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

Anonymous Referee #2

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Part 1. General comments.

The discovery of ammonia oxidation within the domain Archaea has challenged century-old paradigms about the biological control of nitrification and nitrous oxide production. Several recent studies have demonstrated that particularly in the marine water column putative ammonia oxidizing archaea (AOA) are significantly more abundant than ammonia-oxidizing bacteria (AOB). As such, AOA may have a significant impact on the nitrogen cycle of the coastal and open oceans. Indeed, very recently it was shown that AOA release N₂O, an important greenhouse gas, that has long been known to be linked to nitrification. To this end, the study by Loescher et al. provides further insights into the distribution and potential sources of nitrous oxide in the oceans. The authors report nitrous oxide concentrations along with archaeal and bacterial ammonia

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monooxygenase gene phylogeny and abundances in the water column of the Eastern Tropical North Atlantic (ETNA) and Eastern Tropical South Pacific (ETSP). Furthermore, the authors provide long awaited data on the production of nitrous oxide by *N. maritimus*, the only marine ammonia-oxidizing archaeon available in pure culture. The authors show that *N. maritimus* produces N₂O in pure culture, thus, corroborating previous measurements by Santoro et al. in AOA enrichment cultures. By comparison of laboratory and field data, the authors conclude that N₂O in the tropical oceanic areas may derive predominantly from ammonia-oxidizing archaea.

I really enjoyed reading the manuscript by Loescher et al. The manuscript provides exciting data, is reasonably well edited, and the figures are clear and provide easy access to the data. However, as I have outlined in my detailed comments, most of the data remain correlative and particularly the presentation of the molecular data and culture experiments require significant improvement in order to support the conclusions drawn by the authors. Thus, the high potential for novelty remains at least somewhat unfulfilled.

Part 2 Detailed comments.

Page 2099 line 12ff: Based on the data shown in Fig. 3, I somewhat disagree with the statement “High copy numbers of archaeal *amoA* genes and high N₂O concentrations co-occurred in the ETNA”. Based on Fig. 3 elevated archaeal *amoA* gene copy numbers were associated with moderately high N₂O concentrations. However, only rarely N₂O maxima directly correlated with maximum *amoA* gene copy numbers. Please rephrase.

Page 2099 line 14ff: In the Methods section the authors describe PCR assays for *nirS*, *nirK*, *nosZ*, and *hzo*. Were neither of them detected, or were *nirK* and *nosZ* not tested in the ETNA samples? Please clarify!

Page 2099 line 15f: According to Schmidt et al. (1998) in anammox bacteria the *hzo* gene codes for the hydrazine oxidoreductase and *hao* for hydroxylamine oxidoreduc-

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tase. Here, I think you would like to refer to hydrazine oxidoreductase, correct?

Page 2099 line 23ff: Please correct reference: Loescher et al. 2011 or 2012?

Page 2099 line 26f: Beta- and gammaproteobacterial amoA gene sequences were detected in separate qPCR assays. This is a rare and valuable dataset! Very few data are available on the distribution of gammaproteobacterial amoA genes. Thus, please report separately the numbers for beta- and gammaproteobacteria in ETNA and ETSP samples!

Page 2100 line 6ff: The authors present amoA gene and transcript copy numbers for both ETNA and ETSP samples, respectively. The authors nicely discuss the potential pitfalls for interpretation of transcript copy numbers from environmental samples! Nonetheless, albeit no transcript numbers are reported for *N. maritimus*, one should be able to assume that higher transcriptional activity is associated with higher metabolic activity. Can such a correlation also be found between amoA transcript numbers and N₂O production? Or may N₂O production be associated with lower gene copy to transcript ratios?

Page 2100, line 16ff: The phylogenetic analysis of the clone library data remains very superficial. The authors state that DNA samples from 15 stations with 12 depths each were included in the clone library analysis. Thus, from about 180 individual samples a total of ~300 clones were sequenced, i.e. on average < 2 sequences per sample. This is definitely not a representative clone library for any sample and the conclusions withstand no statistical scrutiny whatsoever. I believe the point the authors are trying to make, that cluster B sequences are preferentially associated with low oxygen concentrations, is demonstrated more clearly in the following way: The authors could pool all clone sequences obtained from stations and depth within the OMZ of the ETNA, and ETNP, respectively, and those from outside the OMZ (e.g. based on oxygen concentrations below and above 20 μ M) and in a table summarize the numbers and percentage of cluster A and cluster B sequences in each group. This pooled analysis should reveal

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with much higher statistical confidence whether cluster A or B may be associated with high or low oxygen concentrations, respectively.

Page 2100, line 16ff: It remains unclear how the DNA samples were processed. Were the DNA samples pooled and then a single clone library was constructed? Or were 180 cloning reactions performed and between 1-3 clones selected from each library for sequencing? Please clarify.

