Mdutyana et al. Controls on nitrite oxidation in the upper Southern Ocean: insights from winter kinetics experiments in the Indian sector

Response to reviewer's comments:

We thank the reviewers for their thoughtful comments and suggestions, which will definitely improve our paper. Both reviewers are positive about the manuscript and stress the significance of our unique dataset. Although reviewer 1 supports the ultimate publication of this work, they have raised a few larger concerns that we address in detail below. Reviewer 2 mainly had minor suggestions, which we also respond to below. Our responses to the reviewers are in blue text (with changes to sentences indicated in bold) and their original comments are in black.

Comments from reviewer 1

Mdutyana et al. conducted a series of NO2- oxidation kinetics experiments on the surface waters along one section across the western Indian sector, as well as depth-profile NO2- 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 CO2 fixation, which is logically incoherent with the second half-sentence "weakening the ….CO2 sink". It seems that the authors need to explain CO2 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.

Response: In a mass balance sense, nitrification in the surface layer *does* weaken the CO_2 sink. However, we take the reviewer's point about the semantics and will modify the sentence to read "Across the Southern Ocean in winter, nitrification is the dominant mixed-layer nitrogen cycle process, with some of the nitrate produced therefrom persisting to fuel productivity during the subsequent growing season. **Because this nitrate constitutes a regenerated rather than a new nutrient source to phytoplankton, it will in net support no removal of atmospheric CO_2**".

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.

Response: We will remove the concluding sentence from the abstract and replace it with the following: "Our findings have implications for understanding the controls on nitrification and ammonium and nitrite distributions across the global ocean". We have responded to the reviewer's other comments regarding nitrite undersaturation of the enzyme in detail below where the reviewer has elaborated on their concerns.

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

Response: Apologies for this typo. The superscript on carbon dioxide will be removed, here and in other relevant places.

4. L51 and L59: Clarify the removal of CO2 from the atmosphere (not from the ocean) throughout the paper for a smooth understand.

Response: We do not fully understand the reviewer's concern here. We repeatedly refer to the removal of **atmospheric CO_2** in the introduction (and now also in the abstract). For instance, "The cycling of nitrogen (N) in the upper ocean is central to the role that phytoplankton and bacteria play in **atmospheric** carbon dioxide (CO_2) consumption and production" (p. 2 of the original submission); "Over appropriate timescales, new production is equivalent to "export production", the latter referring to the organic matter produced by phytoplankton that escapes recycling in surface waters and sinks into the ocean interior, thereby sequestering **atmospheric CO_2** at depth" (p. 2); and also in the discussion "Since phytoplankton consumption of regenerated NO_3^- yields no net removal of **atmospheric CO_2**" (p. 18).

5. L63–66: Again, iron-deplete conditions may restrict nitrification and thus weaken the overestimation of new productivity but not weaken the biological CO2 sink itself. It is not recommended to use such an ambiguous term "biological CO2 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.

Response: We will amend the sentence as follows: "If this limitation is verified and proves widespread in the environment, one implication is that the iron-deplete conditions of the surface Southern Ocean may restrict mixed-layer nitrification and by extension, **may decrease the extent to which phytoplankton growth is fueled by regenerated nitrate**".

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.

Response: The sentence will be modified as follows: "Generally, NO_2^- concentrations in the low-latitude oxygenated ocean reach a maximum near the base of the **euphotic zone**".

It should also be noted that nitrite concentrations in the Southern Ocean are not only high because the mixed layers are deeper than the euphotic zone; the Southern Ocean also hosts high mixed-layer nitrite concentrations in summer when the mixed layers can be shallower than euphotic zone (e.g., Mdutyana et al. 2020).

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.

