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 will strengthen the two and a half pages of discussion of the denitrification vs. anammox findings (see detailed comments/responses below), and will also directly address 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 will make 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 will be 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 will add 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 will add 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 shall make 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 will clarify 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 will make 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 will thoroughly check a 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 original L180.

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 will add 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 will simplify the figure by removing the second-best fit. We will also add to the discussion that the finding that the main drivers of denitrification were nitrite and organic matter availability suggest that heterotrophic denitrification rather than chemolithoautotrophic 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 will change 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^- concen-

trations 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...'

L204: 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 will clarify 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 will add 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 NO2ˆ2.

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 will re-format the table, as suggested by the reviewer, and remove colours, re-organise 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 will make 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 will 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 will streamline and simplify the figure.

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

RESPONSE: We will add 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.