Response to Reviewers 6/4/2024
Submission: "Isotopomer labeling and oxygen dependence of hybrid nitrous oxide production" [Manuscript ID egusphere-2023-2642]

We thank the editor and two reviewers for their positive re-evaluation of our work. We address below the editor's recommendation and a few minor technical corrections suggested by anonymous reviewer #1.

Editor

Regarding your conclusions ("we posit a two–step process for hybrid N2O production involving an initial bond–forming step that draws nitrogen atoms from each substrate to form a symmetric intermediate, and a second bond–breaking step that breaks an N–O bond in the symmetric intermediate to form N2O"), I would like to refer your attention to the paper Wei et al. 2019, Geochimica et Cosmochimica Acta 267, 17–32, https://doi.org/10.1016/j.gca.2019.09.018, where in section 4.2 we discussed possible pathways ("end members") of hybrid N2O formation, i.e. via cis-hyponitrous acid, trans-hyponitrous acid and nitramide, all leading to N2O with different SP values. We have cited in this section the relevant papers in which the respective SP values were either measured or predicted. You may want to include these hypothetical pathways in your discussion or conclusions, but do not feel obliged to do so.

Thank you for pointing out this excellent work on potential $\delta(^{15}N^{sp})$ values of hybrid N_2O formation. We have added two references to this work to our discussion of hybrid $\delta(^{15}N^{sp})$:

The question, then, is what reaction would be specific enough to have one N derived from each substrate, but not specific enough to govern ^{15}N placement in the resulting N_2O? One such reaction could be the combination of NH_4^+ and NO_2^- to form a symmetrical intermediate such as hyponitrous acid (HONNOH, or hyponitrite $^-ONNO^-$ in its deprotonated form), which has been discussed as a possible intermediate in hybrid nitrous oxide formation (Wei et al., 2019). Hyponitrous acid may react to form N_2O via breakage of one of the N–O bonds, resulting in N_2O that contains a 1:1 ratio of NH_4^+:NO_2^-. With a precursor such as hyponitrite or hyponitrous acid, equal formation of $^{45}N_2O^{\alpha}$ and $^{45}N_2O^{\beta}$ could be achieved with non–selective N–O bond breakage.
[…]
This means that a $\delta(^{15}N^{sp})$ endmember could potentially be established for hybrid N_2O production, even though hybrid N_2O production draws from different substrate pools. Wei et al. (2019) discuss possible pathways or end members of hybrid N_2O formation, i.e. via cis-hyponitrous acid, trans-hyponitrous acid and nitramide, all leading to N_2O with different $\delta(^{15}N^{sp})$ values. More studies are needed to determine the $\delta(^{15}N^{sp})$ of N_2O produced by ammonia–oxidizing archaea under a range of conditions.

Reviewer 1

L198. "… was added to each sample a final concentration of …" I think "a" between "sample" and "final" should be "at".
Corrected.

L682. “Fig. 8g-h” must be “Fig. 8a-d” or “Fig. 8a-b”.
Corrected.

L684. “Fig. 8h” should be “Fig. 8b”.
Correct.ed