Response: We cannot rule out the possibility of underestimation of nitrification rates due to phytoplankton outcompeting nitrifiers in the absence of zooplankton grazers, although we think this is highly unlikely in the case of our experiments, for two major reasons: 1) the South Ocean's winter mixed layer hosts quite high concentrations of NH_4^+ and NO_2^-, suggesting that neither phytoplankton nor nitrifiers should be substrate limited, and 2) the nitrite oxidation experimental design was such (i.e., bottles were dark) that phytoplankton activity should have been minimized. In addition, recent studies from our group (Smith et al. 2022 and Mdutyana et al. 2022) have investigated the potential drivers of elevated NH_4^+ in the mixed layer of the Southern Ocean during winter. Smith et al. (2022) concludes that NH_4^+ production outpaces NH_4^+ removal in the mixed layer, which is inconsistent with limitation of NH_4^+ oxidation (or phytoplankton NH_4^+ uptake) by NH_4^+ availability. Mdutyana et al. (2022) finds that NH_4^+ oxidation is limited by substrate availability only at NH_4^+ concentrations <90 nM (typically far below those encountered in the Southern Ocean mixed layer in winter). This study further shows a positive relationship between the rates of NH_4^+ uptake by phytoplankton and NH_4^+oxidation by nitrifiers, which is the opposite of the relationship expected if there were competition between the two groups for NH_4^+ substrate.

8. L226: The nitrification rate calculation based on the difference between two time-points values may be biased, especially when the added $^{15}NO_2^-$ tracer concentration (final concentration 200 nM) is higher than the in situ NO2- 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 $^{15}NO_3^-$ content with incubation time helps to assess the stability of nitrite removal and the potential influence of $^{15}NO_3^-$ uptake by phytoplankton on the nitrite oxidation rate in the incubation system.

Response: We agree that a linear fitting of 3 to 4 timepoints may have been a more appropriate methodological approach and one that we will endeavour to adopt in future. However, given the number of experiments conducted during this study, such an approach was unfeasible. Additionally, the main purpose of this research was to investigate whether NO_2^- oxidation in the winter Southern Ocean followed a Michaelis-Menten curve. In the case of these Michaelis-Menten experiments, the goal was to stimulate the oxidation rates via higher and higher additions of substrate. For the vertical profiles (to which we believe the reviewer is largely referring in their comment here), we go to great lengths in the manuscript to address the issue of potential overestimation of the oxidation rates due to the high concentration of ^{15}N-nitrite added, demonstrating that nitrite oxidation rates can indeed be quite strongly affected by the addition of concentrations of ^{15}N-nitrite tracer in excess of the K_m for nitrite oxidation (p. 8 of the original submission). This is exactly the reason why we calculate corrected nitrite oxidation rates. As to phytoplankton assimilation of ^{15}N-nitrate, this is unlikely to be an issue in the Southern Ocean at any time of year, and particularly in winter. The ambient concentration of the mixed-layer nitrate pool is high year-round (4-28 uM) meaning that phytoplankton assimilation of that pool will have a negligible effect on the small fraction of it that is ^{15}N-labelled due to nitrite oxidation. Moreover, the rate of phytoplankton nitrate uptake in winter is extremely low (Philibert et al. 2015; Mdutyana et al. 2020), rendering phytoplankton removal of ^{15}N-nitrate even less likely. Finally, the nitrite oxidation experiments were conducted in opaque bottles, in order to minimize phytoplankton activity (see methods; p. 5 of the original submission).

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.
Response: The information mentioned by the reviewer is now cited properly; we have changed the citation from the graduation thesis to our recently published paper on NH_4^+ uptake and oxidation kinetics in the Southern Ocean (Mdutyana et al. 2022). Additionally, we will add a brief summary of the methods used to determine the ammonia oxidation rates and iron concentrations to the amended version of the manuscript.

10. L347: delete a “from”.
Response: The word “from” will be removed.

11. L351–353: This is a very important conclusion. Please give the correlation coefficient and statistical significance (r and p values).
Response: Correlation coefficient and statistical significance will be included in the text; these values are $r^2 = 0.59$ and $p = 0.045$.

12. L357: Fig. 2e showed 56°S for St 05.
Response: This error will be corrected in the caption of figure 2, which will read as “e) St 05: 56°S (OAZ)”.

13. L440–444: This is a discussion and should be moved to the discussion section.
Response: We will move this sentence to the discussion.

14. L447–450: These statements seem repeated with the content in the Introduction section.
Response: We will remove the repetition by deleting the first two sentences of the discussion so that the section will begin with our findings, as such “Across all the major zones of the wintertime Southern Ocean, the addition of NO_2^- to samples of surface seawater stimulated NO_2^- oxidation following a Michaelis-Menten relationship…”

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)”.
Response: Redundant citation “(i.e., 27-506 nM; Zhang et al., 2020)” will be removed and the sentence will be amended as suggested by the reviewer.

