

***Interactive comment on: “Vertical segregation among pathways mediating nitrogen-loss (N<sub>2</sub> and N<sub>2</sub>O production) across the oxygen gradient in a coastal upwelling ecosystem”***

**Referee report to version 2.**

Overall this is a major improvement since the last draft. I recommend acceptance after minor revision.

After reviewing the revised manuscript and the authors' response, I think there is one major problem that the authors should clarify: The inconsistency between N<sub>2</sub> production and gross N<sub>2</sub>O production with acetylene, which is demonstrated in figure 3d and 4d. Current understanding is that, N<sub>2</sub>O is an intermediate in the sequence of denitrification. When N<sub>2</sub> production is measured by nitrite reduction, N<sub>2</sub>O production with acetylene should have similar rates. This was not observed in the result; quite surprisingly, N<sub>2</sub> production and N<sub>2</sub>O production decoupled. The authors can add some comments in line 30, page 10.

Although we expected to obtain similar rates of N<sub>2</sub> and the N<sub>2</sub>O production by nitrite reduction without and with acetylene, respectively (Figs., 3d and 4d), a mismatch between these two products was observed that we cannot explain as denitrification is the only mechanism known to produce N<sub>2</sub>O in the presence of acetylene.

Three minor issues concerning the nitrite production experiments that I would hope the authors clarify.

First, in section 3.3.3 (page 10-11), the authors claimed major sources of nitrite as either nitrate reduction or ammonium oxidation in September sampling. Drawing this conclusion should be careful because the oxygen condition was manipulated, at least for 25 m samples, in the incubation experiment.

Done. P11, L5-6. A note on the differences between oxygen conditions during the incubations with samples from the oxycline was included.

Second, the authors should point out that ammonium oxidation to nitrite occurred under helium-purged samples, in section 3.3.3 and section 4.2.3...

Done. P11, L5-6. A note on the differences between oxygen conditions during the incubations with samples from the oxycline was included.

...And use some of their 'response to reviewers' in the main text to support their experimental findings (i.e. high oxygen affinity during ammonium oxidation in cultures and natural waters). In page 6, line 31 – 34 the authors stated the procedure of the incubation. Unless the authors transferred the water in oxygen-free environment (e.g. glove bag or anaerobic chamber), oxygen contamination is highly likely. I think it more important to validate the oxygen concentration in the 12 ml vials, rather than the 250 ml bottle.

Done. P13, L31-34. A paragraph about the detection of nitrification during anoxic incubations was included.

Third, I would encourage the authors to acknowledge their GC7 and ATU inhibitor experiments provided internally consistent data, and both inhibitors were working well (P14, L6 – 8). As I have stated earlier, the sum of nitrite production rates of ATU- and GC7-treatments matches, within the scale of error bars, the rate using 15-ammonium without inhibitor. Because ATU experiments yielded very low rates, nitrite production during the GC7 experiment was close to the rate from the control. If GC7 was not an effective inhibitor, then ATU would not be effective as well, and the experimental results would become a big question mark. I think the central Chilean upwelling system is unique in that bacterial contribution to ammonium oxidation is more significant because of influence from shelf sediment. For this case, it is not an issue that the authors found different things than the other investigators. It is an issue treating their own data unfairly.

Based on the perspective presented by the Referee here, it is true that the sum of the nitrite rates from the ATU and CG7 treatments is close to the rate found in the control treatment. However, these experiments were developed in different incubations; so, the correct way to interpret the results is to compare each inhibitor treatment with the control separately. In this case, the GC7 results were not significantly different from the control. Therefore, at the beginning of the paragraph (P. 15, L 28-29), it was stated that bacteria play a major role modulating the ammonium oxidation relative to archaea does. Nevertheless, considering the biogeochemical and molecular background reported in this system we propose the possibility that GC7 could not affect archaeal metabolism in incubations of short duration. Then, no changes were made in the document.

Other wording issues:

P2, L 2-3: Confusing sentence. Do you mean the oxycline, from oxygen saturation to anoxia, spans only a few meters, located in 30 – 50 meters depth?

Done. P2, L2. Sentence was modified.

P2, L4: Nitrification is not considered as nitrogen removal process. Please revise accordingly

This is true in the classical view, but in the context of lower oxygen conditions this process participates in the nitrogen loss due the reaction of ammonium oxidation could produce  $N_2O$  as will be demonstrated through the manuscript. In this sense, no changes were made in the document.

P2, L15: What do you mean by ‘main subsidiary processes’? Wordiness and confusing.

Done. P2, L15. Sentence was modified.

Also, define DNRA because in the main text there are terms like 'DNaRA' and 'DNiRA'. Please make it easier to the readers. I would recommend using the full name of these processes.

Done. P2, L15, and throughout the text.

P4, L3: Delete 'mechanism or'

Done. P4, L3.

P4, L4: What is 'almost completely'? Wordiness and confusing. Under anoxia (no oxygen), N<sub>2</sub>O is completely reduced to N<sub>2</sub>.

Done. P4, L4. Sentence was modified.

P6, L30: Change to 'purged with He for 15 min'

Done. P6, L30.

P10, L25: I would choose 'September' rather than 'spring' here. I request once again about naming of the seasons and sampling times throughout the text. When referring to sampling period, which happens in a short time scale, I suggest using "September" and "January". When discussing seasonal features on a longer time scale, please use "spring" and "summer".

Done. P10, L25, and throughout the text.

P14, L6: Please change: 'related to'.

Done. P14, L19.

P17, L11 – 15: Here, I would suggest adding some simple calculation, just as they showed in the 'response to reviewers', to demonstrate the N fixation is a source of nitrate that should not be neglected.

Done. P17, L19-24. A sentence about it was included.

Caption in figure 4: State that the N<sub>2</sub>O production rate when measured under C<sub>2</sub>H<sub>2</sub> addition, is 'gross production rate'.

Done. P36, L7. An annotation was included.