Response to Reviewer #1

We thank Reviewer #1 for their insightful comments and suggestions. Below please find our detailed point-by-point response.

General Comments

1. '... While this study is worthy of publication, I do have some concerns, mostly regarding purging of the samples during the 15 N-labelled incubations, which removes H_2S - an electron donor during chemolithoautotrophic denitrification. Furthermore, since mostly all O_2 is removed during purging with He prior to incubating the samples, their measured rates are not representative of in-situ conditions (O_2 concentrations in bottom waters were generally higher than 20 $\mu molL^{-1}$, which is too high for N_2O conversion to N_2....'

RESPONSE: We have addressed the issues raised by the reviewer in the specific responses below.

Specific comments of Reviewer #1

Line 9: This was in contrast to realized rates in the surrounding Pacific". This sentence is unclear as the author later claim that rates measured as part of the mesocosm experiment and in surrounding waters were comparable.

RESPONSE: We have clarified this statement with 'In the surrounding Pacific measured denitrification rates were similar, although no indications of substrate limitation were detected.'

Lines 39-43: This sentence about ocean acidification seems a bit out of place since the connection with OMZs and nitrogen cycling is not clear. I suggest removing.

RESPONSE: We have clarified the connection by adding the following 'Changes in upwelling frequency/intensity, oxygen availability, temperature and pH can influence planktonic food web functioning in EBUS, with repercussions for nitrogen loss processes.'

Line 73: How long were the samples stored before analysis?

RESPONSE: It was about 2-4 hours. This information has been added.

Line 74: O_2 concentrations should ideally be monitored during 15N-labelled incubations using non-invasive O_2 measurement technology .... This is also to ensure that no O_2 is infiltrating during the incubations from the stoppers.

RESPONSE: We did not monitor the oxygen inside the exetainers in this experiment. However, data from other very similar exetainer experiments, in which O_2 was monitored with the Lumos sensors (Sun et al. 2020), show that the O_2 level varied between essentially zero and up to 100 nM over a period of two days. Even that highest concentration is below the lowest thresholds reported to inhibit anammox and denitrification (>200 nM Dalsgaard et al. 2014; see discussion in the manuscript, section 4.2.2).

Line 75: I think such a high O_2 offset is problematic and not representative of anoxic coastal

waters off Peru – where O_2 concentrations are generally well below 10 μM. I suspect that O_2 is also introduced during sampling from a Niskin bottle (de Brabandere et al.. 2012). Furthermore, O_2 in the nanomolar range has been shown to influence N_2 production rates (Dalsgaard et al., 2014). Since their samples are purged with He, the estimated rates are only putative and likely not representative of real in-situ conditions. I understand the limitation, but in-situ incubations would be preferable.

RESPONSE: The reviewer is correct, it would be wonderful to be able to do in situ incubations in order to assess true rates of denitrification and anammox. Unfortunately, at present, we are not aware of any realistic way to do that. The in situ incubation methods are cumbersome and allow at most a few measurements per day (e.g., Collins et al. 2018, Ward et al. 2019), and at present would be difficult to deploy in a mesocosm and hard to scale up to the numbers of experiments required for the experimental design used here. The non incubation approach of measuring in situ gas concentrations (gas tension device, Reed et al. 2018) has great attractions. This method was published after the study under review had been completed. The GTD has not been widely used yet, but based on the presentation by Altabet et al. at the Ocean Sciences meeting in 2020, it promises to be very useful on the oceanographic scale, although with substantial assumptions and constraints. So much as we would like to, making in situ rate measurements in the mesocosms is not yet feasible.

The purging approach reduces the gas concentrations for all gases prior to the incubations (except helium), including $N_2, N_2O, O_2, CO_2, H_2S$, etc. By lowering the oxygen concentration, purging may enhance the rates of anaerobic processes such as denitrification and anammox. But as observed by de Brabandere et al. (2013), the fact that the processes occurred in anoxic incubations in water collected from oxic layers indicates the presence of viable populations of microbes capable of those processes. That implies the definite potential for in situ activities.

