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Interactive comment on “Production of oceanic nitrous oxide by ammonia-oxidizing archaea” by C. R. Loescher et al.

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Received and published: 22 May 2012

Biogeosciences Discuss., 9, C498–C500, 2012 www.biogeosciences-discuss.net/9/C498/2012/ © Author(s) 2012. This work is distributed under the Creative Commons Attribute 3.0 License. Biogeosciences Discussions Interactive comment on “Production of oceanic nitrous oxide by ammonia-oxidizing archaea” by C. R. Loescher et al. Anonymous Referee #1 Received and published: 28 March 2012

We thank the anonymous reviewer #1 for considering our manuscript worthy of publication in Biogeosciences and for the very helpful discussion on our manuscript. We modified our manuscript accordingly and added some additional discussion on the potential role of nitrifier-denitrification. The general and specific comments were addressed as

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follows:

General Comments R1: This manuscript adds to the growing body of evidence for the dominant role of ammonia-oxidizing archaea in nitrous oxide (N₂O) production in the ocean. The authors provide field data from the eastern tropical North Atlantic (ETNA) and the eastern tropical South Pacific (ETSP) on N₂O concentration as well as abundance and expression of bacterial and archaeal amoA genes, which show that the much more abundant archaeal amoA genes generally covary with N₂O concentration especially in the ETNA. These data are supplemented with incubation experiments with seawater where inhibition of archaeal activity led to decrease in N₂O production. Finally, a pure culture of an archaeal ammonia oxidizer is demonstrated to produce N₂O with the enhancement of N₂O yield at low oxygen levels. The manuscript is worthy of publication in Biogeosciences.

Q1: The only substantial comment I have is on Section 7 (potential pathways of N₂O production). Based on the 15N site preference data from the culture the authors conclude that N₂O production occurs during the oxidation of ammonium to nitrite and not from the reduction of nitrite to N₂O. However, I believe that the latter pathway (nitrifier denitrification) cannot be ruled out as an important mechanism. In fact, using labeled nitrite Nicholls et al. (2007) concluded that reduction of nitrite is the dominant metabolic pathway responsible for N₂O production in the Arabian Sea. Moreover, the isotopic data presented by Santoro et al. (2011) also suggest that nitrifier denitrification may be a significant contributor to N₂O formation, particularly at low oxygen levels. The sensitivity of N₂O yield to the ambient oxygen concentration itself points to a reductive pathway.

Author response: We agree that to certain extend nitrifier-denitrification could impact on N₂O production especially in the field studies when oxygen was very low in the OMZs. However, in our pure culture laboratory experiment were O₂ concentrations were still relatively high; we propose that the impact of nitrifier-denitrification can be neglected. Nevertheless, we consider it highly likely, that under low O₂ conditions such

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as present in the Arabian Sea or in the ETSP, this process might have a strong impact. We thus changed the paragraph as follows:

‘Thus, our dataset points towards a production of N₂O via the oxidation of NH₄⁺ to NO₂⁻, potentially via an unknown intermediate as we were not able to detect NH₂OH in *N. maritimus* cultures using the method described in Schweiger et al. (2007). However, taking $\delta^{18}\text{O}$ data into account, Santoro et al. suggested a reduction of NO₂⁻ to N₂O (Santoro et al., 2011). As we have not performed O₂ isotopomeric studies, we cannot exclude N₂O production via nitrifier-denitrification, particularly, when O₂ becomes limiting as previously described for the Arabian Sea (Nicholls et al., 2007) where O₂ concentrations drop far more than in our experiments (lowest O₂ concentration $\sim 112\mu\text{M}$).’

Q2: Are the site preference data available for the experiments the results of which are presented in Fig. 7?

Author response: Unfortunately, the detection limit for measuring the N₂O isotopes has a much higher detection limit as the gas chromatographs. This requires higher incubation volumes, which means that we had to perform a parallel experiment in 2 L cultures, in order to perform the N₂O isotope experiment. The isotopic data presented, are resulting from a parallel experiment using the lowest O₂ concentration of the three chosen ($112\mu\text{M}$). The two other incubation experiments in high culture volumes with concentration of 223 and 287 μM O₂ did not result in a sufficient N₂O production to give a clear result.

