

## Comment on bg-2021-280

Anonymous Referee #1

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Referee comment on "Controls on nitrite oxidation in the upper Southern Ocean: insights from winter kinetics experiments in the Indian sector" by Mhlangabezi Mdutyana et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-280-RC1>, 2021

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Mdutyana et al. conducted a series of NO<sub>2</sub>- oxidation kinetics experiments on the surface waters along one section across the western Indian sector, as well as depth-profile NO<sub>2</sub>- oxidation rates determination along another section during a winter cruise in July 2017. This work provides reliable data/evidence that nitrite oxidizing bacteria require a minimum (threshold) nitrite concentration to produce nitrate. This result is a highlight of the paper. Yet, I have a few concerns that the authors need to deal with before I can recommend publication.

1. L16–17: This sentence is not easy to understand lacking explanations. Normally, “fuel productivity” means more CO<sub>2</sub> fixation, which is logically incoherent with the second half-sentence “weakening the ....CO<sub>2</sub> sink”. It seems that the authors need to explain CO<sub>2</sub> sink meaning export production or new production, which can be overestimated by nitrification. I agree nitrification complicates new production estimates but does not weaken new production (or carbon sink) itself.

2. L31–33: I do not agree with the authors about the understanding of "nitrite undersaturation of the ... enzymes" in this paper. Please see below my comments on the relevant issues. In addition, the speculative conclusion should not be included in the abstract without the support of research data.

3. L39–42: Carbon dioxide has no superscript “-”

4. L51 and L59: Clarify the removal of CO<sub>2</sub> from the atmosphere (not from the ocean) throughout the paper for a smooth understand.

5. L63–66: Again, iron-deplete conditions may restrict nitrification and thus weaken the overestimation of new productivity but not weaken the biological CO<sub>2</sub> sink itself. It is not recommended to use such an ambiguous term “biological CO<sub>2</sub> sink” unless it has already been defined/explained in the preceding part of the text. The use of more specific terms such as new production, export production, etc. helps readers easier to understand.

6. L97: Lomas and Lipschultz (2006) reported that PNM appeared at the base of the euphotic zone rather than the bottom of the mixed layer, which is different from this study. This study showed that the mixed layer of the Southern Ocean was much deeper than the euphotic zone. The authors should clarify these differences.

7. L175, L183, and L198: The seawater in these incubation experiments was prefiltered through a 200 µm nylon mesh to remove zooplankton grazer. This operation may result in an overestimation of the phytoplankton uptake rate relative to the in situ rate and thus an underestimation of nitrification rates due to substrate competition with phytoplankton.

8. L226: The nitrification rate calculation based on the difference between two time-points values may be biased, especially when the added 15NO<sub>2</sub><sup>-</sup> tracer concentration (final concentration 200 nM) is higher than the in situ NO<sub>2</sub><sup>-</sup> concentration (average 168±48 nM), the incubation time is long (23–30 h), and the inferred nitrification rate is relatively high. A linear fitting of at least 3 to 4 time-point values showing the variation of 15NO<sub>3</sub><sup>-</sup> content with incubation time helps to assess the stability of nitrite removal and the potential influence of 15NO<sub>3</sub><sup>-</sup> uptake by phytoplankton on the nitrite oxidation rate in the incubation system.

9. L300–302, 375–383, 529–531, 537–539, 661–662: Ammonia oxidation rates and kinetic parameters were mentioned and shown throughout the paper, including the results, Figure 3g–j, Figure 6, and a lot of discussions, but there was no description of the methodology. Similarly, the dissolved iron concentrations (L595–597) were shown in Figure 5, but the corresponding measurement methods were not given. The cited literature is a graduation thesis and cannot be retrieved. Please include these necessary contents in the paper so that the readers can fully understand the entire story.

10. L347: delete a “from”.

11. L351–353: This is a very important conclusion. Please give the correlation coefficient and statistical significance (r and p values).