We have also re-calculated oxygen concentrations as measured by the optical CTD sensor by applying a 1 second response time hysteresis correction as described in Fiedler et al. (2013), rather than just mentioning the issue that 'raw' CTD data will over-estimate oxygen concentrations in a particular depth during a down cast, when moving from high to low oxygen concentrations. Now, corrected oxygen concentrations at the sampling depth are hovering around 20 $\mu\mathrm{molL}^{-1}$ for most of the time. Together with the +13 $\mu\mathrm{molL}^{-1}$ offset in comparison to Winkler titrations in these samples, this suggests that in-situ oxygen concentrations reached indeed below 10 $\mu\mathrm{molL}^{-1}$, being representative for anoxic coastal waters off Peru.

Lines 80-82: Why is this treatment referred to as a moderate treatment if the N:P values between this and the extreme treatment are similar? I would rename this treatment as it is clearly not representative of moderate N loss conditions.

RESPONSE: We have re-named the treatments to 'low N/P' and 'very low N/P', respectively, which will also keep it consistent with the terminology introduced in the accompanying paper by Bach et al. (2020).

Lines 82-83: How did they prevent gas exchange and minimize O_2 contamination in these

waters during collection/injection? As this is an important detail for their experiment, a brief explanation should be added here (without having to refer to the Bach et al. (2020) paper).

RESPONSE: We have added the following piece of information: 'The deep waters were collected into 100 m^3 bags without headspace at the respective depths and sealed once brought back to the surface. Deep water was added by first removing about 20 m^3 from each mesocosm and replacing it with the respective deep water that was injected into the bottom layer between 14-17 metres on day 11, and the surface layer between 1-9 metres on day 12. To minimise changes to deep water gas concentrations during injections, water was pumped from two metres depth out of the deep water bags.'

Lines 87-88: Why not use 15 N-labeled $\mathrm{NO_3^-}$ to measure denitrification rates? Nitrate concentrations are generally higher than $\mathrm{NO_2^-}$ and $\mathrm{NH_4^+}$ (thus, the substrate is less limiting).

RESPONSE: Previous experience has shown repeatedly that lower rates of $\mathrm{N_2}$ production result from parallel incubations with $^{15}\mathrm{NO_3^-}$ vs $^{15}\mathrm{NO_2^-}$. We have interpreted that difference to the exchange of $\mathrm{NO_2^-}$ as an intermediate in the complete denitrification pathway, i.e. $^{15}\mathrm{NO_2^-}$ produced from $^{15}\mathrm{NO_3^-}$ is diluted with residual $^{14}\mathrm{NO_2^-}$ in the medium and reduces the amount of label that makes it all the way to $\mathrm{N_2}$. Thus, the rates measured from $^{15}\mathrm{NO_2^-}$ are a better estimate of the actual rate of $\mathrm{N_2}$ production. The fact that we routinely detect $^{15}\mathrm{NO_3^-}$ reduction (as $^{15}\mathrm{NO_2^-}$ production) in these same incubations shows that $\mathrm{NO_2^-}$ dilution does indeed occur.

Line 89: Why 3 $\mu\mathrm{molL^{-1}}$? It seems to be a bit arbitrary.

RESPONSE: 3 $\mu\mathrm{molL^{-1}}$ tracer addition is pretty standard. For $\mathrm{NO_2^-}$, in particular in ODZ conditions, it is a level commonly occurring in natural waters, so does not represent a big perturbation, but ensures enough substrate to obtain a signal in the product. At the beginning of the experiment similar concentrations were present in the bottom layer. However, being closed systems, $\mathrm{NO_2^-}$ concentrations decreased over time, so the tracer addition could have stimulated measured rates, in particular after the N-depleted deep water addition. This is consistent with the statements in L134-138 (original MS) about the measured rates exceeding the rates that would be possible at in situ substrate concentration.

