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Biogeosciences Discussions

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

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Biogeosciences Discuss., 9, C770–C771, 2012 www.biogeosciences-discuss.net/9/C770/2012/ Biogeosciences Discussions Interactive comment on "Production of oceanic nitrous oxide by ammonia-oxidizing archaea" by C. R. Loescher et al.

Anonymous Referee #3 Received and published: 24 April 2012

Referee 3 This manuscript describes a series of field observations, manipulations and culture experiments which are performed to investigate which group of organisms, ammonia oxidizing archaea or bacteria are the dominant producers of nitrous oxide in the

marine environment. This study is very timely, and following a small number of edits is entirely appropriate for publication in Biogeosciences.

We thank the anonymous reviewer #3 for the comments on our manuscript and moreover for considering our study as timely, well presented, and appropriate for publication in Biogeosciences after careful revision. We considered the comments during revision and believe we have thereby further improved the manuscript.

Q1: The manuscript is generally well presented, though I would suggest that the position of the methods section is wrong and should be re-positioned after the introduction. The present arrangement means that one is consistently looking forward to find out what, where and why something has been done.

R1: We agree with the reviewer and shifted the methods section following the introduction.

Q2: I would also suggest that the current title is not entirely representative and should be altered to reflect the coastal and shelf seas component of this study.

R2: The expression 'oceanic' represents for us a combination of coastal, shelf and open ocean regions. We thus consider the chosen title as adequate as our study includes oceanic regions reaching from the coast (with water depth below 150m in the ETNA and 100m in the ETSP) over the shelf region out to the open ocean (with water depth over 1000m in the ETNA and ETSP), thus we think the title is appropriate and like to keep the title.

Q3: In Section 2 – vertical distribution. . . I find that this discussion is not particularly easy to follow and a better approach might be to separate out the description of the two ocean areas.

R3: We presented the results and discussion section in a combined way as we considered it most focused. We chose this structure to have the possibility to directly compare the two different OMZs with regard to the strongly differing N2O and O2 conditions. If

separating the description of the two areas, this paragraph would contain too many repetitive statements. Thus we like to keep the combined comparative description.

Q4: Further to this the interrogation of the relationship between numbers of amoA genes and N2O should be more rigorous than a simple comparison of two contour profiles, which do not actually match up as well as is described. A correlative relationship does not prove a direct link, but some statistical investigation should be performed here.

R4: We agree with the referee. Regression analysis have been applied on the overall dataset of N2O and number of amoA genes in the ETNA resulted in a Pearson correlation coefficient of r = 0.63 indicating a linear correlation of the two variables. See also response to reviewer 2 question 1.

This information has now been included into the revised manuscript (Vertical distribution. . .): 'A comparable pattern of the distribution of archaeal amoA genes and N2O was observed in the water column of the ETNA (Fig. 3) suggesting a correlation between AOA abundance and N2O accumulation (Pearson correlation coefficient r = 0.63; statistical significance is indicated) in the layers with low O2 (Fig. 4). . .

Q5: The description of "certain depths at some stations" is very vague and this should be tightened up, I can not tell from this whether the "key genes" for denitrification and anammox where determined in the Pacific study. The lack of a relationship between deltaN2O and AOU in the Pacific study merits further discussion.

R5: We agree that the expression 'certain depth at some stations' is too vague and rearranged the sentence:

'A co-occurrence of N2O and archaeal amoA genes was detected at certain depths, e.g. at profile V at 100m water depth (Fig.1) in the ETSP, but was not a general feature possibly resulting from additional N2O production via other processes such as denitrification, nitrifier-denitrification or anammox (Kartal et al., 2007) at present suboxic

conditions (see Fig. 1).'

Key genes for denitrification and anammox were assayed in the Atlantic and Pacific studies; however, they were only detectable in the ETSP. The respective sentence was rearranged to clarify, that the respective genes were present and that most likely mixed processes contribute to N2O formation in this area. The contribution of e.g. denitrification to N2O production may explain the lack of relationship between delta N2O and AOU in the ETSP:

'The presence of key genes of anammox and denitrification assayed and predominantly detected at coastal stations of the ETSP but also present in large parts of the area off Peru points further to an active contribution of mixed processes to N2O production in the ETSP (the complete dataset of the ETSP can be seen in Löscher (Löscher, 2011)). N2O production by mixed processes may explain the lack of correlation between $\triangle$N2O and AOU as well as NO3– in the ETSP.'

Q6: In Section 4 – Potential importance . . . I do not understand the statement: . . .AOA might dominate the production of N2O and the balance between reduced and oxidised nitrogen species in the ocean, gradually.

Authors comment: We changed this sentence in the revised manuscript in order to make it clearer and better understandable:

'Regarding the on-going decrease in dissolved O2 concentrations in tropical ocean areas (Stramma et al., 2010), we hypothesize that activities of cluster B affiliated AOA might dominate the production of N2O and the balance between reduced and oxidized nitrogen species in the ocean, as those organisms are likely more adapted to low O2 concentration.' Q7: Section 7 is much too short and lacking in detail. Description of the methods should be more involved as should the discussion. It would seem that culture conditions are likely to affect the mechanism by which N2O is produced, though these are not described.

Authors comment: We agree that the description of the methods used is to short and details are missing thus we changed the section with regard to the methods and discussion and added some more details in the revised manuscript:

'AOB can produce N2O from NH2OH during nitrification or from NO2– during nitrifier-denitrification (Kool et al., 2010;Shaw et al., 2006). In AOA however, the pathway of ammonia oxidation is yet not understood. So far, no equivalent to the hydroxylamine-oxidoreductase, which catalyses the oxidation of NH2OH to NO2– during nitrification, has been identified (Könneke et al., 2005;Martens-Habbena et al., 2009) which means/indicates that AOA likely use a different pathway than AOB do, when producing N2O. The detection of the nitrite reductase gene nirK in the sequenced genomes of cultured Thaumarchaeota (Walker et al., 2010) led to the theory that AOA might produce N2O by nitrifier-denitrification, which might particularly impact at low O2 concentrations. To identify the origin of N2O formation isotopomeric studies were performed with N. maritimus pure cultures. Using the lowest O2 concentration of the three chosen (112μM), a 15N site preference (SPN2O) in N2O of 34 $\pm$ 12 ‰ was detected, consistent with results from AOA enrichments (Santoro et al., 2011), which is in agreement with the SPN2O of $\sim$33‰ typically found in AOB cultures performing ammonia oxidation (Sutka et al., 2006) (for comparison: nitrifier-denitrification of AOB results in a SPN2O of about 0‰. 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. (Schweiger et al., 2007). However, taking δ18O 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$112μM).'

We additionally added more specific details concerning the methods description to the

methods section.

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