequences related to beta-subgroup ammonia-oxidizing bacteria. Appl Environ Microbiol 62: 4147–4154.

Takai, K., and Horikoshi, K. (2000) Rapid detection and quantification of members of the Archaeal community by quantitative PCR using fluorogenic probes. Appl Environ Microbiol 66: 5066–5072.

Tournas, M., Stieglermeier, M., Spang, A., Könneke, M., Schintmeister, A., Urlich, T., et al. (2011) Nitrosopumilus viennensis, an ammonia oxidizing archaeon from soil. Proc Natl Acad Sci USA 108: 8420–8425.

Valentine, D.L. (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat Rev Microbiol 5: 316–323.

Walker, C., De La Torre, J., Klotz, M., Urakawa, H., Pinel, N., Arp, D., et al. (2010) Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaeota. Proc Natl Acad Sci USA 107: 8818–8823.

Wenzhöfer, F., and Glud, R.N. (2002) Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. Deep Sea Res Part 1 Oceanogr Res Pap 49: 1255–1279.

Wuchter, C., Abbas, B., Coolen, M.J.L., Herfort, L., van Bleijswijk, J., Timmers, P., et al. (2006) Archaeal nitrification in the ocean. Proc Natl Acad Sci USA 103: 12317–12322.

Zabel, M., Boetius, A., Emeis, K.-C., Ferdelman, T.G., and Spieß, V. (2008) METEOR Cruise report – Cruise No. 76, Leg 1–3 [WWW document]. URL https://www.marum.de/Binaries/Binary12275/Fahrtbericht_M76_1-3a_b.pdf.

Zhang, L.-M., Hu, H.-W., Shen, J.-P., and He, J.-Z. (2011) Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. ISME J 6: 1032–1045.

Zheng, Y., Hou, L., Liu, M., Lu, M., Zhao, H., Yin, G., and Zhou, J. (2013) Diversity, abundance, and activity of ammonia-oxidizing bacteria and archaea in Chongming eastern intertidal sediments. Appl Microbiol Biotechnol 97: 8351–8363.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Fig. S1. Pairwise comparison/correlation plot of 16S rRNA and bacterial amoA gene identities of cultivated AOB species. amoA genes and corresponding 16S rRNA genes of the same species of AOB were retrieved from GenBank (Benson et al., 2010) and JGI’s IMG (Markowitz et al., 2006). The gene pair for a given AOB was compared with gene pairs of all other AOB; plotted are the corresponding similarity values of that comparison. Only pairs were the amoA gene was ≥600 bp long and the 16S rRNA gene ≥1300 bp long were considered for the analysis. Dotted lines indicate the amoA or 16S rRNA threshold values below which sequences are indicative of novel AOB species.

Fig. S2. Phylogenetic affiliation of archaeal amoA sequences retrieved in the present study. Shown is a consensus tree of maximum likelihood (ML; RAxML version 7.4.2; Stamatakis et al., 2008), maximum parsimony (MP; PHYLIP 3.69; Felsenstein, 2005) and neighbour joining (NJ; PHYLIP 3.69; Felsenstein, 2005) analyses of archaeal amoA gene sequences. The tree is based on the 635 bp gene fragment that was obtained with the primer set Arch-amofAT and Arch-amofAR (Francis et al., 2005) and publicly available amoA gene sequences of AOA. The tree was rooted against the amoA gene sequence of the AOB Nitrosomonas europaea (AF058692; not shown). Branching patterns that were not supported by all three methods were collapsed to multifurcations. For branching patterns obtained with all three
methods, bootstrap support values (1000 re-samplings) are shown: Circle pie pieces from 8 to 12 o’clock represent bootstrap values of the NJ analysis from 12 to 4 o’clock those of the MP analysis, and finally from 4 to 8 o’clock those from the ML analysis. Bootstrap support of branching > 90% is depicted with black pie piece colouring, while bootstrap support > 50% and < 90% are depicted with grey pie piece colouring. Representative sequences for each OTU and station are shown in bold, along with their EMBL Nucleotide Sequence Database accession numbers. OTU class and number of clones for a given station are indicated in parenthesis. Sequences marked with an asterisk (*) were retrieved from JGI’s IMG website (Markowitz et al., 2006). Scale bar represents 10% estimated sequence divergence. Naming of the phylogenetic clusters (except for unaffiliated clusters A–E) is based on Pester and colleagues (2012).