Lines 86-93: On a cautionary note, other studies (de Brabandere et al., 2013; Chang et al., 2014), observed that more $^{29}\mathrm{N_2}$ is sometimes produced than could be accounted for assuming a binomial distribution after taking the production due to anammox. They propose that "nitrite shunting" where $\mathrm{NO_3^-}$ is converted to $\mathrm{N_2}$ completely intracellularly without exchange with the external ambient $\mathrm{NO_2^-}$ pool could lead to that $^{29}\mathrm{N_2}$ excess. I am curious to know if such $^{29}\mathrm{N_2}$ excess was also observed in this study. Using $\mathrm{NO_3^-}$ as a tracer could help to account for this process.

RESPONSE: As we have only made incubations with labelled $\mathrm{NO_2^-}$ we have no comparison, or binomial expectation. Please also refer to our response to the Lines 87-88 comment.

Lines 93-101: I understand that purging with He is necessary since these are anoxic incubations, but since $\mathrm{H_2S}$ was present in bottom waters, removing all gases (including $\mathrm{H_2S}$) would underestimate chemoautotrophic denitrification. I strongly recommend complementation of

stripped gases involved in N cycling metabolisms.

RESPONSE: The reasoning behind He purging is to reduce background $^{14}N_2$ concentrations (to more easily pick up label incorporation into the N_2 produced during denitrification or anammox), not to strip the solution off dissolved gases. And indeed, we saw in earlier studies that we can observe massive rates of chemoautotrophic denitrification using this approach (Kalvelage et al., 2013), suggesting that a significant portion of dissolved gases such as H_2S is retained (please also see our response to the next comment). Hence, this method doesn't appear to necessarily underestimate rates resulting from He bubbling.

Line 99: What is the recommended flow rate? I assume purging time is adjusted to leave enough background N_2 for GC-IRMS measurements?

RESPONSE: As mentioned previously, the idea of purging is to reduce background N_2 in order to enhance the ability to detect the small isotopic signal of the labelled N_2 produced during the incubation. Since N_2 concentration doesn't vary much in seawater at the level we can detect with these methods, we used a standard flow rate (monitored by assuring 1 – 2 psi at the exetainer level, i.e. 3 psi at the cylinder level) and purging time, previously calculated to assure a specific replacement volume for the exetainers (unpublished data). At this pressure and with 16 exetainers being purged at the same time, the volume would get replaced about 20 times. This is lower than the 24 times reported in Holtappels et al. 2011 (a total of 15 min at a flow rate of 0.4 L min^{-1} and a serum bottle volume of 250 ml), which reduced oxygen concentrations by about 20%. A similar reduction would then be observered for most other gases, except those buffered by conjugate acid-base pairs, such as H_2S or CO_2, for which the reduction would be even less.

To clarify the influence of He sparging on incubation gas concentrations we have added the following to the methods section: 'To reduce large background $^{28}N_2$ levels and facilitate detection of the small isotopic signal of labelled N_2 being produced during incubations, ... Based on previous calculations and measurements, such setup will replace about 20 times the volume of each exetainer (unpublished data). This is lower than the 24 times, reported to ensure that the reduction in O_2 concentration is less than 20% compared to in-situ conditions (Holtappels et al. 2011). A similar reduction would also be observered for most other gases (Wanninkhof 1992), except those buffered by conjugate acid-base pairs, such as H_2S or CO_2, for which the reduction would be even less.'

Lines 102-103: At what temperatures were samples equilibrated?

RESPONSE: Samples were equilibrated at room temperature. This is now mentioned in the text.

Lines 110-114: This approach could potentially affect their rate calculations if "nitrite shunting" produces $^{29}N_2$ excess. See above comment (lines 86-93).

RESPONSE: Please see our response to the previous comment(s).

Lines 113-115: In figure A1, it seems like there might be an exponential increase from time

point 6 hrs and 20 hrs. In this case, I would only use the linear portion for rate calculations. Is this observed for all other incubations? It would have been useful to obtain another time points somewhere in between (at 12 hrs) to better disregard this possibility.

