

Reviewer report

Peer review report on “Nitrification in the oligotrophic Atlantic Ocean”

1. General comments

The manuscript ‘Nitrification in the oligotrophic Atlantic Ocean’ by Clark et al. investigated the NO_2^- production rate in the euphotic zone across a large area of the Atlantic ocean. Additional environmental variables were presented to examine the potential controls on this key process in the sunlit ocean.

They found NO_2^- production was active at all depths, and across the study areas with large spatial variability, a striking feature of rate difference between the two hemispheres was highlighted. The authors identified that Chl-a, duration of the light phase, and Si concentration best explained the observed rates.

While such large-scale observation on the biogeochemical rate is encouraging and inspiring, there are several key issues that need to be further clarified and resolved before drawing the conclusions presented in the manuscript:

1.1 Methodology

Instead of using the $^{15}\text{NH}_4^+$ labeling and dark (or paired light-dark) incubation, the authors chose the $^{15}\text{NO}_2^-$ dilution method, and the incubation was carried out under light conditions, this inevitably induces several issues:

- 1) The measured rate stands for the total NO_2^- production rate rather than ammonia oxidation rate. Specifically, in the sunlit ocean, it would be expected that phytoplankton also play a significant role in producing NO_2^- , particularly in the upper euphotic zone where NO_2^- production might be dominated by phytoplankton rather than nitrifier. For this reason, I would suggest re-consider the measured rate as ‘ NO_2^- production rate’ rather than ‘nitrification rate.’
- 2) The lack of rates been measured in dark conditions would also cause a significant bias of NO_2^- production rates in the sunlit ocean as both phytoplankton and nitrifiers are sensitive to light.
- 3) I am also concern about the potential effect of NO_2^- consumption by phytoplankton in the nutrient-deprived surface ocean on the rate measurement and calculation.

1.2 Result and discussion

- 1) The lack of in-situ NH_4^+ measurement is another major flaw of the current study. The substrate concentration is one of the most fundamental controls for ammonia oxidation in the marine environment, so it would be of great relevance in understanding the environmental control on this process. Is that possible for the authors to
- 2) The main finding—a significant hemispheric difference of the NO_2^- production rate is exciting. More detailed information and discussion are encouraged further to explore the potential mechanism of this novel finding. Either it is attributed to spatial or seasonal variation, the production, and remineralization of organic matters might be a fundamental control. Thus, I suggest expanding the discussion on this point to examine this hypothesis, i.e., to examine the productivity (satellite, Bio-Argo, literature...), the strength of remineralization, etc.
- 3) The authors declared that NO_2^- production was enhanced in the mesoscale eddies. While I appreciate that mesoscale processes exert profound influences on marine biogeochemistry, more evidence is expected to bolster this argument. For instance, mesoscale eddy is a ubiquitous feature in the global ocean, have the authors examined whether they sampled in other eddies aside from these two eddies reported here? Can the authors expand a bit to examine the underlying causes? i.e., Increased primary production? Intensified remineralization? Alleviated competition between nitrifier and phytoplankton etc.
- 4) I am also curious about the effect of light, i.e., any explanation for the lack of correlation between the measured rate vs. light intensity, but the duration of light can well explain the observed rates? Also, it is counterintuitive to see the positive correlation between light duration and NO_2^- production rate.

2. Specific comments

Line 1: You do not need the full stop at the end of the title.

Lines 26-27: Why not NH_4^+ ?

Lines 91-95: I encourage the authors to expand to clarify the method, particularly the detection limit and the accuracy of the trace level of nutrient measurement.

Line 106: What criteria are used to define the euphotic zone, 1% PAR or 0.1 PAR%?)

Line 109: Taken the extremely low level of NO_2^- outside the PNM layer (i.e., less than 10nM in many samples?), is that mean that only <1nM of $^{15}\text{N-NO}_2^-$ (<10%) was added into the incubation system? Would that cause bias if the trace amount of NO_2^- was assimilated by the

phytoplankton and/or bring a significant challenge in measuring the NO₂- isotope at such low concentration?

Line 116: What is the reason for performing a ten h day-time incubation? Taking the inhibition of ammonia oxidizers by light in the sunlit ocean and their ability to recover under dark, ten-hour day-time incubation rather than a full day incubation covering the light-dark cycle would cause underestimating ammonia oxidation rate. In another aspect, NO₂- release during assimilatory NO₃- reduction appears to be another key source of NO₂- in the euphotic zone. The dilution method used in this study cannot distinguish these two sources of NO₂- and thus would overestimate the ammonia oxidation rate.

