Interactive comment "Regulation of nitrous oxide production in low oxygen waters off the coast of Peru"

Response to Referee #1:

We are grateful to the reviewer for the positive feedback and constructive suggestions which greatly helped us in preparing a revised manuscript.
We addressed the specific suggestions below (our replies in bold).

Abstract: Another important finding is that hybrid N_2O formation represented 70-86% of the N_2O production during ammonium oxidation, regardless of the ammonium oxidation rate or O_2 concentrations. One sentence about this should be added to the abstract.

We added: "Hybrid N_2O formation (i.e. N_2O getting one N atom from NH_4^+ and the other from other substrates such as NO_2^-) was the dominant species, comprising 70 – 86 % of total produced N_2O from NH_4^+, regardless of the ammonium oxidation rate or O_2 concentrations."

Introduction Lines 70-75: The distinction between hybrid N_2O production by ammonia oxidizing archaea and chemodenitrification (e.g. nitrite reduction coupled to iron II oxidation) should be better made. Hybrid N_2O formation (mediated by AOA) has been observed in the ODZ water-column, but not chemodenitrification (also referred to as abiotic N_2O production; Wankel et al., 2017), likely due to substrate limitation (Fe, Mn).

In line 74, we added: Abiotic N_2O production, also known as chemodenitrification, from intermediates like NH_2OH, NO or NO_2^- can occur under acidic conditions (Frame et al. 2017), or in the presence of reduced metals like Fe or Mn and catalyzing surfaces (Zhu-Barker et al. 2015, Wankel et al. 2017), but the evidence of abiotic N_2O production (chemodenitrification) in ODZs is still lacking.

line 78: Correct nitrifier-denitrifiaction for denitrification.

We added (line 80) denitrification, but did not replace nitrifier-denitrification because this is what the paragraph is about.

Lines 79-81: It should be noted that Frame and Casciotti (2010) only observed higher yields at decreasing O_2 concentrations for high starting cell densities. At lower cell densities (closer to values found in ODZs), the impact of decreasing O_2 on N_2O yield was much lower than observed in other studies.

We re-wrote the sentence as follow (line 83-87): Overall, the yield of N_2O per NO_2^- generated from AO is lower in AOA then AOB (Hink et al. 2017, 2018) but it should be noted that the degree to which N_2O yield increases with decreasing O_2 concentrations is variable with cell densities in cultures or field sites, (Cohen & Gordon 1978; Yoshida 1988; Goreau et al. 1980; Frame & Casciotti 2010, Santoro et al. 2011, Löscher et al. 2012, Ji et al. 2015a, 2018a).

Lines 102-104: Charpentier et al (2007) also suggested that nitrifier-denitrification is enhanced by high concentration of organic particles, which creates high NO_2^- and low-O_2 microenvironments.

We added a sentence in line 81-83. "It has also been suggested that high concentration of organic particles create high NO_2^- and low-O_2 microenvironments enhancing nitrifier-denitrification (Charpentier et al. 2007)."

Lines 113-114: It would also be relevant to look at *nor* genes which are encoding nitric oxide reductase.

The reviewer is correct, but the goal here was to distinguish between nitrifiers and denitrifiers and for that the *nor* gene is not ideal as it is present in both. Furthermore, in Fuchsmann et al. (2017) (doi10.3389/fmicb.2017.02384) the canonical forms of the gene *norB* and *qnorB* were very low abundant, suggesting that there might be other genes encoding enzymes mediating NO reduction to N_2O.

Materials and methods:
Line 136: It is not clear why a 3 mL He helium headspace is created before incubating, since it will impact in-situ O_2 concentrations.

The headspace was added for several reasons: 1) to avoid diffusion of oxygen from the septum into the liquid directly, headspace provided an additional barrier, 2) to be able to purge the serum bottles and 3) to avoid artificial differences by different treatments, all bottles received a headspace. We added (line 148 – 150): "He purging removed dissolved oxygen contamination which is likely introduced during sampling and the headspace prevents direct oxygen leakage from the rubber seals (DeBrabandere et al. 2012)."

Line136-137: I assume purging is done to avoid O_2 contamination? What is the O_2 threshold defining anoxia here? One potential problem with purging is that it also removes other gases (e.g., H_2S) involved in autotrophic denitrification (for instance, see Callbeck *et al*., 2018).

Yes, purging was done to decrease oxygen contamination during sampling. We rewrote the sentence as such (line 147-148): A 3 mL helium (He) headspace was created and samples from anoxic (O_2 < below detection) water depths were He purged for 15min. We also added line 148 – 150, as written as answer to your previous comment. The point of H2S removal during purging is added into the discussion section line 511- 521: "In addition, sampling with Niskin bottles and purging can induce stress responses (Stewart et al. 2012) and shift the richness and structure of the microbial community from the *in situ* community (Torres-Beltran et al. 2019), which can be one potential explanation for the different responses between manipulated O_2 and *in situ* O_2 experiments. The removal of other gases like H_2S during purging introduces another potential artefact. However, this is unlikely because measurable H_2S concentrations have mostly been found at very shallow coastal stations (< 100 m deep) (Callbeck et al. 2018), which have not been sampled in this study. On the contrary, high abundances (up to 12 %) of sulfur oxidizing gamma proteobacteria, like SUP05 can be found in eddy-transported offshore waters where they

actively contributed to autotrophic denitrification (Callbeck et al. 2018). This study cannot differentiate between autotrophic or organotrophic denitrification, but a contribution of autotrophic denitrification in the eddy center is likely."