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

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Response: We will amend the sentence as follows: "In the low- to zero-oxygen waters of the ETNP ODZ, similarly low K_m values have been reported (254 ± 161 nM; Sun et al., 2017), **although some** have values >5 µM (Sun et al., 2021)".

17. L505–506: Table 2 did not show Vmax values. Please add them.
Response: V_{max} values will be added to the table.

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.
Response: We do not completely understand the reviewer's comment here given that the line numbers to which they refer detail a finding from other studies (Sun et al. 2017; Zhang et al. 2020). However, in the amended version of the manuscript we will take care to add in-depth discussion of the trends and drivers thereof wherever appropriate and remove "blanket" statements.

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.
Response: We agree that it would be better to use directly measured PAR for the discussion of light availability; however, surface PAR was not measured during the cruise, which is why we use latitude as a proxy for light. We will add a sentence to the methods clarifying that PAR was not measured, such that we use latitude as a proxy for light. Given how far apart our stations are, this is not an unreasonable assumption.

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.
Response: In a mass balance sense, surface layer nitrification **does** weaken the biological pump. This is particularly true in the Southern Ocean where the ambient mixed-layer nitrate pool remains high throughout the year. The fact that phytoplankton do not drawdown more of the shallow nitrate reservoir amounts to a "leak" in the biological pump (i.e., CO_2 released to the atmosphere is not compensated for by photosynthetic CO_2 removal; Sarmiento and Toggweiler 1984; Sigman and Boyle 2002). If nitrate is additionally added to the mixed-layer pool by recycling (which in net, means CO_2 production even though we recognize that nitrification is an autotrophic pathway), then the biological pump will be further weakened because some amount of the nitrate consumed by phytoplankton is associated with CO_2 production (despite its consumption by phytoplankton removing CO_2).

21. L604: What does "It" mean here? Iron-limiting nitrification? or iron-limiting condition? Clarify it.
Response: "It" means iron-limiting nitrification, which will be clarified in the text: "Since phytoplankton consumption of regenerated NO_3^- yields no net removal of atmospheric CO_2 (Yool et al., 2007), an iron-related control on mixed-layer nitrification would help to limit the extent to which this process can weaken the Southern Ocean's biological pump **and** would lead to enhanced competition between phytoplankton and nitrifiers **for iron**".

22. L628-632: Deep mixing events cannot explain the results of this study. The discussion does not make sense.
Response: We agree that deep mixing cannot explain our results; however, mixing has been suggested by others (e.g., Zakem et al. 2018) as the reason that nitrite accumulates throughout the mixed layer in the high latitudes (versus at the base of the euphotic zone in the subtropical ocean). We thus feel it necessary to address whether such as explanation is the reason for our observations of near-invariant nitrite in the Southern Ocean mixed layer (and rapidly conclude that it is not). We will amend the paragraph as follows to clarify our meaning: "The persistence of elevated NO_2^- concentrations in the mixed layer at high latitudes has **previously** been attributed to the inability of iron- and/or light-limited phytoplankton to fully consume NO_2^- transported to the surface with NO_3^- during deep mixing events (Zakem et al., 2018). However, subsurface NO_2^- concentrations in the Southern Ocean are typically below detection (Figure 1b and 3a; Olsen et al., 2016), so it is unclear how deep mixing could supply

measurable NO_2^- to the euphotic zone. **We thus discount subsurface mixing as an explanation for the elevated mixed-layer NO_2^- concentrations observed across the Southern Ocean, during our study and in other seasons (e.g., Fripiat et al. 2019; Mdutyana et al. 2020)**".

23. L655–657: "while in other cases, NH4+ oxidation is dominant …" seems redundant. This sentence needs to be reorganized.
Response: We are uncertain what the reviewer objects to here. The sentence is evaluating past suggestions of which of the two nitrification steps is rate-limiting: "However, rate measurements from numerous ocean regions show contrasting results, with NO_2^- oxidation sometimes outpacing NH_4^+ oxidation while in other cases, NH_4^+ oxidation is dominant".