Lines 120-128: The method used here for NO₂- isotope analysis is quite complex that involves multiple steps and reagents. Extreme care should be taken to avoid any contamination for the isotope analysis of the trace amount of NO₂-. For this reason, some introductions on the detection limit and accuracy will benefit the audience on the accuracy of the result.

Lines 135-139: Similarly, I suggest further clarifying the calculation process and detection limit of the rate.

Lines 175-176: Citation required?

Lines 187-189: Perhaps some description on the integrated light intensity (dose)? And the potential reason for the absence of a corresponding increase of irradiance?

Lines 213-214: Any reason for not showing the results of *Synchronous* and *Prochlorococcus*? Given that these cyanobacteria are the most representative and abundant phytoplankton in the oligotrophic ocean and the main competitors for NH₄⁺, information on the distribution of *Synchronous* and *Prochlorococcus* should be relevant of interpreting the observed ammonia oxidation rate.

Lines 220-221: Since the vertical resolution is relatively coarse, it would not be robust to do the depth-integrated rate calculation.

Lines 305-308: Although there is a clear spatial pattern of the light duration of the investigated area, the light dose did not show a similar pattern. Additional analysis between the rates and light dose might also be helpful.

Lines 318-320: Numerous studies have discussed the effect of light and the competition between nitrifiers and phytoplankton in the sunlit ocean. It deserves to discuss further the potential effect of the diel cycle and the role of phytoplankton on consuming and /or producing NO₂-.

Lines 336-342: Regretfully, that NH_4^+ concentration was not measured in this study! The substrate acts as the most critical factor in regulating the ammonia oxidation rate in the ocean. The significant correlation between substrate concentration and ammonia oxidation rate is more often reported in the literature, so it would be better to point it out. Also, the NH_4^+ concentration is usually very low (i.e., lower than the reported K_s of the ammonia oxidizers) in the oligotrophic gyres; saturation kinetics appears to be very unlikely in the study area.

Line 361-362 This is another key issue of the study. A main weakness of the dilution method lies in its unavailability of discerning the potential sources. It is very likely that the rate of NO_2^- production by ammonia oxidizers in the euphotic zone is overestimated, particularly at the upper euphotic zone where nitrifiers are inhibited by light and outcompeted by the phytoplankton, the NO_2^- production rate measured here is more likely attributed to phytoplankton rather nitrifiers.

Lines 385-386: See my comment above. I suggest showing the profiles of *Synchronous* and *Prochlorococcus*.

Lines 386-387: Doesn't that mean the potential of NO_2^- released by phytoplankton might occur throughout the sampling depth and thus should be taken into account in the discussion?

Lines 415-430: It is interesting to see the systematic rate difference between the hemispheres. The authors have proposed several potential causes for explaining the phenomenon.

Nevertheless, I would like to see more detailed information on bolstering these hypotheses, i.e., does the productivity/ biomass (either from Argo or satellite) show a similar spatial pattern between the hemispheres during the cruise? Counterintuitively, the duration of light increases from the northern hemisphere to the southern hemisphere. As light is an inhibitor for the nitrifiers, is there any explanation for the apparent positive correlation between ammonia oxidation rate and light duration?

Lines 434-435: What does this sentence mean?

Lines 471-472: Do the bacterial abundance indicate higher remineralization rates in the South hemisphere?

Lines 483-505: It is also quite interesting to see the significantly enhanced rate in 28°S and 39°S , which was attributed to the mesoscale process. While it deserves to expand a bit more to discuss it: 1) it is true that the prominent high rate occurred in these two eddies, worth noting that mesoscale eddy is a ubiquitous feature in the ocean, are there any other eddies at the sampling stations during the cruise? If so, is there any evidence of stimulation of ammonia oxidation by the eddy? 2) Is that possible to look into the biomass, productivity,

heterotrophic activities, directly or indirectly, to bolster the argument that the high rate was due to the eddy?

Line 795: I suggest plotting the depth of the mixed layer in Fig. 2a.

Line 805: I suggest presenting nitrite and silicate (Fig. 3e and 3f) in the same way with Fig. 3a-3d, because the vertical resolution (only three depths) is too coarse for the extrapolation.

Line 825: How was the NO_3^- profile derived, given that only three depths were sampled for nutrient analysis in each station?

Line 830: Some statistical analysis (i.e., if the difference between the hemispheres is significant) would further bolster the difference between the two hemispheres in Fig. 7d.

Line 840: Again, the depth-integrated rate in Fig. 8 was based on the rates measured at three depths, which was insufficient for extrapolation.