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Biogeosciences
Discussions

# *Interactive comment on* "Nitrification and growth of autotrophic nitrifying bacteria and Thaumarchaeota in the coastal North Sea" *by* B. Veuger et al.

**Anonymous Referee #2**

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This study represents an important contribution to our understanding of nitrification and the roles of ammonia-oxidizing bacteria and archaea. This work provides a nice complement to the Pitcher et al. (2011) genetic study of ammonia oxidation, and goes a step beyond the norm by also examining autotrophic growth of ammonia oxidizers. The authors report ammonia oxidation and nitrite oxidation rates, autotrophic DIC incorporation rates, as well as ammonia uptake rates in the North Sea during the winter months. The pairing of ammonia oxidation rates with ammonia uptake and nitrite oxidation provides an unusually thorough look at nitrogen dynamics during a time of year when non-phytoplankton dynamics may be most important. The authors report that although ammonia oxidation and nitrite oxidation are tightly coupled, the rates are not



equal, a frequent assumption given the lack of available data. Additionally, they show that, during the winter when phytoplankton are less abundant, ammonium oxidation is by far the dominant ammonium sink (rather than uptake). However, the study relies heavily on inhibitors to examine ammonia oxidation and nitrite oxidation separately. While the inhibitors are proven to (and do) efficiently block ammonia oxidation and nitrite oxidation separately, I would caution against inferring anything about non-targeted processes, given that inhibitors often have unintended consequences as well. Which is to say that chlorate may also inhibit Thaumarchaeota even without the ability to oxidize nitrite. A further discussion of the specific targets of the inhibitors and alternate explanations is warranted. For these reasons, I recommend that this paper be published with minor revisions.

Specific comments:

P2, line 20-24: How can Thaumarchaeota contribute less than expected to nitrification from their gene abundance when ammonia-oxidizing bacterial abundance was not also measured? Yes, Thaumoarchaeotal genes were abundant compared to other environments, but the study did not measure bacterial amoA or 16S abundance. Also note that amoA gene abundance does not necessarily equate activity, especially if the Thaumoarchaeota in question are mixotrophic.

P2, line 22: Please specify 16S and amoA instead of 'gene.'

P2, lines 26-27: The authors should expand on this point in the discussion and offer potential reasons why the ratio of NH4 fixed to C incorporated into the lipids is so high.

P3, line 18: It is my understanding that while a few ammonia-oxidizing bacteria (AOB) have been shown to be capable of heterotrophy, those are not the groups abundant in the ocean. However, there is evidence that Thaumarchaeota may be mixotrophic in the open ocean (Hansmen et al. 2009, Ward et al. 2010)

P4, line 5: The evidence that archaea oxidize ammonia in the ocean is compelling; it is

their contribution relative to bacteria that is unresolved.

P4, line 16: Report the actual abundance values from Pitcher et al. (2011) for 16S and amoA.

P5: lines 20-23: If the filters were rinsed with sample water before collecting water samples, it would be worth noting (as many filters are contaminated with ammonium).

P5, line 22: As rates of nitrification and ammonia uptake are high, the better choice of filter would have been 0.2 uM to ensure all ammonia-impacting activity ceases as soon as possible. Were the samples frozen at -20 or -80?

P8, lines 17 and 20: My interpretation may be backward here, but shouldn't the calculations look at excess 15N or 13C x the proportion of the pool labeled? I.e. added/total rather than total/added?

P10, line 8: While the authors' point that the bubbling simulates the turbid ocean environment, my understanding is that oxygen is a direct substrate for ammonia monooxygenase and not for the C-fixation pathways in aerobic autotrophic bacteria and archaea. This is outside my area of expertise, however.

P11, section 3.4: Further discussion of the inhibitors is warranted here, especially the mechanism of inhibition and discussion of any studies showing the impact on archaea specifically.

P12, line 27-30: Have there been any reports of PUFA sequences in the known AOB or thaumarchaeotal genomes? While it is suggestive, I caution against assuming that the inhibitors block only the intended targets. It seems more likely that other, PUFA-containing bacteria were also impacted by the inhibitors.

P13, lines 19-21: Again, I would caution against assuming the inhibitors did not have unintended consequences. While it is certainly possible that there are undiscovered nitrite-oxidizing archaea, it seems equally likely that the inhibitors impact archaea differently from ammonia-oxidizing bacteria. Additional interpretations would be appropriate



here.

P15, lines 5-8: While the interpretation set forth by the authors is certainly possible, it may be worth offering alternative possibilities. For example, perhaps the archaea are diverting more C into enzymes and DNA production than lipids.

P15, lines 12-17: Since AOB were not measured in the Pitcher et al. (2011) study, the claim that amoA copies alone do not indicate actual nitrification activity is overreaching. Perhaps AOB were much more abundant than AOA at the time of sampling. Also, abundance does not equal activity. Again, my understanding is that there is more evidence to suggest that marine, in situ AOA are mixotrophic than AOB. Perhaps the abundance of AOA is not correlated to nitrification rates because they are not all nitrifying. This would be a good opportunity to stress the importance of examining RNA/activity rather than gene abundance as a standard in the field.

Typos:

P2, line 12: add a comma between "nitrification" and "with"

P10, line 15: "an" should be "and"

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Interactive comment on Biogeosciences Discuss., 9, 16877, 2012.