12. L357: Fig. 2e showed 56°S for St 05.

13. L440–444: This is a discussion and should be moved to the discussion section.

14. L447–450: These statements seem repeated with the content in the Introduction section.

15. L481–482: Redundantly cited "(27-506 nM; Zhang et al., 2020)". It can be revised as "oxygenated coastal or open oceans (27-506 nM; Olson, 1981; Zhang et al., 2020)".

16. L482–484: This sentence reads confusing and needs to be reorganized. The  $K_m$  values were high in Sun et al. (2021) (5-11  $\mu$ M), which is not similar to the low  $K_m$  values mentioned earlier (Olson, 1981; Sun et al., 2017; Zhang et al., 2020).

17. L505–506: Table 2 did not show  $V_{max}$  values. Please add them.

18. L513–515: There were several descriptive sentences in the Discussion section, e.g. focusing on the values distribution patterns. It is better to add some in-depth discussion about the causes of these phenomenon in order for a discussion to be effective.

19. L524 and 686: The authors frequently used latitude as an indicator of light throughout the paper. I suggest directly using light intensity data (such as PAR) for analysis.

20. L602–604: The logical process of the sentence is unclear. I cannot understand nitrification weakens the biological pump. Nitrification supports primary production (carbon fixation), but indeed it can cause an overestimation of new productivity. The authors should accurately state the point.

21. L604: What does "It" mean here? Iron-limiting nitrification? or iron-limiting condition? Clarify it.

22. L628-632: Deep mixing events cannot explain the results of this study. The discussion does not make sense.

23. L655–657: "while in other cases,  $\text{NH}_4^+$  oxidation is dominant ..." seems redundant. This sentence needs to be reorganized.

24. L606, 659, 665: The rates in Figures 5 and 6 were the corrected rates of ammonia and nitrite oxidation, right? Please accurately express them on the figure axes and legends.

25. Figure 6: There were no error bars at all in Figure 6b. In addition, SE cannot be given based on two parallel measurements ( $n=2$ ).

Please use unified symbols for the same station in Figures 3, 5, and 6.

26. L660: derived from?

27. L693-694: Why dilute NOB particularly? not dilute AOA? The authors should give an explanation in order for the logic to be understood clearly.

28. L737-744: The discussion does not make sense. The consumption of N producing the same biomass of NOB and AOA and their growth rates cannot explain the results of this study. In another word, the differences in the yield and growth rates (life strategies) of AOA and NOB cannot explain the coupling or decoupling of two steps of nitrification, which only depends on the rates of two steps of nitrification.

29. L771: Nitrite concentration or oxidation rate?

30. L776-787: Normally, the undersaturation by substrate of enzyme means the first-order reaction is occurring. The reaction rate reaches the maximum with substrate saturation. However, the authors used substrate undersaturation to explain the substrate ( $\text{NO}_2^-$ ) concentration threshold of the reaction below which no reaction occurred. The opposite meanings are confusing to readers.

31. L787-794: The logic is confusing too. Nitrospira and Nitrospina with a periplasmic NXR have a higher  $\text{NO}_2^-$  affinity than Nitrococcus and Nitrobacter with a cytoplasmic NXR. That means  $K_m$  should be lower for Nitrospira and Nitrospina, and thus there should be no or lower threshold. But the authors explained the substrate threshold phenomena in the Southern Ocean with the high substrate affinity/low  $K_m$  of Nitrospira and Nitrospina NXR. This is incomprehensible. The discussions about the substrate undersaturation of the enzyme and the response kinetics of the enzymes of different NOB to the substrate are too speculative and some discussions do not make sense.

31. L801: What does “depending on the maximum substrate concentration added during kinetics experiments” mean? Normally a series of concentrations of substrate (not only the maximum substrate concentration) were added during kinetics experiments.

32. L811-815: The findings from Saito et al. (2020) cannot explain/support the nitrite concentration threshold ( $C$  value) for nitrite oxidization here. Nitrospira and Nitrospina

dominance does not necessarily cause the existence of a threshold. Nitrospira and Nitrospina usually distribute in the oligotrophic ocean with low concentrations of N-nutrients. According to the positive correlation between C and nitrite concentration (L351), the C value of Nitrospira and Nitrospina should be very low. This is not consistent with the high values of C observed in this study.