Lines 150-153: How did O_2 vary during the incubations? These data should perhaps be included as part of the supplementary materials.

The oxygen concentrations stayed constant in the low oxygen treatments, while it decreased in higher oxic treatments. That explains the higher standard deviation in higher treatments. Oxygen concentrations over time are added to the supplements Figure S1.

Line 153: Explain the rationale for using particles >50 μm.

It is the fraction that is sinking. This is stated in line 607-608: "However, the particle size (>50 μm) used in the experiments is indictive of sinking particles."

Lines 192-219: Plots showing increase in ^{15}N labeled products over time should be included in the supplementary materials. Were the relationships always linear?

Linear relationships were used to calculate the slopes and only significant slopes were included as written in line 233-235. We added example time plots from the oxygen manipulation experiments into the supplements. See Figure S2.

Lines 228-229: These nirS primers exclude epsilon-proteobacteria (Murdock, et al., 2017). Epsilon proteobacteria are often the dominant portion of autotrophic sulfur oxidizers in sulfidic waters (e.g., Grote et al., 2008), thus this aspect should be discussed.

We added a statement in the methods that we are aware that epsilon-proteobacteria are not captured with the Primer we used. Line 260 - 263: "The *nirS* Primers are not specific for epsilon-proteobacteria (Murdock et al. 2017), but in previous metagenomes from the ETSP epsilon-proteobacteria where below 3-4% or not found, except in very sulfidic, coastal stations (Stewart et al. 2011, Wright et al. 2012, Ganesh et al. 2012, Schunck et al. 2013, Kavelage et al. 2015)."

Line 256: Add accession number.

Added. GEO Accession No GSE142806

Results:

Lines 282-283: Could a contour plot of chlorophyll concentration added to the supplementary material for reference?

We added surface Chlorophyll data to the station map. See Figure 1.

Lines 334-335: This result is a bit puzzling as previous studies (e.g., Dalsgaard et al., 2014), observed fifty percent inhibition of N2O production by denitrification at about 300nM O2. These observations are also unlike results from their *in situ* O2 gradient experiment.

This is not contradictory to Dalsgaard et al. 2014. They were in depths with high NO2- concentration indicative for the core of the anoxic zone, whereas this study took place at the upper part of the anoxic zone and in the oxycline.

Lines 349-350: It is also surprising to observe the highest yield for N_2O production at highest O_2 concentrations, for which N_2O production should be inhibited (Dalsgaard et al., 2014).

This is not to be confused with the N2O yield/N2. The yields are for NO3- and not like in Dalsgaard with 15NO2- which apparently makes a large difference as we can show in this study!!!

Discussion:

Lines 421-426: Some of these are likely causal relationships.

Yes, absolutely.

Lines 425-426: This suggest that when NO_3^- is abundant, denitrifying bacteria are less likely to use NO_2^- (either from their internal pool or outside the cell) for N_2O production during denitrification.

This comment is added to the text line 460 - 461.

Line 441: What is the detection limit for [N_2O]?

The detection limits is 2nM. We added that information into the method section 2.1. line 137.

Lines 441-444: Bourbonnais et al. (2017) used biogeochemical tracers (N_2O concentrations and isotopes) that integrates over longer timescales compared to ^{15}N-labeled incubations, which are more like taking a snapshot in time. Therefore, discrepancies between N_2O production rate is expected and should be discussed in this context.

We rewrote that section: "Previously reported maximum rates were up to 86 nmol L^{-1} d^{-1} (Dalsgaard et al. 2012) based on ^{15}N tracer incubations. Much smaller maximum rates, 49 nmol L^{-1} d^{-1} (Bourbonnais et al. 2017) and 50 nmol L^{-1} d^{-1} (Farìas et al. 2009), were obtained using N_2O isotope and isotopomer approaches which provide time and process integrated signals. Hence, the deviation of maximum rates can be explained by 1) the different approaches and 2) the sampling of the core of the eddy. "

Line 451: Cite Fassbender et al. (2018) that discusses impacts of eddies on biogeochemical processes at different scales.

We did not add Fassbender here, because the recommended paper does not contain information on impact of eddy age on the N2O distribution, which is the point we are trying to make here.

Lines 443: The error on this higher rate estimate seems rather large (in Figure 3, p).

We added the exact rate with the standard deviation.

Lines 458-460: This part is confusing. The O_2 threshold for reductive N_2O production should be higher than for N_2O consumption, not the converse. In other words, nitric oxide reductase should be more O_2 tolerant than nitrous oxide reductase (Dalsgaard et al., 2014). Otherwise, N_2O would not accumulate.

