Anaerobic Ammonium Oxidation (Anammox): A Perplexing Global N Sink

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Anaerobic Ammonium Oxidation

Anaerobic ammonium oxidation (anammox) is drawing increasing attention due to reports of its significant contribution to N losses in a variety of aquatic environments.
Anammox is particularly interesting due to it's unique characteristics:

- Anaerobic, primarily autotrophic process
- Converts ammonium and nitrite to dinitrogen gas
- Produces twice the N_2 per mole nitrite consumed as denitrification
- Can reduce nitrate to nitrite using organic acids, outcompeting denitrifiers in the process
- Can perform dissimilatory nitrate reduction to ammonium (DNRA)
- Produces hydrazine as an intermediate which is stored within an internal anammoxosome
- Responsible for up to 79% of total N_2 removal in marine sediments

Anammox reside within deep phylogenetic branches of the order *Planctomycetales* and appear to be divided into two primary ecotypes and yet undefined species specific niches. The *Candidatus* "Scalindua sp." anammox are primarily marine while the *Candidatus* "Kuenenia", "Brocadia", and "Anammoxoglobus" appear to be limited to freshwater habitats.

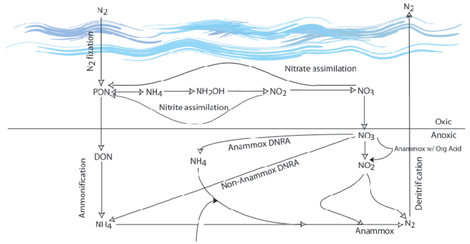
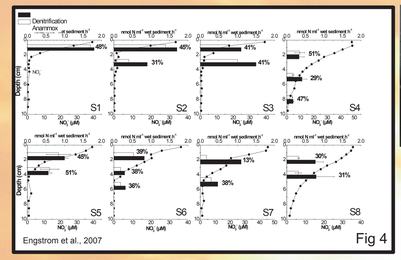


Fig 1



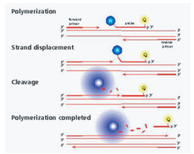
Engstrom et al., 2007 Fig 4

Ammonium porewater profiles show a removal of NH_4^+ in the suboxic zone where accumulation begins only after NO_3^- disappearance, suggesting anaerobic ammonium oxidation processes are responsible for extensive NH_4^+ removal in Cascadia Basin. MnO_2 reduction appears to occur parallel to denitrification which releases NH_4^+ to the porewater. Oxygen penetrated 0.5-1 cm in the surface sediment. Nitrate penetrated 2.5 cm in the stations closest to shore (S1, S2, S3) and up to 10 cm at the stations located furthest out (S2, S4, S6, S8), reflecting sediment reactivity.

Anammox contributed between 13 and 51% of total N_2 production with an average contribution of 38%. Absolute anammox and denitrification rates varied between 0.15-1.7 nmmol N ml^{-1} wet sediment h^{-1} and 0.17-1.8 nmol N ml^{-1} wet sediment h^{-1}, respectively. Anammox and denitrification generally decreased with distance from shore.

Quantification of "Marine" Anammox

Fig 5



Plant Research International, Wageningen, UR

Quantitative PCR was performed using the Taqman assay. Primers and probes were designed to target only the 16S rRNA gene of the *Candidatus* "Scalindua" sp. "marine" anammox bacteria. The taqman probe consisted of a reporter fluorophore and a quencher molecule. Due to the short intermolecular distance the quencher masks the fluorescence of the reporter. During PCR amplification the fluorophore is released by the 5'->3' exonuclease activity of DNA polymerase (Fig 5).

The number of 16S rRNA gene copies were measured as the PCR cycle which fluorescence crosses a certain threshold. Copy numbers were normalized to g^{-1} wet sediment.

