

Reviewer comments are written in bold italics; our answers are kept in plain font.

General comment: This manuscript by Bardhan et al. reports N₂O concentration and multi-isotope abundance from the central Baltic Sea. They found that, in oxic waters, N₂O accumulates with isotopic signatures indicating ammonia oxidation-derived N₂O source; in suboxic or anoxic waters, they found elevated isotopes signatures and attributed the N₂O processes (small concentration) to consumption by denitrification or even chemo-denitrification. Overall, this publication is easy to follow. However, as its current form, this study does not deliver a strong enough conclusion.

We thank the reviewer for their constructive and helpful comments and suggestions about our paper. Following, we reply to each issue individually, and explain the changes we will make to the revised manuscript to meet the reviewer's criticism.

- ***With samples during one cruise, the study heavily relies on the fragmented isotope results (some are not available due to low concentration) for discussion of N₂O processes. Little information from other parameters are implemented for supporting such explanations, including salinity, temperature and even DOC/DOM contents. In addition, more side-by-side comparison with other N₂O isotope studies and summary in figures/tables may be necessary.***
 - Thank you for the suggestion. We have added salinity and temperature data under Supplementary Information. We do not have DOC/DOM data. We have now also included a paragraph on N₂O isotope studies from some aquatic systems to be included in the revised version :
 - Studies on N₂O isotope data are scarce, especially from fresh and brackish water systems. Ho et al. (2023) used a combination of N₂O and NO₃⁻ isotopic data from the urbanized Scheldt estuary in Europe and observed denitrification to be the dominant pathway of N₂O production. Ammonia oxidation, on the other hand, was the most important source of N₂O in the eutrophic Pearl River Estuary in China (Zheng et al., 2024). The isotope ratios of N₂O identified submarine groundwater discharge to deliver N₂O -laden water to the shallow salt-wedge Werribee River estuary in Australia (Wong et al., 2020). Thus N₂O isotopic data can shed light on pathways of production, consumption as well as sources of this trace gas.

References:

1. Ho, L., Barthel, M., Harris, S., Vermuelen, K., Six, J., Bodé, S., Boeckx, P., and Goethals, P.: Unravelling spatiotemporal N₂O dynamics in an urbanized estuary system using natural abundance isotopes, *Water Research*, 247, 120771, 2023.
2. Zheng, Y., Zhan, L., Ji, Q., and Ma, X.: Seasonal isotopic and isotopmeric signatures of nitrous oxide produced microbially in a eutrophic estuary, *Marine Pollution Bulletin*, 204, 116528, 2024.
3. Wong, W. W., Lehmann, M. F., Kuhn, T., Frame, C., Poh, S. C., Cartwright, I., and Cook, P. L.: Nitrogen and oxygen isotopomeric constraints on the sources of nitrous oxide and the role of submarine groundwater discharge in a temperate eutrophic salt-wedge estuary, *Limnology and Oceanography*, 66(4), 1068-1082, 2020.

- ***The discussion was formulated in a simple and thus uncertain way. I am not totally sure hydrogen sulfide detection is enough for identifying specific regions of biogeochemical cycling in the ocean water. Further, the interpretation of the isotope signatures need to be revised: instead of calculating kinetic isotope effects, it is highly important to consider both N₂O production and consumption at every station. Even for large production of N₂O (net concentration), consumption is always happening.***
 - The existing knowledge from Baltic Sea shows chemolithoautotrophic denitrification to be an important process and the presence of H₂S is critical for this pathway to take place. Looking at the data from the suboxic depths, we observed a distinct pattern between the study sites with and without detectable sulfide levels. This is why, it made sense to group the data in this fashion.
 - We agree that the simultaneous production and consumption of N₂O are quite common and frequently observed in oxygen deficient zones. We also observed the possible production of N₂O by fungi and reduction by bacteria in our study site. So we did not calculate kinetic isotope effects, but rather the apparent isotope effects (combining production and consumption) similar to Wenk et al. 2016. We also acknowledge the limitations of the closed-system Rayleigh approach in field studies. We have added this clarification in the revised manuscript.

Reference :

- Wenk, C.B., Frame, C. H., Koba, K., Casciotti, K. L., Veronesi, M., Niemann, H., Schubert, C., Yoshida, N., Toyoda, S., Makabe, A., Zopfi, J., and Lehmann, M. F.: Differential N₂O dynamics in two oxygen-deficient lake basins revealed by stable isotope and isotopomer distributions, *Limnol. Oceanogr.*, 61, 135-1739, 2016.

Specific comments:

Line 12: “ δ values” should not be written as “d”.

Thank you. Corrected.

Line 60-64: Strong reduction of N₂O will also result in enrichment of SP.

Added.

Line 228-230: Keeling plot approach is based on the assumption of simple mixing between source and background, which is clearly not the case here. The production and consumption processes may be quite different from station to station and long the vertical profiles.

We agree. We are using the lack of significant linear relationships in the Keeling plots to stress on the importance of atmospheric N₂O in the oxic waters and to show variability among stations.

Line 379-395: Regarding NosZ I and II genes and the likely regulatory mechanism on N₂O is beyond the scope of the study and the collect observation evidence. I suggest to leave them out.

The reason we wrote this discussion was to include all possible pathways/players that may have an impact on the isotopic signature of N₂O. This highlights the need to include these investigations in future studies. However, taking the reviewer's comment into consideration, we will reduce this paragraph to a few sentences and mention that it is beyond the scope of our study.