This is exactly my point. There is a discrepancy between the thresholds in rates we find and the N2O concentration maxima we measure between 1 – 8uM O2. If N2O production is so sensitive from denitrification then where is all the N2O coming from? Just NH4+ oxidation is unlikely based on the N2O production rates we find from NH4+ oxidation. There might be a higher threshold for N2O production from denitrification?

Lines 445-446: I do not understand this statement.

We did not measure N2 production rates, so we cannot say anything about the N2O/N2 yield during denitrification. This yield is subject to changes and not constant, Because of that, we have no chance to make an estimate on the N2 production rate. Maybe in the Eddy incomplete denitrification to N2O was favored and that is what we measured or complete denitrification was fueled and this is what we measured. We rephrased the sentence (line 481- 485) to "N_2 production measurements were not performed in this study, so it cannot be determined whether the eddy only stimulated N_2O production but not N_2 production from denitrification (i.e. increasing the N_2O/N_2 yield) or if the eddy also increased complete denitrification to N_2 by 10 times compared to stations outside of the eddy. "

Lines 479-481: This hypothesis is also supported by a rather long turnover time for NO_2^- as inferred from the $\delta^{18}O$ of NO_2^-, which is generally fully equilibrated with water in offshore waters (Bourbonnais et al., 2015). This is not the case in coastal waters, where NO_2^- seems to be more dynamic (see and cite Hu et al., 2016).

We added this statement into the manuscript as follow (line 532 – 534): "Long turnover times for NO_2^- have been inferred from $d^{18}O$ of NO_2^-, which was fully equilibrated with water in the offshore waters (Bourbonnais et al. 2015) and more dynamic in the coastal waters (Hu et al. 2016) supporting our hypothesis. "

Lines 495-496: How can these contrasting results be reconciled?

We attribute this to the intensity of the ammonium oxidation rate which exerts a first order control on the N2O production rate. Meaning if the NH4+ oxidation rates would exponential decrease with O2 concentration then we would find that relationship in the N2O production rates. We discuss this further down in line 554 – 556.

Lines 522-524: If hybrid N_2O formation during AOA is purely (or even partly) abiotic, then measured rates would be overestimated as $HgCl_2$ would not stop N_2O production at the end of the incubations. For how long were these samples stored before being measured? This point should be better discussed.

The samples were stored between 2 – 5 month. Abiotic N2O production would take place and continue until we measure the samples, indeed. But it also goes on in all samples raising the N2O baseline (in mass 44,45,46) for all and not just in specific ones. This impact will likely vary with depth, but then all the timepoints are affected by the same abiotic production. The rates are calculated from the increase over time making them independent of the baseline. We added a figure to the supplements S9, where results for abiotic production from 15NO2- tracer are shown from 4 depths from 2 stations. The addition of 15NO2- tracer results in little abiotic production; 0.018 – 0.37 nM 45N2O and 0.009 – 0.026 nM 46N2O up to the point of mass spec analysis, but independent HgCl2 addition. We added this point into the discussion line 281. However, we did not test abiotic N2O from NH4+ tracer, hence this can not be fully ruled out. We added that point in line 588-590: "Additionally, at four depths the potential for abiotic N_2O production in $^{15}NO_2^-$ addition experiments showed variations with depth and no significant impact of HgCl2 fixation (Figure S9)."

Lines 565-566: What was the chlorophyll concentration in the center of the eddy?

Low, below 1mg/m3. We added a map with surface Chlorophyll, see Figure 1.

Lines 641-643: N_2O emission to the atmosphere are possible only if the water is upwelled.

We rephrased the sentence to (line 698): "Regardless of which processes are responsible for N_2O production in the ODZ, high N_2O production at the oxic-anoxic interface of the upper oxycline sustains high N_2O concentration peaks with a potential for intense N_2O emission to the atmosphere during upwelling events."

Lines 649-652: Temporal variability is particularly not well captured in observational studies.

We added a sentence to pick up on that comment (line 705 – 706): "While this study does not help to resolve temporal variability, manipulation experiments give valuable insights on the short-term response of N_2O production to oxygen and particles. "

Figure legends:
Rename Figure 7: N_2O production after additions of...

The figure was renamed accordingly.

Figures 2 and 3 are too small. Legend (station #) is almost impossible to read.

The figure Legend and axis label were adjusted.

Figure 5: Samples impacted by denitrification should be more clearly indicated (by a circle or rectangle and in the Figure legend) in Figure 5b.

In all samples in Figure 5b, N_2O production from 15NO3- was found. If that is what the reviewer means. There was no adjustment done to the figure.

Supplements:
Figures S1: I recommend expanding the scale at lower O_2 concentrations since this is the focus of the paper.

We did not expand the scale here as the focus is the shallowing of the oxycline in the center of the eddy , which is nicely visible in this figure.

Figure S5: Add linear regression and r-square for natural samples in the zoom up plot.

Linear regressions and equations were added to the Figure S7.

Figure S6: Since there are only a few data points for $[NO_2^-]/[NO_3^-]$ higher than 0.10, I don't think the outlier (light gray dot) can be removed. There is much more scatter in Figure 5 in Ji et al. (2018) for the same relationship.

The point was included into the regression.