

SHORT COMMUNICATION

Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia

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Nitrification, the oxidation of NH_4^+ to NO_2^- and subsequently to NO_3^- , plays a central role in the nitrogen cycle and is often a critical first step in nitrogen removal from estuarine and coastal environments. The first and rate-limiting step in nitrification is catalyzed by the enzyme ammonia monooxygenase (*AmoA*). We evaluate the relationships between the abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) *amoA* genes; potential nitrification rates and environmental variables to identify factors influencing AOA abundance and nitrifier activity in estuarine sediments. Our results showed that potential nitrification rates increased as abundance of AOA *amoA* increased. In contrast, there was no relationship between potential nitrification rates and AOB *amoA* abundance. This suggests that AOA are significant in estuarine nitrogen cycling. Surprisingly, more of the variability in potential nitrification rates was predicted by salinity and pore water sulfide than by dissolved oxygen history.

The ISME Journal (2007) 1, 660–662; doi:10.1038/ismej.2007.79; published online 4 October 2007

Subject Category: geomicrobiology and microbial contributions to geochemical cycles

Keywords: nitrification; ammonia oxidation; estuary; salinity; sulfide

The long-held view that ammonia oxidation was restricted to relatively few genera in β - and γ -Proteobacteria has changed following the discovery that *amoA* occurs in Crenarchaeota (Francis *et al.*, 2005, 2007; Treusch *et al.*, 2005; Beman and Francis, 2006). In addition, a Crenarchaeota, *Nitrosopumilus maritimus*, has been isolated that is capable of nitrification and contains the *amoA* gene (Konneke *et al.*, 2005). However, little is known about whether ammonia-oxidizing archaea (AOA) are significant nitrifiers in marine and estuarine environments.

Critical environmental variables known to affect nitrification rates include temperature (Kaplan, 1983; Henriksen and Kemp, 1986), salinity (Jones and Hood, 1980; Rysgaard *et al.*, 1999), dissolved oxygen (DO) concentrations (Kaplan, 1983; Henrik-

sen and Kemp, 1986), NH_4^+ availability (Jones and Hood, 1980; Kaplan, 1983; Henriksen and Kemp, 1986), light (Ward, 2000) and sulfide concentrations (Joye and Hollibaugh, 1995).

We measured potential nitrification rates; characteristics of bottom water and sediments; and abundance of ammonia-oxidizing bacteria (AOB) and AOA *amoA* genes in six different estuaries at multiple sites (Supplementary Table 1). Three estuaries were sampled repeatedly (Supplementary Table 1). Sampling sites differed in salinity of overlying water; and in sediment chlorophyll *a*, extractable NH_4^+ and sulfide concentrations of sediment pore water. Bottom water DO variability ranged from sampling sites that were often hypoxic ($\text{DO} < 2 \text{ mg l}^{-1}$) to sites that never went hypoxic (Supplementary Table 1).

The abundance of AOA *amoA* genes was significantly higher than AOB *amoA* (Figure 1; *t*-test $P = 0.001$). AOB *amoA* dominated only in samples from Weeks Bay, particularly at the Fish River site (Figure 1 inset). Log abundance of AOA *amoA* and AOB *amoA* was significantly correlated with one another ($r = 0.56$, $P = 0.001$; Supplementary Table 2). We found that AOA *amoA* genes were more abundant, often as much as 80 times greater than AOB *amoA* genes. However, this relationship was

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Received 22 May 2007; revised 3 September 2007; accepted 4 September 2007; published online 4 October 2007