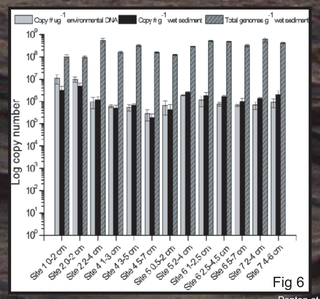


Fig 6

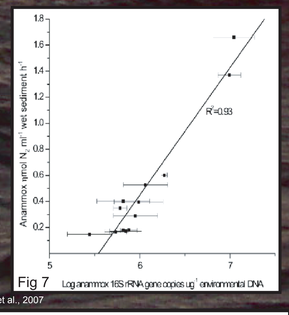
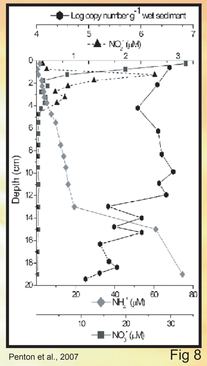


Fig 7

Penton et al., 2007

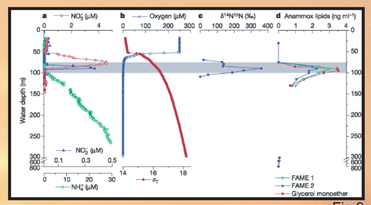
Anammox 16S rRNA gene copies were enumerated at all sites where ^{15}N assays were performed. Mean anammox densities were ~2.0 x 10^6 copies g^{-1} wet sediment. Anammox 16S rRNA gene copy numbers were correlated (R^2=0.93) with anammox N_2 production (nmol N ml^{-1} wet sediment h^{-1}). When the two highest values were removed the correlation decreased (R^2=0.68). Calculated cell specific activities, based on 16S copies, ranged from 1.3 to 11.3 fmol ammonium $cell^{-1}$ day^{-1}.



Penton et al., 2007 Fig 8

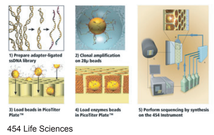
A depth profile of anammox 16S rRNA copy numbers from the sediment surface to -22 cm at station 2 (Fig 8) showed that anammox numbers remained relatively constant to -6 cm, ranging from 9x10^5 to 4x10^6 g^{-1} wet sediment then decreasing to 8.8x10^4 g^{-1} wet sediment at -19 cm. There were no relationships to NO_2^-, NO_3^-, or NH_4^+ profiles.

The significant numbers of anammox 16S rRNA genes found at depths below where NO_2^- was available may be due to bioturbation, a result of possible anammox metabolic flexibility, or inactive or dead bacteria. A similar relationship was observed in the Black Sea water column (Fig 9), where anammox cells quantified by FISH did not wholly decrease after NO_2^- was consumed and anammox N_2 production ceased.



Kuypers et al., 2003 Fig 9

Anammox Evidence Using 16S rRNA gene Pyrosequencing



454 Life Sciences

Ultra-deep sequencing of 16S rRNA genes in a station 2-Cascadia Basin sediment was performed using newly designed primers targeting variable region 4. First, a PCR reaction is carried out on environmental DNA using fusion primers, the products are purified, and ssDNA is immobilized on capture beads. Each bead contains a single ssDNA molecule which is placed in water-in-oil emulsions.

Amplification results in millions of copies of a single sequence on each bead, which is then loaded into the wells of a PicoTiterPlate™ for sequencing, one bead per well. 400,000 reads can be done simultaneously in one run with ~200-250 bases sequenced.

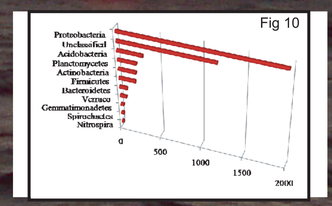


Fig 10

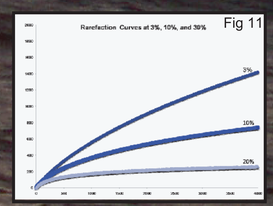


Fig 11

Approximately 4000 16S rRNA gene sequences were obtained using the 454 pyrosequencing method. The Ribosomal Database Project (RDP) classifier was used to predict phylogeny. Proteobacteria was the most abundant phyla (~2000 sequences) while Planctomycetes were represented by ~250 sequences.

Rarefaction curves were constructed at 3, 10, and 20% sequence divergence. At the species level (3%) the current coverage does not approach a full assessment of the diversity. This is especially due to the presence of singletons. However, at the genus level (10%), it appears that the sampling is starting to approach full coverage.