Specific (minor) Comments Q1: Page 2096, line 9: Please change “described” to “reported”. R1: The sentence has been changed accordingly.

Q2: Page 2096, line 12: Please move “(ETSP)” to between “Pacific” and “Oceans”. R2 Changed accordingly.

Q3: Page 2097, first paragraph: Several statements are not supported by references

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(e.g. lines 9-10). R3: The paragraph has been changed accordingly; several references have been put in to support the mentioned statements:

'Atmospheric nitrous oxide (N₂O) is a strong greenhouse gas (Forster, 2007) and a major precursor of stratospheric ozone depleting radicals (Ravishankara et al., 2009). The ocean is a major source of N₂O contributing approximately 30% of the N₂O in the atmosphere (Denman, 2007). Oceanic N₂O is exclusively produced during microbial processes such as nitrification (under oxic to suboxic conditions) and denitrification (under suboxic conditions, (Bange et al., 2010;Codispoti, 2010)) . The formation of N₂O as a by-product of nitrification (oxidation of ammonia, NH₃, via hydroxylamine, NH₂OH to nitrite, NO₂⁻) was reported for ammonia-oxidizing bacteria (AOB) (Frame and Casciotti, 2010;Goreau et al., 1980). In case of nitrifier-denitrification NO₂⁻ can further be reduced to nitric oxide (NO) and N₂O (Poth and Focht, 1985;Shaw et al., 2006). The accumulation of oceanic N₂O is favored in waters with low oxygen (O₂) concentrations, which is attributed to an enhanced N₂O yield during nitrification (Goreau et al., 1980;Stein and Yung, 2003). The frequently observed linear correlation between Δ N₂O (i.e., N₂O excess) and the apparent oxygen utilization (AOU) is usually taken as indirect evidence for N₂O production via nitrification (Yoshida et al., 1989).'

Q4: Page 2100, line 7: Please change "present" to "observed". Q5: Page 2102, line 19: Please change "is also depending on" to "also depends on". R 4+5: The suggested changes have been included.

Q6: Page 2104, lines 4-7: Similar prediction has also been made by Naqvi et al. (2010). R 6: We are grateful for the reviewer's hint; the sentence has been now changed as follows: The predicted expansion of OMZs in the future in many parts of the ocean (Stramma et al., 2008) may lead to an enhanced N₂O production in the ocean (Naqvi et al., 2010) and therefore may have severe consequences for the budget of N₂O in the atmosphere as well.

Q7: Section 8.1 (methods summary): It is unusual (in Biogeosciences at least) to

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describe the methods in the end. Could this be moved forward? R7: We agree and moved the methods part now following the introduction section.

Q8: Page 2104, line 11: Please change “according” to “following”. R8: changed accordingly.

Q9: Page 2104, line 19 (accordingly): According to what? R9: We agree that the sentence was not clearly understandable; we now changed it as follows: ‘N₂O was measured with a GC headspace equilibration method as described in Walter et al. (Walter et al., 2006); Δ N₂O and AOU were calculated as described therein.’

Q10: Page 2106, line 20: Please change “CTD” to “CTD casts”. Q11: Page 2107, line 8: Please remove “with”. R10+11: changed accordingly

Q12: Page 2115 (Fig. 1): Stations corresponding to profiles I-VI may be identified in the map. R12: We apologize and adjusted the map; stations corresponding to the profiles are now indicated with black asterisks and profile numbers corresponding to the vertical profiles, below (the modified version is attached).

Additional References Naqvi, S. W. A., Bange, H. W., Farías, L., Monteiro, P. M. S., Scranton, M. I., and Zhang, J.: Marine hypoxia/anoxia as a source of CH₄ and N₂O, *Biogeosciences*, 7, 2159-2190, 2010.

