Response to Authors from Ref #2

(Reviewer replies in highlighted text)

The authors have done a good job at incorporating and considering comments. I suggest publication after some very minor revisions/additions that I have added where necessary to their replies. Replies that have sufficiently addressed the issues have been omitted from this document.

General comments:

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'.

The reviewer raises a very important point and no, we cannot be sure that the occurrence of DNRA is impacting our results. We added this consideration to the manuscript in line 216 - 232. "Nevertheless, this assumption brings some initial considerations which need to be accounted for. There is a potential for overestimating hybrid N2O production in 15NO2- incubations by 5% in samples with high NO3- reduction rates. But in incubations from anoxic depths with high NO3- reduction rates, no hybrid N2O production is found at all. For example, accounting for a decrease in fN of the NO3- pool by active NO2- oxidation, the process with highest rates (Sun et al. 2017), had an effect of only ± 0.2 % on the final rate estimate. The presence of DNRA complicates 15N-labelling incubations because it can change f in all three tracer experiments. In the vicinity of DNRA in 15NO3- incubations, 15NO2- and 15NH4+ can be produced from 15NO3- which can contribute to 46N2O production by AO. Even when a maximum DNRA rate (20 nM d-1 in Lam et al. 2009) is assumed to produce 0.02 nM 15NH4+ in 24 h with all of it being oxidized to N2O (max. N2O production from AO 0.16 nM d-1, this study), its contribution to 46N2O production is likely minor and within the standard error of N2O production rates from NO3-. Hence an overestimation of the N2O production rates is unlikely. The same applies in incubations with 15N-NO2- when DNRA produces 15NH4+, additional 46N2O can be produced with a hybrid mechanism by AO not accounted for in the present rate calculations. In 15NO2- incubations with high starting f (>0.7) the production of 14NO2- by NO3- reduction (which decreases f) leads to an underestimation by max. 9%, whereas in incubations with a low f (<0.3) the effect is less with max. 3 % underestimation of N2O production rates. In 15NH4+ incubations (f>0.9), max. DNRA rate would lead to an underestimation of 3.5 %. "

Thanks, I realise it will be a small % but important to acknowledge. It would also be good to add a sentence in the discussion to suggest that DNRA(& anammox) are measured in addition in future work to rule out potential artefacts – there are now several (sediment) papers on the artefacts of the coocurrence of NO3- reducing processes on the IPT assumptions.

I think you just need to change "In the vicinity of DNRA in 15NO3- incubations…" to "In relation to DNRA in 15NO3- incubations…"

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.

It is correct, that such set ups can get confusing very quickly, but in table 1 stations, depths, measured variables and the kind of experiment performed are given. However, we added one column with the kind of tracer addition we did. The replicates and time points did not vary between experiments and hence is only stated in the test. We only measured oxygen in one bottle with each incubation per depth or treatment, which was also consistent and written in the text line 168/169.

My apologies – I missed this table but think the added information now makes it clearer.

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)?

In Bristow et al. 2016 a and b the maximal oxygen concentration in their manipulation experiments was 10uM and 20uM dissolved oxygen, so we are not quite sure what the referee means. Dalsgaard et al 2014 performed a really nice microcosm experiment, where oxygen concentrations were monitored online in the flask they subsampled. In our case, each time point was a separate bottle making it impossible to use such an approach. For the experimental design in this study, it was important to choose oxygen levels where we can be sure that oxygen concentrations are different enough from each other that we can differentiate the two treatments (f.e. 100nM and 200nM would be tricky to tell apart with our standard deviations of 180nM and 240nM over 24h). We added a plot of oxygen over time into the supplements Figure S1.

I meant that in Bristow et al. and Dalsgaard et al that a lot of their measurements are concentrated below 1-2 uM oxygen and fewer concentrations in the 'higher' 10-20uM range… i.e. focusing on the concentrations where the inhibition/regulation really 'happens'. But I understand the reasons you describe above given the standard deviations of O2 measurements and without more sensitive sensors it would be difficult to designate concentrations, I agree. I appreciate that Dalsgaard et al do have a nice reactor/microcosm set up which I realise is very specialised for precisely these experiments and with larger volumes than the serum vials – also that it is a lot of work with these types of experiments. I think it would still be good to add a sentence/statement as to why 'your' oxygen concentrations were chosen (e.g. given the reasons above, SD in measurements etc) if possible.

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.

Anoxic micro niches can never be fully ruled out, if not investigated. The Chlorophyll concentrations were in deed low for an upwelling area, max 5mg/m3, but on average 1mg/m3 and less. Figure 1, map of the study site was adjusted with Chlorophyll concentrations. The treatment was identical between depth profile samples and manipulation samples, so if the particles settle, they would settle in all of the bottles and create microniches in the samples from the depth profile as well. There is no plausible explanation why more anoxic micro niches should be in the oxygen manipulations compared to the others.

But if there is the same amount of particles in all vials/O2 manipulations then there is potential for some anoxic processes to be 'unaffected' by O2 additions - with some changes in anoxic microsite volume with O2 diffusion into particles. I realise this is hard to rule out – especially as you collect small particles from the water column to use, indicating that they are there. I think it would be important to write something shortly about why you consider it unlikely that any (significant) anoxic niches occur.

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.

We are not able to say anything about N2 production, we can only assume. We added sufficient amounts of 44N2O carrier prior to the incubation to trap 15N-labelled N2O. If N2O reduction is taking place at high rates, we would see a decrease in the N2O pool over time. A plot with the mass 44, 45 and 46 over time was added to the supplements (Figure S2).

Shortly suggest/indicate benefits of also measuring other end products (e.g. 15N-N2 and maybe also 15NH4+ from DNRA) in the text (i.e. how does the 'efficiency' of denit change with changing O2)

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

The 15NH4+ substrate was not measured on the GC-IRMS because high 15N label/ almost pure tracer is always problematic to analyze. We added the possibility of an overestimation of hybrid production to the method section line 217 and rephrased the wording here to (line 573-575) "Even though, *in situ* NH4+ is below detection in almost all water depths (f > 0.9), there remains the potential for 15NH4+ pool dilution by remineralization and/or DNRA during ~~during~~ the incubation. Despite below NH4+ concentrations, ~~detection limit~~ studies have shown fast turnover for NH4+ (Klawonn et al. 2019)…. However/thus"

Some kind of 'conclusion' is needed at the end of the last sentence in relation to your study. Papers referring to 'cryptic' biogeochemical cycling in ODZ waters would also be nice to include in relation to 'hidden' processes.