Planctomycetes detected by 454 16S targeted pyrosequencing at station 2

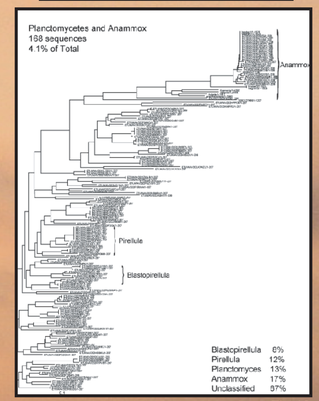


Fig 12

Ca. "Scalindua sp." detected by 454 16S targeted pyrosequencing at station 2

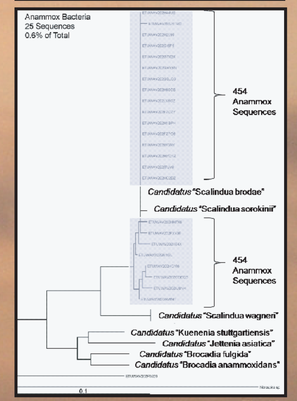


Fig 13

454 FLX pyrosequencing was used to assess whether the "marine" *Candidatus* "Scalindua brodae", *Candidatus* "Scalindua wagneri", and *Candidatus* "Scalindua sorokinii" 16S rRNA gene sequences could be recovered from station 2 sediments in Cascadia Basin in order to validate the specific design of the q-PCR primers and probe. Planctomycetes accounted for 168 sequences, or ~4.1% of the total.

A neighbor joining tree was constructed which showed that anammox sequences formed a relatively deep branch even when based on only ~200 bp (Fig 12). All 25 sequences (0.6% of total 16S rRNA sequences detected) grouped closely with *Ca.* "S. brodae" and *Ca.* "S. sorokinii" and no "freshwater" anammox were detected (Fig 13).

Anammox Activity in deep sea sediments

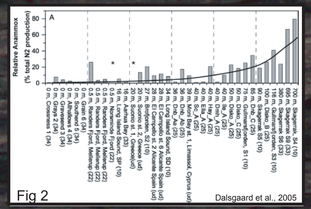


Fig 2

Dalsgaard et al., 2005

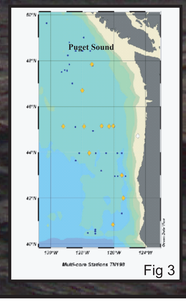


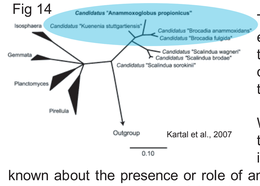
Fig 3

Anammox activity has been measured in a variety of marine sediments and water columns. Percent anammox contribution to total N_2 production generally increases as a function of sediment depth below water surface in various marine habitats (Fig 2). The maximum contribution of anammox to total N_2 production is from the previously deepest reported site (700 m; Thamdrup and Dalsgaard, 2002; Engström et al., 2005). These data suggest that the relative importance of anammox increases with depth (Dalsgaard et al., 2005) and will continue to do so in deeper sediments.

In order to test this, sediment cores were collected from deep Pacific Ocean Cascadia Basin sediments (~3000 m) using a multicorer (Fig 3) during an August 2006 cruise. Anammox and denitrification rates were estimated from the production of $^{29}N_2$ and $^{30}N_2$ in the treatment where $^{15}NO_2^-$ was added (Thamdrup and Dalsgaard, 2002). Porewater O_2, inorganic nitrogen, and Mn^{2+} concentrations were also determined.

Quantification of the "Freshwater" Anammox

Fig 14



Kartal et al., 2007

Taqman primers and probe have been designed to enumerate the "freshwater" anammox. Based on their distinct 16S rRNA outgrouping (Fig 14), a degenerate probe was designed that specifically targets these members of the anammox group.

While much information has been garnered about the ubiquity of the anammox bacteria and process in marine water columns and sediments, little is known about the presence or role of anammox in freshwater habitats. We are currently assessing the abundance of the "freshwater" anammox in several lakes, streams, and wetlands using q-PCR as well as the possible role of anammox in flooded agricultural systems in order to address several questions on the microbial ecology of anammox:

1. Are the "freshwater" anammox as ubiquitous as the "marine" anammox are?
2. How do habitat specific influences, such as organic matter availability, sediment stability, and redox status influence anammox population size?
3. Do anammox play a role in N removal in flooded agricultural systems where soil reactivity is high?

References

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