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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.

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We are very pleased with the three positive reviews of our manuscript and the appreciation of our work. Below, we respond to the individual comments by the reviewers. (our comments are indicated by »> xxxxxx «<).

Anonymous Referee #1 The authors present the results of a very interesting study on nitrification and DIC fixation by nitrifying bacteria and Thaumarchaeota in the North Sea during winter. They present a very nice dataset of activity rates, which questions the role of Thaumarchaeota as nitrifers in coastal waters, in contradiction with previous findings based on the detection of amoA gene copies in the same area. Overall

the paper is well written and will undoubtedly contribute to our understanding on the nitrification in coastal waters. Nevertheless, I have some comments which may help to improve the manuscript. My major concern is related to the use of chlorate as nitrite oxidation inhibitor.

»> See response to specific comments below «<

Abstract -The authors could indicate in the abstract that the study was conducted during a period characterized by both high nitrification and high Thaumarchaeota abundance.

»> High nitrification rates were already mentioned in the abstract (lines 10-11 “Measured nitrification rates were high”). Thaumarchaeotal abundance was not always high, but showed a clear peak within the sampling period, which is mentioned in the abstract (page 16878, line 20). «<

-Lines 16-18. Please, clarify here the implications of 13C-fixation into different PLFAs or delete the sentence from the abstract.

»> The composition of the 13C-labeled PLFA pool is just a basic result here and does not really have any major implications. The presence of the PUFAs was merely included in the abstract as an interesting observation (albeit not essential for the main line of the paper). «<

-Line 18. The authors did not measure cell abundances therefore these are not cell-specific rates.

»> This is correct. What was meant was biomass-independent activity. We removed “cell-specific” from this sentence. «<

-Line 26. The authors did not report DIC fixation by nitrifiers, they only provide data for bacterial nitrifiers.

»> The term “nitrifiers” was used here because the contribution by Thaumarchaeota

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was negligible, meaning that nitrification was dominated by bacteria (see section 3.5). For correctness, we changed “nitrifiers” to “bacterial nitrifiers”. «<

Introduction -Page16879, Line 18. Please provide references for heterotrophy among nitrifiers.

»> We added a general reference (Ward 2008). Also see related comment by reviewer #2. «<

Materials and methods -Please, indicate the periodicity of sampling for each measurement.

»> This information has been added for the general sampling (“Water was sampled weekly . . .”) and for the 15N-ammonium incubations (“Every week, two glass 250 ml bottles were filled . . .”). For the 13C-DIC incubations, this information was already present in the original text (lines 13-14 “water was collected approximately every second week . . .”) «<

Results and discussion -Page 16885, lines 25-28. Please clarify this sentence.

»> We are not entirely sure what needs to be clarified here. The purpose of this sentence is to indicate that the aeration treatment, which was necessary to keep the conditions in the incubation bottles as representative for the field situation as possible, may have had an effect on the nitrification rates as nitrification is dependent on O2 availability (which may have been enhanced by the aeration). However, as indicated in the following sentences, this effect was probably not very large and does not alter the main results from this study. «<

-Page 16887, lines 22-27. The authors should discuss why the inhibition of 13C-fixation is similar with both inhibitors (Figure 3). This can only occur if chlorate is also inhibiting ammonium oxidation. This fact has been demonstrated by Hynes & Knowles (1983, AEM 45:1178-1182). Chlorate may indirectly inhibit ammonium oxidation in mixed communities, through the reduction of chlorate to chlorite mediated by nitrite ox-

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idizers. Therefore, the inhibition of 13C-fixation by chlorate must be taken with caution, and I suggest removing these data from the manuscript as they do not provide any useful information.

