Review of Frey et al; 'Regulation of nitrous oxide production in low oxygen waters off the coast of Peru'

The authors combine 15N labelling experiments with analyses of gene abundances in the ODZ of the Peruvian coast in order to assess the pathways contributing to the production of the potent greenhouse gas, N2O. This is an interesting and overall well-written paper that adds to the relatively small amount of studies investigating the effects of small-scale O2 variations on N cycling processes in ODZs. However I would like to highlight some minor corrections/clarifications and several points in the (biogeochemical) methods that I think need to be shortly addressed/discussed in the paper before publication. Given the points already highlighted by R1, I will try not to repeat their comments

General comments:

Check nitrite/NO_2^- throughout manuscript

You note differences in process rates and between the communities exposed *in situ* to O2 gradients and in the O2 manipulation experiments (e.g. Line 463-4). I think at least some discussion is needed as to the potential effects of purging the samples with gas as described in refs below (e.g. Dalsgaard et al, deBrabandere et al, Holtappels et al, Stewart et al.)

Can you be sure that there is no DNRA occurring in your experiments – in particular given the Lam et al. 2009 'Revising the N cycle…' paper also off the Peruvian coast. The presence of DNRA would complicate your isotope pairing experiments with 15NO3- and 15NO2- by transferring 15N into the NH4+ pool and you would get 'hybrid'N2O' of 15nh4+ and 15no2- forming 46N2O and be wrongly assigned. DNRA would also potentially dilute your 15NH4+ pool with 14N from background NO3- and alter the assumed 99% labelling in these experiments. I realise the contribution of AO to N2O production is small relative to denit, but the artefacts of DNRA on the rates/data should be discussed as it could lead to some N2O from AO being 'hidden'.

Specific comments:

Section 2.1: As with other papers with many sites, sampling points and manipulation experiments a written methods text quickly becomes very complicated with different additions, concentrations, replicates, time points etc. I think as a result of the text being quite confusing some information has been missed/is unclear. Adding a table of experiments, stations, variables, sampling routine (e.g. time points), number of replicates, other factors (e.g. whether O2 was measured in vials) would be informative/helpful to readers who are interested in comparing/replicating experiments.

Also Section 2.1: Missing info on NO3- and NO2- analyses (e.g. shown in Fig 2).

Line 145 (O2 manipulation experiments): Why was such a 'coarse' O2 range used compared to previous studies which use O2 manipulations generally below 1-2µM (e.g. Dalsgaard et al 2014, Bristow et al 2016)?

Line 145 (O2 manipulation experiments): This is a bit confusing: '…headspace volume was adjusted depending on the amount of site water added…'. Do you mean that after the addition of different oxygenated water volumes you also wanted to end up with a 3mL headspace as in the 'natural gradient' O2 experiment? Please rephrase and explain more clearly.

Line 153 (OM experiments): So only total N2O was measured in the OM experiments? Or were 15N substrates also added. Unclear as it is written now.

Line 166: Do you mean ‘Ascarite’ instead of Ascarid?

Line 186-8: Rephrase to: ‘If more single labelled N2O is produced than expected (…), a hyrid formation of one nitrogen atom from nh4+ and one from no2- (…) is assumed to be taking place se found in archaeal ammonia oxidizers’

Line 191: What about 45N2O formed from dilution from background 14NO3- and 14NO2- in samples? Then you will get 45N2O from 15NOx + 14NOx … You note earlier (ca Line 140) that there is likely substantial 14NO3- (at least in some samples/depths) which will be reduced to 14NO2- and dilute your 15NO2- pool. Perhaps there is something I have missed in the text but this doesn’t make sense to assume all 45N2O in incubations with 15NO2, especially in anoxic/low O2 manipulations where NOx can be respired.

Section 3.3: Could the % inhibition of processes be plotted to help comparison to other relevant studies on O2 manipulation on AO/no2- ox/denit (e.g. Kalvelage et al 2011, Dalsgaard et al 2014, Bristow et al 2016). I think at least some short discussion is warranted in relation to O2 effects on processes in these previous papers.

Section 3.4: This is a little confusing, additional 14NH4+ was also added along with the POC to experiments? Or was the POC filtered/rinsed after autoclaving?

Line 466: In relation to the ‘Unchanged N2O production with higher O2 levels in NO3- treatments…’ sentence: Can anoxic niches be ruled out in these experiments? You do note the sampling being during low upwelling and chl period but the settling of small particulates during experiments may create anoxic/low O2 zones to sustain anaerobic processes.

Sentence line 470-472 Bristow et al 2016 should also be a ref here in relation to kinetics of multi-step processes

Line 477: How can you be sure none of the N2O was consumed without further measurements (e.g. 15N-N2)? Production may just be much faster than consumption.

Line 512-515: Confusing sentences, consider rephrasing.

Line 521: This is a bit of an oversimplification - because something is below detection doesn’t necessarily mean nothing is happening, more likely a tight coupling between consumption and production (e.g. see Figure 4 in Klawonn et al 2019 and Figure 3 in Olofsson et al 2019 references). Could there be a dilution of your 15NH4+ pool to consider due to rapid cryptic cycling on shorter scales than your experiments? Ideally 15NH4+ and total NH4+ would be followed through the time series to check for dilution effects. Both show very rapid NH4+ turnover (within ~5h) in oligotrophic waters

Line 532: If measured, the accumulation and consumption of intermediates (e.g. NO2-) could also be used to imply biotic vs abiotic mechanisms (e.g. Betlach and Tiedje 1981 reference).

Line 560-3: Could a 15N recovery/inventory be calculated for the experiments (e.g. 15N recovery from initial substrate, measured intermediates and 15N-N2O?) This could help infer a % N2O production from denitrification which is important for putting the N2O production from denit in context – i.e. how do variations in O2 impact the proportion of N2O produced by denit relative to N2?

Fig 4 b, c & Fig 6 b: consider zoomed-in insert of x-axis (e.g. similar to Fig S5)

Figure S5: Seems to be two different slopes here from manipulated vs natural O2 gradients – could also be discussed in relation to purging artefacts.

Ref suggestions

Betlach, M. R., and J. M. Tiedje. 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. 42: 1074–1084.

Bristow et al 2016 Ammonium and nitrite oxidation at nanomolar oxygen concentrations in oxygen minimum zone waters. PNAS

Dalsgaard, T. et al. Oxygen at nanomolar levels reversibly suppresses process rates and gene expression in anammox and denitrification in the oxygen minimum zone off Northern Chile. mBio 5,

De Brabandere, L., Thamdrup, B., Revsbech, N. P. & Foadi, R. A critical assessment of the occurrence and extend of oxygen contamination during anaerobic incubations utilizing commercially available vials. J. Microbiol. Methods 88, 147–154 (2012).

Holtappels, M., Lavik, G., Jensen, M. M. & Kuypers, M. M. M. in Methods in Enzymology: Research on Nitrification and Related Processes, Part A, Vol. 486 (ed. Klotz, M. G.) 223–251

Kalvelage et al 2011' Oxygen sensitivity of anammox and coupled N-cycle processes in oxygen minimum zones.' PlosOne

Klawonn et al 2019 'Untangling hidden nutrient dynamics: rapid ammonium cycling and single-cell ammonium assimilation in marine plankton communities' ISME

Olofsson et al 2019 Nitrate and ammonium fluxes to diatoms and dinoflagellates at a single cell level in mixed field communities in the sea. Scientific Reports

Stewart et al 2012 'Experimental Incubations Elicit Profound Changes in Community Transcription in OMZ Bacterioplankton' PlosOne