

The authors present a supremely well executed study of N cycling rates in an oxygen deficient zones from well-controlled tracer incubations, from which they derive the relative contribution of respective processes to N<sub>2</sub>O production, and from which they document the sensitivity of said production pathways to dissolved oxygen concentrations. Their tracer incubations rely in part on site-preference measurements of isotopocules in order to determine pathways of production. Their data corroborate a dominance of denitrification in N<sub>2</sub>O production within the anaerobic regions of the water column, whereas multiple pathways operate concurrently in oxyclines. N<sub>2</sub>O production from ammonium, presumed to be catalyzed by nitrifiers, occurred dominantly through a hybrid pathway reliant on both ammonium and nitrite as substrates, whereas the hydroxylamine oxidation pathway (both N's in N<sub>2</sub>O from ammonium) was relegated to the well-oxygenated upper water column. The results and interpretation are highly informative, providing important constraints on pathways of N<sub>2</sub>O production and their respective sensitivity to oxygen.

I found the manuscript generally well written but, perhaps necessarily, a challenging read. I read it multiple times. The “cognitive challenge” arises from the inherent complexity of the topic and study design. It is also exacerbated by some structural elements of the manuscript that would benefit from revision: (a) The motivations for the study are not made clear in the introduction; (b) the general “order of operation” keeps jumping around in the results and discussion (I explain what I mean below), (c) there is a heavy reliance on supplementary materials, requiring a lot of back and forth.

I suggest a number of modifications that I think could improve ease of understanding by readers peripheral to the field of N<sub>2</sub>O isotopes who want to understand the findings and who also want to have a sense of the limitations of the findings.

The introduction does not effectively motivate the study. This study appears to be a companion to a published study where net rates of N cycling were determined from bulk tracer additions. I suppose that is why the bulk rate estimates figures were relegated to the supplements even though they are highly informative in the current context. Regardless, questions evidently emerged from the previous study that are presumably addressed herein, but these questions are not articulated in the introduction. I suggest the following paragraph sequence, which would make the intro more seamless:

The first paragraph alerts us that the study deals with nitrous oxide in oxygen deficient zones, with a justification of why N<sub>2</sub>O matters. In the second paragraph, the reader expects to learn where N<sub>2</sub>O is believed to come from in ODZ's. Instead, the paragraph otherwise begins with what seems a separate (but related) topic, N<sub>2</sub>O production by archaea, ocean-wide, not necessarily in ODZ's. In lieu, I suggest moving up the third paragraph to the second, to explain the current understanding that most N<sub>2</sub>O in ODZ's appears produced by denitrification. This would lead into a third paragraph that explains that nonetheless, a significant fraction appears to be produced by archaeal nitrification. I would present the current evidence that supports this hypothesis, in order to motivate “looking” for hybrid production, which is where this paper ultimately brings us.

The fourth paragraph should be explicit in whether it is referring to naturally occurring isotopes or tracer isotopes, since the subsequent paragraph jumps into tracers. To better motivate the study, perhaps this section can explain what naturally occurring isotopocules have divulged about N<sub>2</sub>O production in ODZ's specifically, and which questions remain unanswered – in order to link to the last paragraph of the intro.

IN the last paragraph, the motivation for measuring site preference on tracer experiments needs clearer articulation. What additional insights can it provide that natural abundance or bulk tracer experiments did not? And your results, as I see them, inform on more than a dependence of oxygen on hybrid production, correct? They (a) corroborate previous findings on relative pathways of N<sub>2</sub>O production (b) uncover that the hybrid pathway dominates production by nitrification and (c) production from hydroxylamine is not a thing except at the surface. Importantly, do the results confirm inferences from natural abundance tracers in the same system? These can be posed as questions to which the authors can return in the discussion.

Methods:

Line 200: I would rephrase to "... contribution of <sup>15</sup>N<sup>15</sup>NO to masses 46 and 31, which, while negligible at natural abundance, becomes important in tracer experiments."

Equations 1-4: I think it would be wise to define ALL the terms in equations 1-4, for readers peripheral to this field who may still strive to understand the equations.

Line 245: Nitrate IS produced from nitrite when sulfamic acid (or any acid) is added to nitrite, due to the acid decomposition of nitrous acid. See Granger and Sigman 2009, Equations 6 and Figure 2. And <sup>15</sup>N nitrate is a probable contaminant of the <sup>15</sup>N nitrite solutions.

Line 274: what is N exchange between substrates?

Line 280: These "pathways" were not discovered by Wan et al. 2023. The citations are unclear to me.