»> The results for the inhibitors are discussed in section 3.5. Hynes and Knowles (1983) is a very useful reference, we have now included this reference, and the possible inhibition of ammonium oxidation by chlorite as an additional explanation for our results in the revised manuscript. Moreover, a sentence was added in section 3.4 referring to further discussion of this issue in section 3.5. We have considered removing the chlorate results but decided to keep these results because we feel that, even though we cannot fully explain them, they still contain potentially valuable information. Note that these results are interpreted with great caution. Also see response to related comments by the other reviewers below. «<

-Page 16889, lines 14-21. I would delete this speculation about the potential role of Thaumarchaeota as nitrite oxidizers, as chlorate appears to be inhibiting also ammonium oxidation.

»> This is directly related to the previous point. See our comments above. «<

-Page 16892, lines 11-13. Please clarify this sentence. It is not clear why this calculation was possible for 13C-fixation into PLFAs but not for 13C-fixation into bulk SPM.

»> This is because the 13C-enrichment level (δ13C) of the PLFAs was higher than that of the bulk SPM. This is a normal phenomenon in this type of studies and is the result of a relatively large background pool of unlabeled C in the SPM pool which effectively dilutes the δ13C. This is accounted for when calculating absolute 13C incorporation. In this study, dilution resulted in bulk $\Delta\delta$13C values for the inhibitor treatments being too low for detection while values for the PLFAs in the same treatments were still above the detection limit. We summarized this with "... the 13C enrichment of SPM for incubations with inhibitors was below the limit of detection" because we feel that a more elaborate explanation distracts from the main line of the discussion here while it does

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not add useful information to the discussion. «<

Anonymous Referee #2 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.

»> We agree that interpretation of the results for chlorate inhibition, in particular the similar level of inhibition by both inhibitors, is not straightforward. This is why we discuss this with caution and do not derive any major conclusions from this. Note that the main results and conclusions are primarily based on the inhibition by nitrapyrin of which the inhibitory mode of action is more clear and established than that of chlorate. Also see our response to similar comments by reviewer #1. «<

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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.

»> We agree that abundance alone does not necessarily equate activity. The purpose of this sentence in the abstract is to emphasize that great care should be taken with translating abundance measurements into conclusions about the potential biogeochemical impact of the organisms and stressing the need to actually measure this impact, like we did in this study. Also see response to related comments by the other reviewers below. «<

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

»> changed as suggested«<

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.

»> This point is discussed in detail in the Results and Discussion (section 3.6). However, since we cannot provide a full explanation for these results, we chose not to elaborate on this in the abstract. «<

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).

»> It is certainly true that there is evidence that Thaumarchaeota may be heterotrophic. This is actually addressed in detail in section 3.6 of the manuscript. However, we feel that this much detail is not appropriate at this point in the Introduction. Instead, we

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added a general reference (Ward 2008) for possible heterotrophy amongst nitrifiers as suggested by reviewer #1. «<

P4, line 5: The evidence that archaea oxidize ammonia in the ocean is compelling; it is their contribution relative to bacteria that is unresolved.

»> We completely agree. This is actually one of the points we are trying to make in this paper. «<

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

»> A sentence has been added with archaeal and bacterial amoA numbers from Wuchter et al. (2006) as this paper included both archaeal and bacterial 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).

»> Filters were not rinsed before filtering samples. However, this is a good suggestion for future work. «<

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?

»> Filtered water samples we frozen directly after sampling so we expect the effect of continued activity after filtration on DIN concentrations to be negligible. Samples were frozen at -20. «<

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?

»> In these calculations, the excess 15N or 13C is scaled up to total (labeled + unlabeled) NH4 assimilation and 13C-fixation respectively. To this end, the assimilated

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excess 15N and 13C are multiplied by total/added (e.g. when the labeled substrate was added at 10% of the ambient substrate concentration, resulting total rates were 10x higher than those for the labeled substrate alone). «<

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.

»> Even when DIC fixation by nitrifiers was not directly coupled to oxygen availability, we expect it to be directly coupled to nitrification and thereby to be indirectly coupled to oxygen. «<

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.

