Review of Schulz et al.

**General comments**

The authors addressed some of my concerns during their revisions. However, I am still a bit dissatisfied with the quality of their oxygen measurements, which should have been more carefully done *in-situ*, and also during their incubations. Denitrification and anammox are highly dependent on oxygen concentrations, even at nanomolar levels. I do not quite understand why they would observe such a high offset (+13 $\mu$mol $L^{-1}$) between Winkler titrations and their optical CTD sensor. Were Winkler titrations performed at all depths? Oxygen concentrations are such important measurements for their study, it is a pity that more attention wasn't dedicated to calibrating their CTD sensor better. Second, I would like to see a more thorough assessment of how important gases ($O_2$, $H_2S$) influence nitrogen loss. They added a short discussion in their manuscript, but perhaps back of the envelope calculations would be more helpful to understand the effect of purging with He on the concentration of these gases. In the case of $H_2S$, a reduction of 80% after purging is quite a lot and is most likely to affect chemoautotrophic nitrogen loss ($H_2S$ oxidation coupled to $NO_3^-$ reduction). As was pointed out by reviewer #2, I think the authors need to be more careful in interpreting these rates at the larger scale, especially since it was recognized that rates were stimulated due to substrate limitation during the $^{15}N$-labeled incubations. These are **potential** rates that provide information about processes; hence these rates should be interpreted with caution and the limitations of this study should be better acknowledged. I agree with reviewer 2 that the authors should focus on the importance of different processes (denitrification versus anammox) rather than trying to compare their rates to the full nitrogen budget in the mesocosms (any strong agreement between the two is likely fortuitous). I also agree with reviewer #2 that $NO_2^-$ cannot be deemed to be "more important" than $NO_3^-$ for denitrification. The substrate addition during $^{15}N$-labeled incubations relative to *in-situ* concentrations would generally be higher for $NO_2^-$ than $NO_3^-$, which could further stimulate rates. The authors need to cite relevant study that observed clear discrepancies between $^{15}N$-labeled rate measurements using both $NO_2^-$ and $NO_3^-$ and perhaps briefly discuss these differences. On this note, I do not think the authors understood my argument regarding the production of excess $^{29}N_2$ during $^{15}N$-$NO_2^-$ labeled incubations (see Chang et al. 2019 paper). This process needs to be discussed in the manuscript (in connection with their data). Finally, there were quite a few typos in the original version of the manuscript and quite a few typos also were introduced during revisions (see below). The authors should be more careful when revising their manuscript.
Despite all these concerns, I still think that this is an interesting and novel study, as few such mesocosm experiment exist (if at all) for the ETSP ODZ.

**Specific comments:**

Line 40: Adding "of deep waters" after frequency does not make sense as they are referring to upwelling frequency.

Lines 46-48: at least one reference needs to be added at the end of this sentence.

Lines 63-64: What were measured $H_2S$ concentrations using this sensor for incubated waters? That could help address the He purging issue if $H_2S$ concentrations were negligible.

Lines 77-82: This section is still a bit vague. Oxygen concentration is very important in controlling nitrogen loss rates. Were oxygen concentrations also measured at all depths using Winkler titrations? If so, I think these values should be used instead if they experienced issues with their CTD sensor calibration. I don't think it is sufficient to say: "Hence oxygen concentrations …. are likely to have been significantly lower". How much lower? Are they sure that conditions were truly anoxic, and conducive to nitrogen loss?

Line 86: Remove "However" at the beginning of sentence.

Line 103-106: Also cite the new manuscript by Bourbonnais et al. (2020) in Frontiers that describes these types of incubations in detail as well as provide calculation templates: Bourbonnais, A, C. Frey, X. Sun, L. A. Bristow, A. Jayakumar, N. E. Ostrom, K. L. Casciotti, and B. B. Ward. (2021), Protocols for assessing transformation rates of nitrous oxide in the water column, *Frontiers in Marine Science* 8, 293.

Lines 102-103: I think this sentence is ambiguous. Change to "Our calculations have shown that exchanging the bottle volume at least 24 times is required to reduce the $O_2$ concentration to less than 20% of *in-situ* conditions.

Line 103: "Observered" is misspelled!

Lines 113-115: This sentence is a bit vague. Could they provide a back of the envelope calculation to better estimate how He purging would affect $H_2S$ concentrations? This is important to assess the role of chemoautotrophic versus heterotrophic denitrification.

Lines 118-122: This sentence is too long – I suggest breaking in two.

Line 125-131: Why was $^{30}N_2$ noisy? I think that their rates are high enough to get a good $^{30}N_2$ signal. Calculating denitrification rates using mass 29 can be problematic as other studies reported production of excess $^{29}N_2$ that could not be accounted by assuming binomial distribution (after considering the contribution from anammox). I strongly recommend the authors to read de Brabandere et al. (2013) and Chang et al. (2014) for more information regarding this process. These authors attributed the production of excess $^{29}N_2$ during $^{15}N\text{-}NO_2^-$ incubations to "nitrite shunting" where unlabeled $NO_3^-$ is converted to $N_2$ completely intracellularly without exchange with the external ambient $NO_2^-$ pool. The paper by Chang et al. (2019) needs to be discussed/cited.
Chang, B. X., Rich, J. R., Jayakumar, A., Naik, H., Pratihary, A. K., Keil, R. G., ... & Devol, A. H. (2014). The effect of organic carbon on fixed nitrogen loss in the eastern tropical South Pacific and Arabian Sea oxygen deficient zones. *Limnology and oceanography*, *59*(4), 1267-1274.

Lines 168 and 177: Remove the word "please"

Lines 226-230: I think this similarity is fortuitous since only **potential** rates are measured using $^{15}N$-labeled incubations.

Lines 261-263: I do not think complete nitrogen loss occurs at such high $O_2$ concentrations (30-40 μmol $L^{-1}$).

Lines 269-278: It would have been best to construct the Michaelis-Menten curves as in Michiels et al. (2019) or use published Michaelis-Menten parameters (for the same or similar environments – as published in Michiels et al. (2019)) to estimate denitrification rates at *in-situ* $NO_2^-$/$NO_3^-$ concentrations (rather than using a maximum nitrogen loss rates based on nutrient concentrations). The authors did not well address this point in their response to my previous review.

Line 286-287: Would that rather be an upper boundary estimate (relative to true environmental conditions), since orni-eutrophication and using maximum-sustainable denitrification rates would artificially increase their nitrogen loss rate estimates?

Line 343: Change to "over-consumption"

Lines 364-375: The authors need to acknowledge that purging with He before their $^{15}N$-labeled incubations would reduce the $H_2S$ concentrations. Hence, these rates should be interpreted with caution.