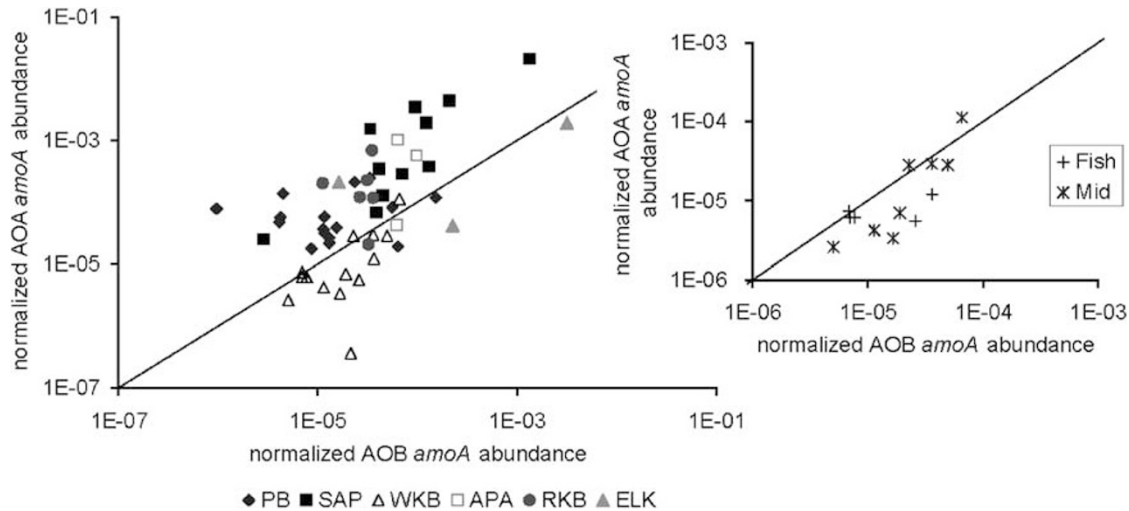


Figure 1 Abundance of *amoA* genes for ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) normalized relative to prokaryotic DNA for six different estuaries (see Supplementary Table 1). The relative abundance of *amoA* genes is expressed by normalizing *amoA* gene abundance to the number of copies of prokaryotic (bacteria + archaea) 16S rRNA genes in each sample. A one-to-one line is included for reference. Inset shows abundance of *amoA* genes for AOA and AOB relative to prokaryotic DNA for Weeks Bay.

not consistent, with one of the estuaries having a higher abundance of AOB *amoA* than AOA *amoA*. Our results contrast with previous studies showing that AOAs were two to three orders of magnitude more abundant than AOBs in the open ocean (Wuchter *et al.*, 2006; Mincer *et al.*, 2007) and in soils (Leininger *et al.*, 2006).

Potential nitrification rates were significantly, positively correlated with AOA *amoA* gene abundance in samples from Pensacola Bay and Sapelo Island (Figure 2). There was no relationship between potential nitrification and abundance of AOB *amoA* genes at any site ($P > 0.13$ at all sites). Across all sites, abundance of AOA *amoA* genes was positively correlated with salinity ($r = 0.51$, $P = 0.04$), while AOB abundance was not ($r = 0.16$, $P = 0.39$). Salinity at the study sites ranged from 0 to 38, with two sites being relatively oligohaline, while two sites were euryhaline (Supplementary Table 1). Abundances of both AOB and AOA *amoA* genes were greatest at low temperatures (Supplementary Table 2). AOA *amoA* was negatively correlated with pore water sulfide ($r = -0.46$, $P = 0.02$). Potential nitrification rates were not significantly correlated with any single environmental variable. However, a multiple regression model revealed that potential nitrification could be best predicted by sediment chlorophyll *a*, salinity, bottom water DO and AOA *amoA* gene abundance, with sediment chlorophyll *a* explaining the most variability and AOA *amoA* explaining the least (Supplementary Table 3; $R^2 = 0.51$, $P = 0.001$). The conditions leading to the highest rates of potential nitrification occurred when salinity and bottom water DO were low, and sediment chlorophyll *a* and AOA *amoA* abundance were high. In contrast to previous studies, potential nitrification rate was not related to AOB *amoA*

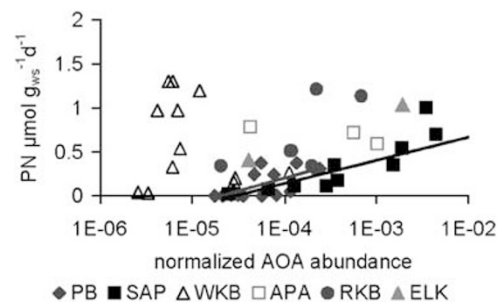


Figure 2 AOA *amoA* abundance normalized to prokaryotic DNA versus potential nitrification (PN) in micromoles per gram_{ws} per day, labeling same as Figure 1. Least-squares regression lines are shown for Pensacola Bay ($r = 0.66$, $P = 0.01$) and Sapelo Island ($r = 0.80$, $P = 0.003$).

abundance (Cebren *et al.*, 2003; Dollhopf *et al.*, 2005).

Salinity is important in controlling the abundance of AOB (Stehr *et al.*, 1995; Cebren *et al.*, 2003) and nitrification rates (Rysgaard *et al.*, 1999; Cebren *et al.*, 2003), with higher abundances of AOB and greater nitrification rates in freshwater than marine end-members. We were surprised that the relationship between potential nitrification rates and AOA *amoA* abundance was not stronger as was observed in Wuchter *et al.* (2006). This may be due to the small-scale spatial heterogeneity of sediments compared to the open ocean. Because potential nitrification rates are measured under optimal conditions, we expected rates to scale with abundance of the nitrifying community. This suggests that environmental variables related to salinity, DO and sediment chlorophyll *a* are as and sometimes more important than genetic potential in controlling potential nitrification rates.