»> We added a reference (Park et al. 2010) in which the inhibitory effects of chlorate and nitrapyrin on archaeal ammonia oxidation and growth is reported. These inhibitors are considered as being process-specific rather than organism-specific. The inhibitor results are discussed in more detail in section 3.5. Also see our response to the general comment on the inhibitor results above and to similar comments by reviewer #1. «<

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.

»> We are not aware of any such reports. See response to comment below and to related comments by reviewer #1.«<

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

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nitrite-oxidizing archaea, it seems equally likely that the inhibitors impact archaea differently from ammonia-oxidizing bacteria. Additional interpretations would be appropriate here.

»> We agree that interpretation of inhibitor-based results in general always contain some uncertainty about specificity and efficiency of the inhibitors. Results are therefore presented with some caution. Based on this comment and related comments by reviewer #1, we added the following text: “Another explanation for the strong inhibition by chlorate may be direct inhibition of ammonium oxidation by chlorite produced from the reduction of chlorate by nitrite oxidizers (Hynes and Knowles 1983). Moreover, we cannot exclude the possibility that non-nitrifying autotrophs were also inhibited by chlorate.” «<

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.

»> This is an interesting line of thought. However, given that the general biochemical composition (proteins, carbohydrates, and lipids) of bacterial and archaeal cells is similar, we would also expect a similar portion of fixed C to be used for synthesis of biomass lipids. Even if such a difference was present, it would not be sufficient to explain the measured large difference in absolute 13C fixation. Moreover, this should not affect the delta13C values for the lipids of both groups. Altogether, this will not change the interpretation of our results. «<

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

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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.

»> We agree that abundance alone does not provide a good indication of activity. This is actually one of the points we are trying to make in this paper. It is correct that bacterial amoA was not measured in the present study. Instead, we compare the abundance of bacterial- versus archaeal amoA from previous years (from Wuchter et al. 2006) and assume a similar ratio for the present study (based on the very consistent seasonal patterns of AOA over several years, Pitcher et al. 2011). The possibility of mixotrophy or heterotrophy amongst the nitrifiers is discussed in detail in section 3.6. «<

Typos: P2, line 12: add a comma between “nitrification” and “with”

»> corrected «<

P10, line 15: “an” should be “and”

»> corrected «<

Anonymous Referee #3 This is a great paper that presents some interesting and much needed data about the relationship between nitrification, autotrophy, and the stoichiometry between N oxidation and C fixation in the coastal ocean. The investigation of the relative roles of ammonium uptake and nitrification are also interesting and timely.

General comments: One potential problem with the methods that could significantly influence the interpretation of their results is that environmental Thaumarchaea may not be captured by the 0.7 μm filter they used to measure both bulk DIC and GDGTs. In culture, N. maritimus is very small–a 0.45 μm filter was used to purify the culture and separate the archaea from the bacteria (Konneke et al. 2005) and in nature we might expect them to be much smaller. Nitrification is even detectible in 0.2 μm filtered seawater (Santoro et al. 2010), suggesting that AOA can pass through even this small pore size. So the C fixation rates presented here could be significant underestimates.

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Is there a way to estimate capture efficiency by using the authors' existing data from N.maritimus culture work, the lipid yield, and the amoA gene abundance data here to estimate a % of archaea captured? At the very least, a statement about this potential consequence of their method should be made.

»> This is a good point. However, we do not expect this to be a major issue in this study. It may be of more importance in open ocean settings with very low SPM concentrations and microbes occurring primarily as free living individual cells. However, in the turbid coastal waters of the present study, a large fraction of the bacteria are particle associated, meaning that they will be trapped on filters with pore sizes greater than the size of individual cells. We would expect this to be similar for Thaumarchaeota. Moreover, due to rapid clogging of the filters, the effective pore size decreased during filtration, meaning that < 0.7 μm particles were also trapped. This is supported by results from Herfort et al. (2006) who analysed GDGT concentrations in the North Sea with sequential filters of 0.7 μm and 0.2 μm pore size and only recovered < 5% on the 0.2 μm filters. Moreover, Pitcher et al. (2011) who found good correlation between AOA DNA concentrations trapped on 0.2 um filters with crenarchaeol concentrations (trapped on 0.7 um filters). Finally, cell size ranges of bacteria and archaea overlap, meaning that there was likely no strict difference in cell size between bacteria and archaea. Therefore, even if part of the microbes were not retained by the filters, we do not expect this to have resulted in a strong bias towards either group. We have included a statement, including the arguments above, about this issue in section 3.5. «<