RESPONSE: Given the clear increase between 0 and 2 hours, we would rather argue for the lower 7 hour data point to be off. Furthermore, deviations from a linear increase over the entire incubation period was rather observed at times to be resulting from a lag phase during the first 2 hours, as acknowledged in the figure caption. We agree that having an extra time point between 8 and 20 hours would have been ideal in hindsight, but there are always logistical constraints. Furthermore, having the majority of samples in the first half of the incubation proved to be quite valuable as the lag phase issue could be clearly detected and accounted for, when necessary. Finally, restricting the incubation period to just under one day ensured to avoid what is usually considered to introduce potential bottle-effects. We will make reference to the latter issue in the methods section.

Lines 134-138: A more correct approach would be to construct Michaelis-Menten curves and calculate the half saturation constants and maximum denitrification rates from the measured in-situ rates (see Michiels et al., 2019).

RESPONSE: There is surprisingly little information on the dependence of denitrification on the concentration of nitrate or nitrite. This is probably because denitrification is often shown to be limited by organic matter concentration in oceanic systems. Again, in the coastal mesocosms, this may not have been the case – OM may not have been the limiting factor – and as noted above, the tracer addition could have stimulated measured rates. Lacking direct experiments on the MM kinetics of denitrification in this system, the approach used here is probably a reasonable compromise.

Lines 146-147: Change to: "24 hrs per day x 38 days x 2 (conversion between N2 to N) divided by 3 (contribution of bottom layer water to overall mesocosm volume), ..."

RESPONSE: We agree and have made the suggested changes.

Lines 189-191: The oxygen concentrations shown in Fig. 3E are generally above 20 $\mu\mathrm{molL}^{-1}$, which would be too high for $\mathrm{N_2O}$ conversion to $\mathrm{N_2}$ (see Dalsgaard et al., 2014 and Frey et al., 2020). Therefore, their measured rates are potential and denitrification was likely only observed because the samples were purged with helium - removing mostly all $\mathrm{O_2}$.

RESPONSE: We agree, like in any assay incubation, measured rates should be taken with a grain of salt when extrapolating to in-situ conditions. Please also see our replies on oxygen response time hysteresis correction (suggesting oxygen levels below 10 $\mu\mathrm{molL}^{-1}$ for most of the experiment), as well as helium purging.

Line 194: I don't think it makes sense to call this a "moderate" treatment (see above comment lines 80-82).

RESPONSE: As suggested, we have changed the terminology.

Lines 199-200: Again, these relatively high $\mathrm{H_2S}$ concentrations indicate that chemoauto-

trophic denitrification might be an important process that was not measured (since samples were purged with He before the incubations).

RESPONSE: Please see our response to the Lines 93-101 comment. Furthermore, please also see our response to the L226 comment from reviewer #2 on nitrite and organic matter being the main drivers of denitrification.

Lines 211-214: The near perfect agreement between the two approaches is a bit surprising considering that measured rates are potential and likely not representative of in-situ conditions.

RESPONSE: Good point. We have wondered about that ourselves. Considering the many caveats for incubation-based rate measurements, how can they make so much sense in comparison to processes estimated from several other independent in-situ measurements? The only conclusion we can come up with is that the rates measured in the essay incubations must have been similar to what was happening in-situ.

Line 266: The calculated overall nitrogen loss could also be overestimated since in-situ denitrification rates were likely lower. Samples collected in the mesocosms and surrounding Pacific waters were purged before the incubations, removing mostly O_2 and thus creating conditions more conducive to N_2 loss. The O_2 concentrations observed in bottom waters were too high for N_2O conversion to N_2 (see Dalsgaard et al., 2014).

RESPONSE: Please see our responses to a number of comments above, in particular the one on the new CTD-oxygen-optode response time correction - Line 75 comment.

Lines 313: Why did C/N values not increase in that one mesocosm?

RESPONSE: Deep water additions were followed by a bloom of the dinoflagellate *Akashiwo sanguinea*, fixing carbon without significant net nitrogen assimilation, in all except this one mesocosm. We now mention this fact, which is described in more detail in the accompanying Bach et al. (2020) paper, in the discussion.