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.
Response: Thank you for the suggestion. The figures and legends will be amended to ensure that they are correctly labelled as "corrected rates".

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.
Response: Error bars will be included in Figure 6b. The station symbols in Figure 3 will be unified with the symbols in Figure 5 and 6. We will change all references to SE where n = 2 to instead refer to the range of values.

26. L660: derived from?
Response: The sentence will be amended as follows "Additionally, the maximum rates of NO_2^- oxidation (V_{max}) **determined** for the surface NOB community **in this study** (~5 to 13 nM d^{-1}; Figure 2)…".

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.
Response: The effect appears not be the direct result of dilution but rather the variable response of AOO versus NOB following dilution due to mixing. We will amend the sentence as follows: "In coastal waters, deep winter mixing has been shown to dilute the nitrifier community, **with AOO subsequently recovering more rapidly than NOB**. **This differential rate of recovery has been hypothesized to result in a period of** low NO_2^- oxidation rates while NH_4^+ oxidation rates remain elevated, ultimately causing NO_2^- accumulation in the upper layer (Haas et al., 2021)".

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.
Response: We agree with the reviewer's concern and will remove this section of text from the manuscript.

29. L771: Nitrite concentration or oxidation rate?
Response: We are referring to nitrite concentration. The sentence will be amended as follows: "Practically, our findings suggest that Southern Ocean NOB require a minimum (i.e., "threshold") NO_2^- concentration below which, **the ambient NO_2^- concentration** becomes severely limiting."

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 (NO2-) concentration threshold of the reaction below which no reaction occurred. The opposite meanings are confusing to readers.
Please see our response below item 31.

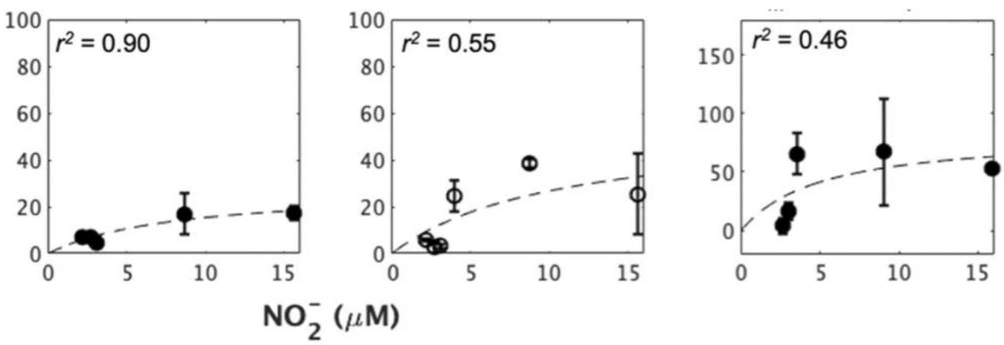
31. L787-794: The logic is confusing too. Nitrospira and Nitrospina with a periplasmic NXR have a higher NO2- affinity than Nitrococcus and Nitrobacter with a cytoplasmic NXR. That means Km should be lower for Nitrospira and Nitrospina, and thus there should be no or lower threshold. But the authors explained the substrate threshold phenomena in the Southern Ocean with the high substrate affinity/low Km 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.

We agree with the reviewer that much of the text to which they refer in comments 30 and 31 was overly speculative and/or confusing, and we thank them for their insights in this regard. In the amended manuscript, we will shorten and alter the text that was previously at lines 776-815, along the lines of:

