Review of Frey et al.

General comments:
Frey et al. used a combined biogeochemical and microbial ecology approach to investigate N_2O production in the ETSP off Peru. More specifically, they used ^{15}N-labeled incubations to measure N_2O production during ammonium oxidation as well as denitrification and their regulation by O_2 and particulate organic matter. They also measured *archaeal amoA* and *nirS* genes abundances and activity. Overall the manuscript is well written and will make a great contribution to the field as the exact mechanisms regulating N_2O production in ODZs are still not well understood. Of particular importance, few studies have looked at the effects of organic matter addition on N_2O production. I recommend publication after addressing the generally minor considerations below.

Specific comments
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.

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).

line 78: Correct nitrifier-denitrifiaction for denitrification.

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.

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.

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

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.

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).

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

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

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?

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.

Line 256: Add accession number.

Results:

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

Lines 334-335: This result is a bit puzzling as previous studies (e.g., Dalsgaard et al., 2014), observed fifty percent inhibition of N_2O production by denitrification at about 300 nM O_2. These observations are also unlike results from their *in situ* O_2 gradient experiments.

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).

Discussion:

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

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.

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

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.

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

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

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.

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

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).

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

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.

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

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

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

Figure legends:

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

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

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

Supplements:

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

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

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.

Other references:

Callbeck, C. M., Lavik, G., Ferdelman, T. G., Fuchs, B., Gruber-Vodicka, H. R., Hach, P. F., ... & Schunck, H. (2018). Oxygen minimum zone cryptic sulfur cycling sustained by offshore transport of key sulfur oxidizing bacteria. *Nature communications*, *9*(1), 1729.

Charpentier, J., Farias, L., Yoshida, N., Boontanon, N., & Raimbault, P. (2007). Nitrous oxide distribution and its origin in the central and eastern South Pacific Subtropical Gyre. *Biogeosciences Discussions*, *4*(3), 1673-1702.'

Fassbender, A. J., Bourbonnais, A., Clayton, S., Gaube, P., Omand, M., & Franks, P. J. S. (2018). Interpreting mosaics of ocean biogeochemistry. *Eos*, *99*(10.1029).

Hu, H., Bourbonnais, A., Larkum, J., Bange, H. W., & Altabet, M. A. (2016). Nitrogen cycling in shallow low-oxygen coastal waters off Peru from nitrite and nitrate nitrogen and oxygen isotopes. *Biogeosciences*, *13*(5), 1453-1468.

Murdock, S. A., & Juniper, S. K. (2017). Capturing compositional variation in denitrifying communities: a multiple-primer approach that includes Epsilonproteobacteria. *Appl. Environ. Microbiol.*, *83*(6), e02753-16.