Lines 319-321: It is also possible that the measured DON pool was mostly recalcitrant, with fast cycling of labile DON.

RESPONSE: We agree and, as stated in the text, it would require preferential N over C remineralisation.

Lines 340-341: Denitrification/anammox linked to microenvironments around particles would not be captured by 15N-labeled incubations, especially if these are not performed in situ.

RESPONSE: As the incubation seawater was not filtered and hence contained particles, microenvironments around those are likely to have been re-established during the 20 hours of incubation.

Lines 343-345: It is unclear how H_2S would inhibit anammox in their incubations, since samples were purged (hence H_2S was removed).

RESPONSE: Please see our responses to various comments above.

Lines 350-353: It would be relevant to include these data (i.e., anammox functional marker gene hzo) in the manuscript.

RESPONSE: Unfortunately, no genomic data can be presented at this stage, as of ongoing Nagoya Protocol negotiations. However, as there is no discrepancy between our rate measurements and described gene abundance observation, there shouldn't be a need to explicitly show the latter.

Line 375: Why is the contribution from the Arabian Sea, where significant N-loss occurs, not taken into account here?

RESPONSE: We now include reference to the Arabian Sea and Bay of Bengal in a revised version of our manuscript. This does not change the main findings and conclusions of our calculations.

Lines 376-379: I don't think there is anything new in this statement. Due to the large uncertainties associated with these estimates, it is still unclear if the majority of the N-loss occurs in the water-column or sediments.

RESPONSE: The discussion in the last (now second-last) paragraph is only about water column denitrification, i.e. a comparison of globally assembled in-situ estimates with our mesocosm-derived measurements.

Lines 379-380: Why is export production projected to decrease if upwelling intensity and frequency (and thus nutrient supply) is expected to increase (Hauri et al., 2013 and Wang et al., 2015 papers cited in the introduction)?

RESPONSE: This is a valid point raised by the reviewer. We are now more specific here and explain that projected reductions in global export production are thought to result from changes to community structure. Furthermore, in regards to export production in ODZs and OMZs, as of a potentially counter-acting increase in upwelling intensity and frequency, we are now more cautious about the expected sign of change.

Table 2: I would rename the "moderate" treatment to "extreme" since the degree of N-loss is similar in both treatments. It is odd to express individual N-budgets for each mesocosms as negative values and present the mean as a positive value. I suggest renaming these columns N-loss from 15N-labelled incubations and N-loss from N-budget.

RESPONSE: We agree and have changed the terminology to 'low' and 'very low' N/P. We have also changed the N-budget mean to a negative number to match the individual mesocosm values.

Figure 2: What was bottom depth at the mooring site?

RESPONSE: The depth at the mooring site was between 25 to 30 metres. This information has been added to the figure caption.

Figure 1A: It is difficult to tell if the last time point around t = 20 hrs represents an exponential

increase (as often observed for 15N-labelled incubations).

RESPONSE: Please see our response to the Lines 113-115 comment.

Technical corrections

Line 49: define nm (i.e., nautical miles).

RESPONSE: We agree.

Line 63: change acoording to "according"

RESPONSE: We have.

Response to Reviewer #2

We thank Reviewer #2 for their insightful comments and suggestions. Below please find our detailed point-by-point response.

General Comments

1. It would help if the authors identified more clearly the goal of the study. They say it was to "quantify the importance of nitrogen loss processes," but that's a bit vague.

RESPONSE: In an ideal world we would have been able to assess nitrogen loss processes following the upwelling of two much more distinct deep waters, in terms of their N-deficit. We had identified waters at two locations but, unfortunately, by the time we did collect them, their signatures were quite similar. Hence, the focus of this paper is to more generally 'quantify the importance of nitrogen loss processes in overall nitrogen cycling following simulated deep-water upwelling in the Humboldt Current System'. This is a first-time study and the unique closed-system mesocosm budgeting approach has revealed interesting conclusions that fit broader scale in-situ observations.

2. The paper has a lot about the nitrogen budget and about comparing the mesocosms to the real Pacific, but I think all that should be minimized. The mesocosms were contaminated by birds and the added 15N apparently stimulated rates.