"The existence of a NO_2^- concentration threshold may indicate limitation of the membrane-bound NXR enzyme, either by NO_2^- or by another essential nutrient. Recently, using NXR concentrations, estimates of NXR specific activity, and direct measurements of *in situ* NO_2^- oxidation rates, Saito et al., (2020) deduced that *Nitrospina* NXR is undersaturated with NO_2^- in the tropical Pacific, possibly due to iron limitation. The authors suggest that under iron-scarce conditions, it becomes increasingly difficult for NOB to synthesize NXR and thus to oxidize NO_2^-. A similar dynamic may be at play in the Southern Ocean, with limited synthesis of NXR at low iron concentrations resulting in a decrease in the efficiency of the NO_2^- oxidation pathway that manifests most strongly when the ambient NO_2^- concentration is also low. This inefficiency could be alleviated at higher NO_2^- concentrations since NOB (even with a paucity of NXR) are less likely to experience diffusion limitation with respect to the NO_2^- substrate when there is more of this substrate available (Pasciak and Gavis 1974). Regardless of its mechanistic basis, limitation of NOB NXR would help to explain the perennially elevated concentrations of NO_2^- in the Southern Ocean mixed layer. Moreover, environmental factors unique to the Southern Ocean such as limited iron availability may be instrumental in setting the NO_2^- threshold and associated elevated mixed-layer NO_2^- concentrations.

Our observations raise the question of why a similar NO_2^- concentration threshold has not been reported from other ocean regions, particularly those characterized by similar conditions to the Southern Ocean. This may partly be due to the very limited number of NO_2^- oxidation kinetics experiments that have been conducted in the open ocean and/or to the fact that a classic Michaelis-Menten function is usually imposed upon kinetics data, with V assumed to increase as soon as $S > 0$. Additionally, depending on the maximum substrate concentration added during kinetics experiments (i.e., the maximum concentration on the x-axis of a Michaelis-Menten V versus S plot), it can be difficult to discern a possible threshold NO_2^- concentration by simply examining the resultant plots. Inspection of published Michaelis-Menten curves does reveal the possibility of a non-zero C value in some cases, including in the ETNP ODZ (Sun et al., 2021) and associated with the PNM in the South China Sea (Zhang et al., 2020). However, there are other published curves that clearly do intercept the origin in V versus S space (Olson, 1981a; Sun et al., 2017), underscoring the need for further investigation of the conditions that lead to a threshold NO_2^- concentration requirement of NOB."

32. 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.
Response: We are referring to the x-axis scale of the plots presented in some previous studies. If the x-axis scale goes linearly from 0 to 10 μM nitrite, for instance (see below), it is easy to ignore a <1 μM threshold because it appears close to the origin of the x-axis (although in the case of the figures below, a standard Michaelis-Menten relationship has been imposed on the data when it is fairly clear that the intercept should not be 0,0). We will amend the sentence as follows: "Additionally, depending on the maximum substrate concentration added during kinetics experiments **(i.e., the maximum concentration shown on the x-axis of a Michaelis-Menten V versus S plot)**, it can be difficult to discern a possible threshold NO_2^- concentration on the order of 0.2 μM by simply examining the resultant plots."



33. 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 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.
Response: This entire section will be re-written, as outlined above, with the intimation regarding the findings of Saito et al. (2020) removed.

Comments from reviewer 2

In the manuscript Controls on nitrite oxidation in the upper Southern Ocean: insights from winter kinetics experiments in the Indian sector, Mdutyana and colleagues present strong evidence for nitrite oxidizing bacteria requiring a threshold nitrite concentration to produce nitrate in the mixed layer of the Southern Ocean in winter. Overall, the manuscript is well written, with a great set of figures, and the key findings and any associated limitations/caveats are clearly presented and thought through. Prior to publication I just have a few comments to enhance the clarity of the presentation in places.

Line 31 to 33: ending the abstract on a hypothesis / speculation seems out of place, I would suggest the authors consider instead a sentence focusing on the broader perspective of their work.
Response: We will remove the speculative concluding sentence from the abstract and replace it with the following: "Our findings have implications for understanding the controls on nitrification and ammonium and nitrite distributions across the global ocean".

Line 39 to 41: the superscript on CO2 needs to be deleted.
Response: This will be fixed, here and elsewhere in the manuscript.

Line 96 to 98: for clarity I think it would be important to clearly distinguish between the base of the euphotic zone and the mixed layer here, it is my understanding from Lomas and Lipschultz, 2006 (and other studies) that they have found the PNM at the base of the euphotic zone which sets it apart from your work.
Response: The sentence will be modified to specify that PNM occurs at the base of the euphotic zone; "Generally, NO_2^- concentrations in the low-latitude oxygenated ocean reach a maximum near the base of the **euphotic zone**".