Page 2101, line 1: The authors point out the importance of amoA cluster B-associated archaea for N₂O production. Is there any basis for this distinction? Do the authors have actual evidence for any difference in N₂O production in members of cluster A and B? (Compare also comment page 2102 line 24ff below).

Page 2101, line 17: The authors show that the chemical compound N-guanyldiaminoheptane (GS7) significantly reduces the production of N₂O in samples from ETNA. The authors used 1 mM of this archaeal cell cycle inhibitor GS7 in these water samples. This is a major finding and could strongly facilitate the future analysis of archaeal nitrification. However, in situ doubling times of microorganisms between 5-14 days are not unusual. Does the inhibition of the archaeal cell cycle indeed inhibit ammonia oxidation activity, and thus N₂O production, significantly in 24h incubations? Or alternatively, could the high concentration of this compound also affect other organisms (e.g. eukaryotes) that excrete ammonia, and thereby diminishing the supply of ammonium to nitrification? I am sure the authors are also aware that AOB are sensitive to a wide range of chemical compounds. To the best of my knowledge, there are no data on the impact that GS7 may have on the activity of AOB. Did the authors test any AOB for sensitivity to GS7? Without actual confirmation that 1 mM of this compound does not inhibit N₂O production by AOB this result remains at least somewhat inconclusive. The authors should discuss these data more comprehensively.

Page 2102, line 4ff: The authors report significantly higher N₂O yields in *N. maritimus* than in their tested AOB strains. Judging from Fig. 7, the N₂O production rate was likely

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not constant throughout the experiments? How much variability was there, actually, and under which conditions were the highest rates observed? Goreau et al. report at least one order of magnitude higher N₂O yields in the tested marine *Nitrosomonas* strains than the authors present in Table 1. The statement in line 12f. should be revised accordingly.

Page 2102, line 24ff: This is a great comparison! However, the authors neglected a further very interesting and important comparison: How do the N₂O yields in *N. maritimus* compare to the in situ N₂O production rates shown in Fig. 6? Assuming AOA in situ at the ETNA and *N. maritimus* have the same N₂O yield of 0.026% (Table 1), the GS7-sensitive in situ nitrification rates associated with 1.5 and 3 nM N₂O production per day would amount to ~5,700 to 11,500 nmol ammonia oxidation per liter per day at 20.001W, and 20.999W, respectively (⇒ 140 to 288 fmol per AOA cell per day). Are such high nitrification rates realistically possible in these areas? On the other hand, assuming AOA carry a single *amoA* gene copy, approximately 4x10⁷ cells per liter (= 4 x 10⁴ archaeal *amoA* per ml average in Fig. 1, panel I and II) producing between 1.5 and 3 nmol N₂O per liter per day (Fig. 6) would have a 1.5 to 3-fold higher daily rate of N₂O production than *N. maritimus*. Thus, either the gene copy numbers must be underestimated by at least one order of magnitude, the N₂O production in the samples overestimated by an order of magnitude, or one could speculate that the N₂O yields of AOA in situ at the OMZ may be significantly higher than in *N. maritimus*. Thus, if OMZs are indeed dominated by cluster B-affiliated AOA, these organisms may have significantly higher N₂O yields than cluster A-affiliated AOA (including *N. maritimus*). A future expansion of OMZ and potentially cluster B-affiliated AOA could thus have important consequences for the oceanic N₂O budget.

Page 2102, line 4ff: In Fig. 7 the authors report oxygen concentrations over the course of the experiment. According the methods section, the time course experiments were conducted in 125ml serum vials and oxygen concentrations were determined by Winkler titrations. To the best of my knowledge this can't be possible. Winkler titrations

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would require significantly bigger water samples. Please add more detailed explanation on how the experiment was carried out.

Page 2105, line 19ff: Several conclusions of this manuscript are based on the absence of nirS, nirK, nosZ, and hzo genes in ETNA samples. Please report the detection limits of your quantitative PCR assays for the specific genes.

Page 2106, line 9ff. Please add more detailed description of DNA processing, sample pooling, and number of clone libraries constructed (see comment above)

Page 2107, line 3: Were the experiments shown in Fig. 7 really carried out in 125ml serum bottles? What were the actual culture and headspace volumes? And what were the incubation temperatures for the different strains?

Page 2107, line 4: Check publication years.

Page 2107, line 6f: Were the cell abundances reported in Fig. 7 generated by the DAPI method, the flow cytometry method, or both? Please clarify.

Page 2115, Fig. 1: DNA and RNA profiles are indistinguishable from each other. Please correct. Panels 1-VI are supposed to reflect dots in the maps. There are at least 30 dots. Please clearly mark stations I-VI in the maps. Remove other dots or add further explanation in caption. From which cruises originate the data?

Page 2122, Fig. 7: It should be stated whether a representative growth curve or averages of the three replicates are shown.

END OF REVIEW and evening.

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