RESPONSE: It appears that there is a misunderstanding in regard to the mesocosm nitrogen budget and comparisons with the surrounding Pacific. The onset of the bird faeces contamination was day 40. Hence, we have restricted the budget calculations to the first 38 days of measurements and excluded the rest. Concerning 15N label stimulating measured nitrogen loss rates, in particular heterotrophic denitrification, this study is not the first to make such observation. This can happen at relatively low dissolved inorganic nitrogen and high organic matter availability. We did account for this observation (when making the direct comparison of the full nitrogen budget with rate measurements in the mesocosms and extrapolations to the Pacific) by using 'maximum sustainable rates', derived from in-situ dissolved inorganic nitrate concentrations rather than maximum attainable rates measured during incubations.

3. I think the authors should concentrate on comparing denitrification vs. anammox. As mentioned below in more detail, they don't address why their rates of anammox were low compared with previous studies and why anammox apparently was lower in the mesocosms than in the real ocean.

RESPONSE: We have strengthened the two and a half pages of discussion of the denitrification vs. anammox findings (see detailed comments/responses below), and also directly addressed this issue by adding the following statement in the abstract: 'Both in the mesocosms and the Pacific Ocean anammox made only a minor contribution to overall nitrogen loss when encountered, potentially related to organic matter C/N stoichiometry and/or process specific oxygen and hydrogen sulphide sensitivities.'

Specific Comments

L8: I think "actual" is better than "realized."

RESPONSE: We agree and have made the suggested change.

L9: I suggest removing the comparison of rates in the mesocosm with rates in the real ocean.

RESPONSE: Please see our response to general comment #2.

L28: Note the misspelling, "denitriciation."

RESPONSE: Thank you for pointing out this typo, it has been changed.

L40: Higher temperature explains oxygen loss in the upper water column, but only accounts for about half of the loss in deeper waters.

RESPONSE: We have added the following clarification: 'Furthermore, due to increasing temperatures the ocean looses oxygen (O_2) and OMZs are expanding (e.g. Bopp et al. (2002); Bograd et al. (2008); Stramma et al. (2008); Oschlies et al. (2017)). Together with changes to microbial activity, this modifies biogeochemical properties of upwelled waters including, next to O_2, carbonate chemistry speciation...'

L43: Missing a word like "waters."

RESPONSE: We have added the term 'deep waters'.

L74: and elsewhere: "$\mathrm{umolL^{-1}}$" should be "$\mathrm{umol\ L^{-}1}$"—a space between umol and L.

RESPONSE: Thank you for pointing out this oversight, we have made the necessary changes.

L83: Rather than emphasizing N:P ratios, I think the authors should emphasize that the extreme condition had unmeasurable NO3 and NO2 and a more negative N* than the moderate condition.

RESPONSE: We have clarified the text by the following statement: 'However, both waters had a quite strong N-deficit (N^*), in comparison to a typical N/P of 16/1 required for phytoplankton growth (Redfield et al., 1963; Brzezinski, 1985), and will be referred to as 'low N/P'

and 'very low N/P' treatments in the following (compare Tab. 1).'

L93: Rather than "aka DNRA", the authors should just define DNRA. It's defined much later in the paper, but it should be here when the abbreviation is first used.

RESPONSE: We have made the suggested change.

L95: Note the misspelling here, "failry." I will stop noting other misspelling that the spellchecker on Word or other word processing programs would catch. The authors should assume the journal won't do much copy editing.

RESPONSE: We apologise for yet another typo and have thoroughly checked the revised version.

L143: Rather than "orni-eutrophication," I suggest "avian eutrophication."

RESPONSE: This term was introduced in the accompanying paper by Bach et al. (2020), hence we are inclined to keep it, for consistency.

L180: Fig A3 seems to be referred to before Fig A1 and A2, which is not standard practice.

RESPONSE: The order should be alright, as A1 and A2 are referred to before A3, on L118 and L148 (new ones).