Line 150: Nutrient collection is not discussed in this section, so suggest you update the subheading
Response: We thank the reviewer for spotting this; the subheading has been updated to "Hydrography".

Line 188: the custom built on-deck incubator use for the nitrite oxidation experiments, were these fitted with the neutral density screens mentioned for the nitrate update experiments, or were they carried out in the dark – this has important implications for your findings.
Response: The nitrite oxidation experiments were conducted in the dark in separate bottles from the nitrate uptake experiments. We will ensure that this detail is clear in the methods section. Conducting

nitrite oxidation experiments in the dark (near-)eliminates phytoplankton activity, which one might be concerned could result in an overestimation of the nitrite oxidation rates. However, we recently published a paper (Mdutyana et al. 2020) detailing the results of nitrification and nitrate uptake experiments conducted in the same bottles under simulated light conditions; these rates from the winter Southern Ocean were very similar to those measured in the present study, suggesting that we are not significantly overestimating nitrite oxidation by incubating in the dark.

Line 180 to 205: it would be beneficial for the authors to comment on (here or in the discussion) the potential limitation of only having Tzero and Tfinal samplings for their rate experiments, and thereby assuming a linear relationship over the incubation period (potentially missing any time lags, flattening off, or exponential activity). Also, it would be worth mentioning the reasoning behind running the NO2- oxidation and NO3- uptake experiments for very different incubation periods?
Response: We agree that a linear fitting of 3 to 4 timepoints may have been a more appropriate methodological approach and one that we will endeavour to adopt in future. However, given the number of experiments conducted during this study, such an approach was unfeasible. Additionally, the main purpose of this research was to investigate whether NO_2^- oxidation in the winter Southern Ocean followed a Michaelis-Menten curve. In the case of these Michaelis-Menten experiments, the goal was to stimulate the oxidation rates via higher and higher additions of substrate. For the vertical profiles (to which we believe the reviewer's comment refers) we go to great lengths in the manuscript to address the issue of potential overestimation of the oxidation rates due to the high concentration of ^{15}N-nitrite added, demonstrating that nitrite oxidation rates can indeed be quite strongly affected by the addition of concentrations of ^{15}N-nitrite tracer in excess of the K_m for nitrite oxidation. This is exactly the reason why we calculate corrected nitrite oxidation rates. We will add a sentence to the amended manuscript explaining how collecting just T_0 and T_f samples is a potential limitation.
As to the different incubation periods for nitrite oxidation and nitrate uptake – to some extent, this is experimental convention (e.g., Lipschultz 2008). Experiments involving phytoplankton nutrient uptake can be compromised by the regeneration of the substrate pool if the incubation length is greater than a few hours – this is particularly problematic for ammonium, and potentially also for CO_2 (especially at night when regeneration processes are dominant). So, keeping the incubation time short helps to reduce isotope dilution and by extension, underestimation of the rates (see Mdutyana et al. 2022 for a detailed discussion of this issue). In some of our previous work we have incubated phytoplankton for 24 hours and been subject to extensive criticism from reviewers (e.g., Mdutyana et al. 2020 – notice the numerous caveats regarding potential isotope dilution due to regeneration). For nitrification, the rates are typically so low that 24 hours are required for enough tracer to be transferred to the product pool to make a reliable measurement. Additionally, there was no light/dark cycle to worry about since the bottles were incubated in the dark. Nonetheless, we will add appropriate references to the best practices for these two different experiments to the amended version of the manuscript and ensure that the differences between them are clear.

Line 237: did you directly determine the fraction of the nitrite pool labelled with 15N i.e. concentration measurements before/after addition – this is not clear in the methods as currently written.
Response: Nitrite concentration were determined ashore, therefore the fraction of the nitrite pool labelled with ^{15}N was not known before ^{15}N tracer addition but was known (and used) at the time of rate calculation. The wording in the manuscript will be amended to remove ambiguity as follows: "$f^{15}_{NO_2^-}$ is the fraction of the NO_2^- substrate pool labelled with ^{15}N at the start of the incubation, **calculated following the direct measurement of the ambient NO_2^- concentrations**".