**Fig. S3.** Phylogenetic affiliation of bacterial amoA sequences retrieved in the present study. Shown is a consensus tree of MP (RAxML version 7.4.2; Stamatakis et al., 2008), MP (PHYLIP 3.69; Felsenstein, 2005) and NJ (PHYLIP 3.69; Felsenstein, 2005) analyses of bacterial amoA gene sequences. The tree is based on the 491 bp gene fragment that was obtained with the primer set amoA-1F/amoA-2R (Rotthauwe et al., 1997) and publicly available amoA gene sequences of AOB. The tree was rooted against the amoA sequence of the gammaproteobacterial AOB Nitrosococcus oceani (AF047705; not shown). Branching patterns that were not supported by all three methods were collapsed to multifurcations. For branching patterns obtained with all three methods, bootstrap support values (1000 re-samplings) are shown: Circle pie pieces from 8 to 12 o’clock represent bootstrap values of the NJ analysis, from 12 to 4 o’clock those of the MP analysis, and finally from 4 to 8 o’clock those from the ML analysis. Bootstrap support of branching > 90% is depicted with black pie piece colouring, while bootstrap support > 50% and < 90% are depicted with grey pie piece colouring. Representative sequences for each OTU and station are shown in bold, along with their EMBL Nucleotide Sequence Database accession numbers. OTU class and number of clones for a given station are indicated in parenthesis. The scale bar represents 10% estimated sequence divergence.

**Fig. S4.** Phylogenetic affiliation of 16S rRNA gene sequences retrieved in the present study. Shown is a consensus tree of MP (RAxML version 7.4.2; Stamatakis et al., 2008), MP (PHYLIP 3.69; Felsenstein, 2005) and NJ (PHYLIP 3.69; Felsenstein, 2005) analyses of betaproteobacterial AOB 16S rRNA gene sequences. The tree is based on 16S rRNA gene sequences (465 bp; *E. coli* positions 189–654) of known betaproteobacterial AOB and partial 16S rRNA gene sequences of the Benguela upwelling system (generated in this study with the primer set CT0189fA-B/CT0654r; Kowalchuk et al., 1997). The tree is rooted against the 16S rRNA gene sequence of *Burkholderia hospita* (DQ855426; not shown). Branching patterns that were not supported by all three methods were collapsed to multifurcations. For branching patterns obtained with all three methods, bootstrap support values (1000 re-samplings) are shown: Circle pie pieces from 8 to 12 o’clock represent bootstrap values of the NJ analysis, from 12 to 4 o’clock those of the MP analysis, and finally from 4 to 8 o’clock those from the ML analysis. Bootstrap support of branching > 90% is depicted with black pie piece colouring, while bootstrap support > 50% and < 90% are depicted with grey pie piece colouring. Representative sequences for each OTU and station are shown in bold, along with their EMBL Nucleotide Sequence Database accession numbers. OTU class and number of clones for a given station are indicated in parenthesis. The scale bar represents 10% estimated sequence divergence. Subgroup naming is based on Stephen and colleagues (1996) and Koops and colleagues (2006).

**Table S1.** Transect stations with coordinates, water depth, representative bottom water temperature (data from Mohrholz et al., 2008), oxygen and ammonium data. Values in parentheses for the predicted O2 penetration depth indicate that those stations were within the mudbelt, and the predicted penetration depths are therefore maximum values. The predicted NH4+ production rate in the oxic zone leads to an equal rate of NH3 oxidation.

**Table S2.** Archaeal and bacterial 16S rRNA gene copy numbers, estimated cell numbers per gram of wet sediment and Bacteria/Archaea ratios. Cell numbers were estimated by using averages of 1.64 and 3.98 16S rRNA gene copies per genome for Archaea and Bacteria (for details, see main text) respectively.

**Table S3.** Archaeal and bacterial amoA gene copy numbers, estimated cell numbers and AOB/AOA ratios. Gene copy numbers are presented per gram of (wet) sediment and per gram of extracted DNA. Cell numbers were estimated by using averages of 1 and 3 amoA gene copies per genome for Archaea and Bacteria respectively. Apparent relative abundance was calculated based on estimated AOA and AOB cell numbers g−1 sediment (this table) and total estimated cell numbers of Archaea and Bacteria g−1 sediment as shown in Table S2.

**Table S4.** Ammonium (NH4+) concentration data as displayed in Fig. 2. Uppermost sample is bottom water. Samples that yielded a positive signal but concentrations below the quantification limit of the method are labelled with an asterisk (*). See Appendix S1 for interpretation of these values.

**Table S5.** Pearson correlation coefficients (r) for comparisons between log-transformed qPCR data (gene copies g−1 sediment) and geochemical data of sediment samples. Strong correlations (r > 0.6) are indicated with bold type. Significance of correlations (P < 0.01) is indicated with an asterisk (for detailed P values, see Table S6). Arc, Archaea; Bac, Bacteria; bac-amoa, bacterial amoA; arc-amoa, archaeal amoA; DIC, dissolved inorganic carbon. The used geochemical data of the samples can be retrieved from PANGAEA – Data Publisher for Earth & Environmental Science (http://www.pangaea.de).

**Table S6.** P values for Pearson correlation coefficients (r) shown in Table S5. P values were calculated using a two-tailed Student’s t-test for the Pearson correlation. P values indicating statistical significance are shown in bold type. Arc, Archaea: Bac, Bacteria; bac-amoa, bacterial amoA; arc-amoa, archaeal amoA; DIC, dissolved inorganic carbon.

**Appendix S1.** Supplementary methods.