The line of reasoning that because archaeal abundance and nitrification rates aren't linearly correlated, archaea cannot be responsible for the majority of nitrification is misguided, just as the converse is (as the authors argue).

»> We agree that, in general, abundance does not have to be correlated 1:1 with activity. However, for the present study, where Thaumarchaeotal abundance was very low at the start of the time series and showed a pronounced peak in Jan/Feb, we still would expect to see some effect of this bloom on the nitrification rates if the Thaumarchaeota

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were important contributors to nitrification. Also see response to similar comment by reviewer #2. «<

Is anything known about the effect of chlorate on sulfur oxidation?

»> We are not aware of such an effect and are not really sure how this would fit the context of this paper. «<

Detailed comments: p.16890 lin 25: Why is the Lipp et al. 2008 paper used for a conversion of lipid biomass to total biomass of archaea? That is a paper about sediment archaea from the deep subsurface. Given work by this group with cultures of planktonic archaea (Schouten et al. 2008), is this still an accurate conversion factor?

»> The conversion factor of Lipp et al. is a hypothetical factor based on modeling. The factor estimated there was similar to the conversion factor of Sinninghe Damste et al. (2002, Appl. Env. Micro.), also based on modeling. Both of these studies used models because actual measurements of these conversion factors have not yet been possible due to the limited availability and slow growth of Thaumarchaeotal cultures. However, the conversion factor used in the present study is consistent with results from recent field studies (Huguet et al. 2010 L&O, Schouten et al. 2012 GCA). It should be noted here that some uncertainty regarding this conversion factor (which is inherent to this type of calculations) does not change the results of this study as the difference in DIC fixation between Thaumarchaeota and bacteria was at least 2 orders of magnitude. «<

p.16891, Line 7: Observing a lack of correlation between abundance and rates doesn't mean that activity was zero.

»> See response to general comment above. «<

Table 1. Must there have been some co-inhibition of ammonia oxidation by chlorate? How could these two supposedly specific inhibitors account for the same % of 13C fixation inhibited and or percentages that sum to greater than 100%? In other words, if we interpret the % inhibition as a proxy for the % of C fixation carried out by that process,

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how could C fixation be 45% from ammonia oxidation and 100% by nitrite oxidation? Does this mean nitrite oxidation is totally controlled by the ammonia oxidation rate? Also, do the authors interpret the inhibition of C fixation into crenarchaeol by chlorate as evidence of archaeal nitrite oxidizers, or sensitivity of AOA to chlorate?

»> See response to similar comments by reviewers #1 and #2. Briefly, we cannot fully explain results for inhibition by chlorate. Therefore we discuss these results with caution and added two more possible explanations to the discussion ("Another explanation for the strong inhibition by chlorate may be direct inhibition of ammonium oxidation by chlorite produced from the reduction of chlorate by nitrite oxidizers (Hynes and Knowles 1983). Moreover, we cannot exclude the possibility that non-nitrifying autotrophs were also inhibited by chlorate."). «<

Shouldn't the caption for Fig. 5 say Dd13C values for PLFAs and crenarchaeol NOT inhibited by nitrapyrin? Am I missing something?

»> The caption is correct as it presents the difference between the incubations with- versus without nitrapyrin (as described between brackets in the figure caption). This means that these values were not directly measured but instead derived from the measured values for the treatments with- and without nitrapyrin. «<

In addition to our response to the reviewers:

-Madigan et al. (2006) has been replaced by Ward (2008).

-A recent reference has been added (Alonso-Sáez et al. 2012).

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