Line 362: directly related to my comment above, were these nitrite concentrations measured or assumed? This needs to be clearly documented in the methods section.
Response: Please see the response above, addressing this issue.

Line 440 to 444: this is a nice point, but it belongs in the discussion.
Response: As per the reviewer's suggestion, we will move this sentence to the discussion.

Line 486: 'low ambient NO2-' can you be quantitative here? Also, how applicable are these 'low' concentrations to the rest of the ocean.

Response: We will amend the text as follows: "Our focus is on the K_m values derived under conditions of low ambient NO_2^- **(i.e., <250 nM)** given that (some of) the environmental factors affecting NO_2^- oxidation at high ambient NO_2^- concentrations appear to be unique. For example, oxygen has been shown to decrease the rate of NO_2^- oxidation in the ODZs (Sun et al., 2017, 2021) where novel clades of NOB have been detected (Sun et al., 2021). **Additionally, NO_2^- concentrations in the oxygenated open ocean seldom exceed 250 nM (Zakem et al. 2018).**"

Line 598: I would add in here as you have in the figure caption that this relationship is only for the euphotic zone. With the kinetics experiments only be conducted with surface waters the question remains, how applicable are these numbers/thresholds to deeper waters in the euphotic zone (where the community might be different, different light conditions etc), and while the authors do point this out in the manuscript, I think it also needs be articulated in this section as well and the potential impacts on the conclusions discussed.

Response: We will clarify in the text that this relationship is only for the euphotic zone as follows: "While we have no iron data with which to compare our kinetic parameters, dissolved iron concentrations ([DFe]) were measured **throughout the euphotic zone** at the depth-profile stations…"

We will also add some text to this section regarding the applicability of our findings for the euphotic zone/mixed layer more broadly – for instance, it is possible that the surface experiments were, in some cases, more iron limited than would be nitrifier communities deeper in the euphotic zone given if the iron concentrations were lowest at the very surface. That said, the mixed layer of the winter Southern Ocean, which more than encompasses the euphotic zone, is typically well- (and vigorously) mixed, as is evident in the near homogenous distributions of nutrients (Fig 1b-c), including trace metals (Cloete et al. 2019). While there are no measurements of nitrifier community composition from our region, one would expect them to also be fairly homogenously distributed over the mixed layer because of the strength of mixing in winter. The one non-homogenous parameter, as the reviewer points out, is light, which would have been considerably different (i.e., higher) at the surface than deeper in the euphotic zone. One might thus expect higher rates of nitrite oxidation deeper in the euphotic zone. Our depth profiles of the nitrification rates (Figure 3b-e) do not clearly show this trend, however, instead remaining fairly homogenous over the euphotic zone and only rising considerably (when they do rise) at the base of the mixed layer.

Line 612 / Section 4.3: I largely enjoy this section, there are some really nice discussion points, but in a few places this section becomes a little like a literature review and could benefit from some streamlining to focus on your findings.

Response: We thank the reviewer for the suggestion. In responding to the comments of reviewer 1 regarding this section, we will remove quite a lot of the text in order to limit speculation and clarify our arguments. As such, the section will become significantly more streamlined in the amended version of the manuscript. In net, we will remove approximately 750-1000 words. Please see our response to reviewer 1 above for specifics.

Line 666 (Figure 6): is it the revised rates that are shown? In panel b are the error bars smaller than the symbols?

Response: Yes, these figures are produced from the revised rates, as is indicated on the figure and in the caption. Error bars will be added to panel b as requested.

Line 694: why particularly NOB?

Response: The effect appears not be the direct result of dilution but rather the variable response of AOO versus NOB following dilution due to mixing. We will amend the sentence as follows: "In coastal waters, deep winter mixing has been shown to dilute the nitrifier community, **with AOO subsequently recovering more rapidly than NOB**. **This differential rate of recovery has been hypothesized to result in a period of low** NO_2^- oxidation rates while NH_4^+ oxidation rates remain elevated, ultimately causing NO_2^- accumulation in the upper layer (Haas et al., 2021)".

Line 744: It is not clear how this line of discussion on life strategies links to a potential explanation for the decoupling observed.
Response: We agree with the reviewer that this text is speculative and does not further our arguments. We will remove the text from the amended version of the manuscript.