Results:

I realize some of the data are published elsewhere but they are fundamental to navigating the paper. I suggest moving some of these back to the main text. In particular, the N<sub>2</sub>O production plots (mass 45 for each <sup>15</sup>N substrate).

I suggest presenting the results in order of dominance of rates, and sticking to this pattern in all subsequent text and **figures**. Denitrification is fastest; detailing it first helps contextualize nitrite oxidation rates, which are also very high, and ammonium oxidation rates, which are puny.

Stick with one, NH<sub>3</sub> or NH<sub>4</sub> oxidation. It varies in the text.

Section 3.3 is very difficult to navigate. I read it multiple times. The term "high rates" is meaningless without context. Rates peak or not, but it can't be argued that rates of <sup>45</sup>N<sub>2</sub>O- $\alpha$  are high even in this context, at picomolar per day. In this regard, I suggest using picomolar in lieu of multiple decimals in the text and figures, which are tiresome. And the Figure S8 is nearly impossible to navigate as every panel has a different x axis range. Perhaps homogenize ranges for given isotopocule production? And I'm not sure why these figures are relegated to the supplements. I spent a long time looking at them. A long time...

The line at 215 belongs with the previous paragraph. And it's not clear whether this will be an example of rates varying in concert or not. Wordsmith accordingly.

Equation 13: In the case of nitrite where a higher concentration was added than intended, I would think that the flux derived therefrom, J, is no longer proportional to nitrite (zero order) at these concentrations. Does this matter?

Line 337: Wording of sentence is awkward.

Line 395: How can nitrite oxidation rates possibly be negative?

Line 420: Remind the reader what "f" designates.

Equation 19: "AP" was designated as "15F" in equations above...

Could  $\delta^{15}\text{N}$  excess result from misestimation of the actual atom percent of substrates the incubations?

The rates are very small such having a small error on AP could potentially account for this? Or wrong proportion of carrier? I think Figure S9 may allude to this but the associated uncertainty needs to be better explained in the main text, whether or not the data evince unequal values of “ $f$ ” beyond a reasonable “doubt”

Figure 4: Present in order brought up in text, which is  $\text{N}_2\text{O}$  production from nitrate first.

Is production from  $\text{NH}_4^+$  only necessarily hydroxylamine oxidation? It is called that in some figure captions. If so, it would be much easier for readers if it were called hydroxylamine oxidation throughout.

Section 3.5: I would start with describing  $\text{N}_2\text{O}$  production “as a whole”, followed by nitrate reduction (highest flux), etc... Same order of operation as suggested above.

Figure 4 d: the trace for ammonium oxidation differs from the corresponding trace in Figure 3 a.

Discussion:

Because the study is very complex, it would be beneficial for the discussion to begin with a paragraph that summarizes the dominant findings, rather than jumping into the deep end from the get go. In this regard, I would also get  $\text{N}_2\text{O}$  production from denitrification out of the way first because it was the dominant flux, then discuss hybrid production. I find it interesting as well that production from hydroxylamine was virtually absent except at the surface – I think this merits more emphasis.

Section 4.3: I get that MOST  $\text{N}_2\text{O}$  is produced by denitrification and 1/5 from hybrid production. Is that what is also inferred from natural abundance measurements, in these proportions? Curious minds want to know

Line 6452: What do you mean by “allowed?” Need better wording.

Line 650: qualify “this” , you mean the notion that internal pool are processed, not external...?

Line 600: Reader is left hanging: What are the implications for mechanisms of production? Need a concluding sentence for the paragraph to bridge it to the next, or simply amalgamate with the following paragraph.

Paragraph at line 605: Reads like something that should be in results section.

Line 610: Articulate fully for readers to catch up again “findings of unequal alpha vs. beta production during hybrid pathway have implications for interpretation of the natural abundance isotopes of  $\text{N}_2\text{O}$  produced by hybrid process.”

Paragraph at line 670: I don’t understand why the results here should be different than cited study.

I remain perplexed by the following: In Figure S8, there is NO production of  $\delta^{15}\text{N}$   $\text{N}_2\text{O}$  from addition of  $^{15}\text{NH}_4$  at 100 m at station 1, yet there is reportedly 50 nM/day  $\text{N}_2\text{O}$  production from the hybrid pathway at this depth... Am I fundamentally misunderstanding something about the experimental design? The hybrid pathway requires some input from  $^{15}\text{NH}_4^+$  which should be detected as  $\delta^{15}\text{N}$   $\text{N}_2\text{O}$ ?