L204: The authors emphasize that the "theoretical" sustainable rate of denitrification is based on changes in NO3+NO2 concentrations. But what about nitrification supplying NO3+NO2? The authors seem to imply nitrification didn't occur because of the lack of oxygen, but the gas was measurable, perhaps at levels high enough for nitrification.

RESPONSE: The reviewer makes an important point. Nitrification has indeed been found to operate at the low micro-molar (and even nano-molar) levels observed in our study (Bristow et al. 2016). And it appears that at such oxygen levels there is cyclic nitrogen turn-over by nitrite oxidation followed by nitrate reduction, not contributing to nitrogen loss via N_2 (Babbin et al. 2020), further complicating the picture. However, nitrification (ammonium oxidation) rates measured in Bristow et al. 2016, and by Peng et al. 2016 and Santoro et al. 2021 were usually at least an order of magnitude lower than measured denitrification rates in our study. Hence, it is unlikely that nitrification played a significant role in supplying nitrite for denitrification. We have added this piece of information to the discussion.

L226: The authors have a table and a very complex, four-panel figure (see below) about the multi-variable linear regression work, but all that is accompanied by two short paragraphs. That's an indication that the figure and the table are overkill. Readers will care only (if they do at all) about the best model, not the rest of the stuff given in the figure.

RESPONSE: We have simplified the figure by removing the second-best fit. We have also added to the discussion that the finding that the main drivers of denitrification were nitrite and organic matter availability suggest that heterotrophic denitrification rather than chemo-lithoautotrophic was the dominant N-loss process.

L227 and elsewhere: The authors shouldn't use "measured/maximum" because it's ambiguous. Which is it? The measured rate or the highest one? At the very least they should define what they mean, but I don't think the term should be used at all.

RESPONSE: We agree, this has been ambiguous. We have changed to 'measured/maximum-sustainable rates', making clear that in cases where substrate limitation was encountered, maximum-sustainable rather than measured rates were used for in-situ N-loss estimates.

L256: I think it doesn't make sense that NO2- is more important than NO3- in driving denitrification. This is worth a brief explanation, perhaps.

RESPONSE: As denitrification from NO_3^- to N_2 involves multiple and independent steps and organisms the correlation between N_2 production and a substrate concentration should become better the closer one gets to the end of this chain (Fig. 1). For example, there should be a perfect correlation between N_2O concentrations and N_2 production, and NO_3^- concentrations and their turn-over to NO_2^- are meaningless if the intermediate steps to nitric and nitrous oxide are blocked or constitute a bottle-neck. This also explains the finding that nitrate reduction to nitrite often exceeds the total rate of denitrification to N_2.

L303: The authors end this section with textbook stuff about denitrification vs anammox with a generalization about which can be observed in the absence of the other. I think much of this can be deleted and replaced a more critical discussion of their data.

The authors need to grapple with the more important and novel findings from their study: that anammox wasn't as high as measured in previous studies and that it wasn't as high (I don't believe) in their mesocosms than in the real ocean.

RESPONSE: We agree, that this section ends with textbook knowledge. It is basically setting the stage for the in-depth discussion on what could explain our denitrification/anammox observations in the following sections. Hence, we are inclined to keep it.

The discussion that follows over the next two sections is actually an attempt to explain why anammox wasn't as high as in many previous studies. Finally, we agree that anammox rates were equally low in the mesocosms and the surrounding Pacific, which is highlighted in the abstract as 'Both in the mesocosms and the Pacific Ocean anammox made only a minor contribution to overall nitrogen loss when encountered...'

L304: Not picked up by a spell-checker: it should be "absence," not "absences."

RESPONSE: Thank you for picking up this typo.

L297: What do the authors mean by "anammox dominance"? They didn't see that, and the theoretical maximum contribution by anammox is only 28%.

RESPONSE: We have clarified our point here by changing the sentence to: 'The reason for an anammox dominance in several studies mentioned above,...'

L306: This section about organic matter C/N should be deleted. The authors found a typical Redfield ratio, but then spend several sentences arguing against their data. The entire

paragraph doesn't add enough to the paper to be worth taking up space in the Discussion.