Both phytoplankton and microphytobenthos can contribute to sediment chlorophyll *a* concentrations in shallow, photic estuarine sediments. It represents a labile source of organic nitrogen, which is then mineralized to NH_4^+ . Alternatively, the relationship between chlorophyll *a* and potential nitrification may result from microphytobenthos and nitrifiers, both responding to the same environmental conditions, such as the supply of NH_4^+ . Our results suggest that nitrification may be maximized when DO is present and labile organic matter is high, but pore water sulfide is low, similar to results found in experimental microcosms (Caffrey *et al.*, 1993). Furthermore, our findings suggest that AOA rather than AOB are responsible for much of the nitrification in estuarine sediments as has also been observed in the Black Sea (Lam *et al.*, 2007).

Acknowledgements

This research was supported by grants from the National Science Foundation Ocean Sciences (to JMC and JTH). We thank Lee Edmiston, Apalachicola Bay NERR (National Estuarine Research Reserve); Dorset Hurley, Sapelo Island NERR; Scott Phipps, Weeks Bay NERR; Mike Shirley, Rookery Bay NERR and Kerstin Wasson, Elkhorn Slough NERR for logistical support and water quality data. We thank Kristin Smith, Elizabeth Gage, Angelique Smith, Tanner Martin, Fran Aftanas, Nathaniel Davila, Matt Wagner and Dean Pakulski for their assistance in field and laboratory.

Conflict of interest

The authors state no conflict of interest.

References

- Beman JM, Francis CA. (2006). Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hyper-nitrified subtropical estuary: Bahia del Tobarí, Mexico. *Appl Environ Microbiol* **72**: 7767–7777.
- Caffrey JM, Sloth NP, Kaspar H, Blackburn TH. (1993). Effect of organic loading on nitrification and denitrification in a marine sediment microcosm. *FEMS Microbiol Ecol* **12**: 159–167.
- Cebon A, Berthe T, Garnier J. (2003). Nitrification and nitrifying bacteria in the Lower Seine River and Estuary (France). *Appl Environ Microbiol* **69**: 7091–7100.
- Dollhopf SL, Hyun J-H, Smith AC, Adams HJ, O'Brien S, Kostka JE. (2005). Quantification of ammonium-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. *Appl Environ Microbiol* **71**: 240–246.
- Francis CA, Beman JM, Kuypers MMM. (2007). New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME J* **1**: 19–27.
- Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci USA* **102**: 14683–14688.
- Henriksen K, Kemp WM. (1986). Nitrification in estuarine and coastal marine sediments: methods, patterns and regulating factors. In: Blackburn TH, Sørensen J (eds). *Nitrogen Cycling in Coastal Marine Environments*. John Wiley and Sons: New York, pp 207–250.
- Jones RD, Hood MA. (1980). Effects of temperature, pH, salinity and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *FEMS Microbiol Ecol* **6**: 339–347.
- Joye SB, Hollibaugh JT. (1995). Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* **270**: 623–625.
- Kaplan H. (1983). Nitrification. In: Carpenter EJ, Capone DG (eds). *Nitrogen in the Marine Environment*. Academic Press: New York, pp 139–190.
- Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543–546.
- Lam P, Jensen MM, Lavik G, McGinnis DF, Muller B, Schubert CJ *et al.* (2007). Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proc Natl Acad Sci USA* **104**: 7104–7109.
- Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW *et al.* (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**: 806–809.
- Mincer TJ, Church MJ, Taylor LT, Preston C, Karl DM, DeLong EF. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific subtropical gyre. *Environ Microbiol* **9**: 1162–1175.
- Rysgaard S, Thastum P, Dalsgaard T, Christensen PB, Sloth NP. (1999). Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* **22**: 21–30.
- Stehr G, Bottcher B, Dittberner P, Rath G, Koops H-P. (1995). The ammonia-oxidizing nitrifying population of the River Elbe estuary. *FEMS Microbiol Ecol* **17**: 177–186.
- Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk H-P, Schleper C. (2005). Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crearchaeota in nitrogen cycling. *Environ Microbiol* **7**: 1985–1995.
- Ward BB. (2000). Nitrification and the marine nitrogen cycle. In: Kirchman D (ed). *Microbial Ecology*. Wiley-Liss: New York, pp 427–454.
- Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P *et al.* (2006). Archaeal nitrification in the ocean. *Proc Natl Acad Sci USA* **103**: 12317–12322.

Supplementary Information accompanies the paper on The ISME Journal website (<http://www.nature.com/ismej>)