Nicholls, J. C., Davies, C. A., and Trimmer, M.: High-resolution profiles and nitrogen isotope tracing reveal a dominant source of nitrous oxide and multiple pathways of nitrogen gas formation in the central Arabian Sea, *Limnol. Oceanogr.*, 52, 156–168, 2007.

R Both references were integrated in the text: The respective sentences have been changed:

‘However, we cannot exclude N₂O production via nitrifier-denitrification, particularly, when O₂ becomes limiting as previously described for the Arabian Sea (Nicholls et al., 2007) and suggested by Santoro et al (Santoro et al., 2011).’

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'The predicted expansion of OMZs in the future in many parts of the ocean (Stramma et al., 2008) may lead to an enhanced N₂O production in the ocean (Naqvi et al., 2010) and therefore may have severe consequences for the budget of N₂O in the atmosphere as well.'

Interactive comment on Biogeosciences Discuss., 9, 2095, 2012.

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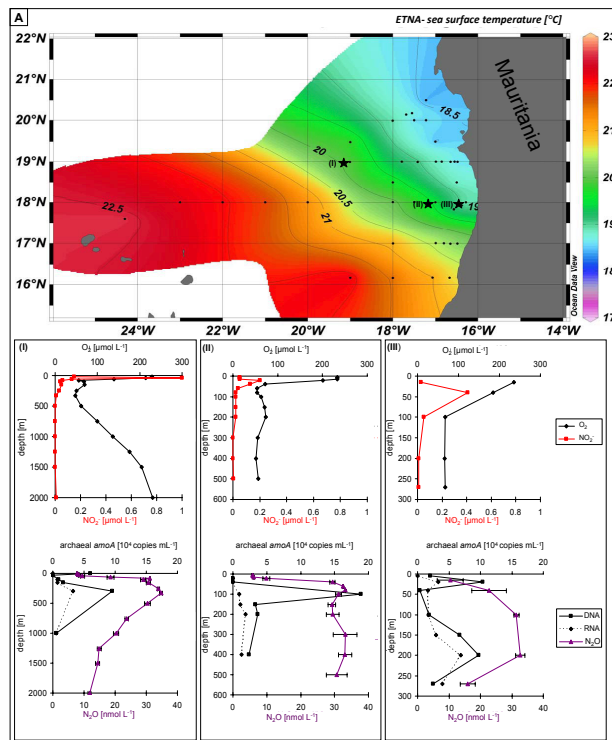


Fig. 1A

Fig. 1. Fig 1A: ETNA

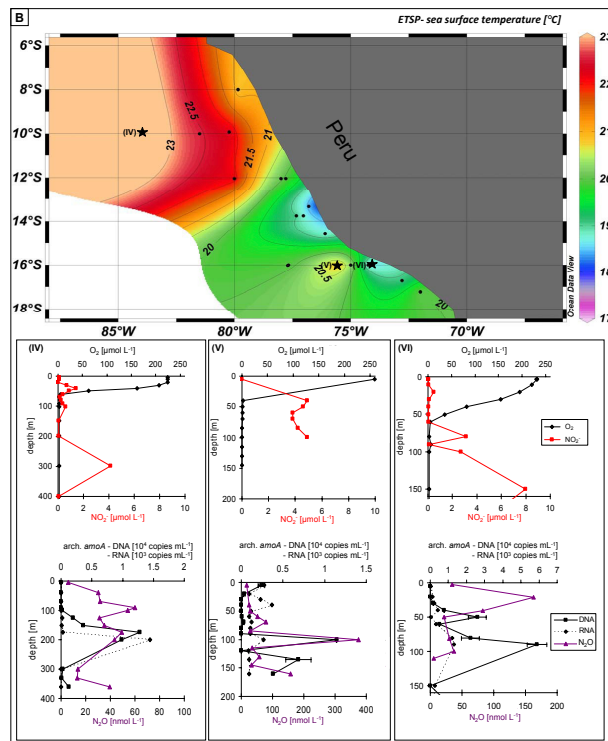


Fig. 1B

Fig. 2. Fig 1B: ETSP