RESPONSE: We are not arguing against our data, but rather try to explain low anammox contributions to overall N-loss. High C/N ratios of organic matter being decomposed by denitrifiers would offer an explanation. And carefully examining the data at hand, a number of possibilities are identified why this might indeed have been the case.

L381: The paper ends very abruptly. I'm not a fan of ending papers with a summary, but it would be nice to see something about the implications of the authors' work for the Big Picture.

RESPONSE: We have added a more general final paragraph, reading: 'Nitrogen cycling in ODZs and OMZs currently plays a very important role in the overall marine nitrogen budget. However, the magnitude and direction of change in the actual nitrogen loss term in response to ongoing climate and ocean change (e.g. ocean stratification, acidification and/or changes in temperature and oxygen levels) is uncertain. This issue is further complicated by uncertainties in future primary productivity and organic matter export. For instance, depending on the representative concentration pathway, future export production could decrease as a result of changes to community structure (see Bindhoff et al. (2019) for details and refs. therein). In summary, future changes in upwelling intensity and frequency, as well as the other potential biotic and abiotic factors mentioned above, could change the nitrogen (im)balance in ODZs and OMZs, having a significant impact on the overall marine nitrogen budget.'

Table 1: Note that NO2- has just one negative charge—it's not NO2÷2.

RESPONSE: Thanks for spotting this typo.

Table 2: Data in this table can be used to make several comparisons, which complicates it: the moderate vs. extreme treatments, 15N rates vs. concentration changes, mesocosms vs the real ocean, and denitrification vs. anammox. I suggest the authors need to re-think the design of this table and use another format, break it up, or put some data in a figure.

If the table is kept, the formatting needs to be improved. Colors and ( ) to denote different types of data should be avoided because the main body of the table can't be understood without looking at the table caption, making the reader work harder than necessary.

I think it's important to give integrated rates for anammox vs. denitrification, so readers can evaluate how the two processes compared for the mesocosms versus the real ocean.

Finally, the overall average and its SD for all mesocosms and the Pacific Ocean are rather meaningless. The authors should report the average and error for the two types of mesocosms alone...

RESPONSE: We have re-formatted the table, as suggested by the reviewer, and removed colours, re-organised the mesocosms into treatments and calculated means and standard deviations separately to facilitate comparisons.

Concerning integrated individual rates of denitrification and anammox we have opted to sum both processes up as anammox was not encountered in most mesocosms and had only a minute

contribution to overall N loss in the others.

Table 3: The caption should say that the regression analysis was done to explain the rate of denitrification.

RESPONSE: We have made the suggested change.

Figure 1: This figure is more appropriate for a textbook or a review paper, not this paper. It should be deleted. Maybe one of the figures now in supplemental materials, such as Fig A2, could be upgraded to the main paper.

RESPONSE: We are inclined to leave the figure in, as it is helpful in understanding certain aspects of the discussion, for example the rationale behind our response to the L256 comment. This will be particularly useful for a non-expert reader.

Figure 3: The authors should say explicitly that M1-M8 are mesocosms. "Bottom" in all of the y-axis labels can be deleted and moved to the figure caption. The labels would be cleaner and easier to read.

RESPONSE: We now explicitly mention that M1-M8 refer to the various mesocosms. We are inclined, however, to keep 'Bottom' in the y-axis labels as a reader will immediately realise where samples were taken, without having to consult the figure caption.

Figure 4: Note that NH4- should be NH4+.

RESPONSE: Thanks for spotting this typo.

Figure 5: Most of this figure doesn't make sense to me, and it seems overkill. It should be deleted. A table summarizing the best model would suffice.

RESPONSE: We have streamlined and simplified the figure.

Figure A4 Explain the symbols and colors, etc. Don't force readers to work and go back to Figure 3.

RESPONSE: We have added a proper description of the colour-coding and symbols to the caption of figure A3 and then refer to it, i.e. all necessary information will be contained in the Appendix.