Dear Mr. Ingeniero,

Thank you for submitting the revised version of your manuscript. Two of the three previous referees have reviewed the revisions and provided overall positive feedback. However, there are still concerns regarding the clarity of the discussion (particularly in section 4.3) and the availability of data. Please review the comments thoroughly and make the necessary revisions.

Additionally, when resubmitting, please include a point-by-point response to the Reviewers' comments.

Best regards, Yuan Shen

Dear Dr. Yuan Shen,

Thank you very much for your time and effort in reviewing our manuscript. We appreciate the constructive feedback provided by the referees and your guidance in enhancing the quality of our work.

We have thoroughly reviewed the comments and made the necessary minor revisions to address these issues. Additionally, we included a detailed point-by-point response to the Reviewers' comments in our resubmission. Our responses are in blue font and in italics.

Thank you once again for your invaluable feedback and support.

Best regards,

Riel Carlo O. Ingeniero On behalf of the Authors

## **Reviewer 1:**

I'm glad the author addressed my previous concerns. And I found the quality of the manuscript has been improved. I think the current form is now acceptable for publication. Only some minor suggestions left:

Thank you very much for your time and effort in reviewing our paper. Your valuable suggestions and comments have significantly enhanced the quality of our manuscript, making it ready for publication. We greatly appreciate your detailed feedback and thoughtful recommendations, which have contributed to the improvement and refinement of our work.

### Main text:

Fig. 7, Fig S4 and S6 The authors replied that they have made separate correlation plots of NO vs other parameters. But I can't find NO vs salinity plot, NO vs. oxygen plot, and NO vs. AOU plot in other places (e.g., Fig. 8) as I commented on the 1st version manuscript. I would still suggest that NO be added to these figures, as NO is at the center of the discussion in this study.

Response: We have added NO in these plots.

Line 45: ranging from  $0.70 \times 10^{-17?}$ 

*Response: This was intended to mean as* 0.70 *to* 45.00 (×  $10^{-17}$  mol cm<sup>-2</sup> s<sup>-1</sup>). We have followed the Reviewer's suggestion and edited the text for clarity

Line 360 R2 ammonium monooxygenase should be ammonia monooxygenase.

*Response: We have followed another Reviewer's suggestion to use the standard abbreviation for the enzymes.* 

Line 473 Ludwig Prandtl should be Italic.

*Response: Thank you for your attention to detail. We have italicized Ludwig Prandtl following the editorial guideline of Biogeosciences on the name of research vessels.* 

Supplementary information (SI): Line 5 I see that the authors have revised the address in the text, keep consistent in the SI.

*Response: We updated the address of our research institution in both the manuscript and supplementary information* 

Line 50 Table S2 Chlorophyll a (mg L-1) the ")" is incorrectly up-scripted.

*Response: We have corrected the typographic error and changed mg*  $L^{-1}$  *to µg*  $L^{-1}$ .

Units of Chlorophyll a appeared as  $(\mu g L-1)$  (e.g., Fig. 2) or (mg L-1) (e.g., Table S2) through text and SI. Check and make them consistent.

*Response: I am really sorry for the oversight. It should definitely be*  $\mu g L^{-1}$ *. We have revised this in the manuscript and supplementary information and checked for consistency.* 

Ensure that revisions/changes in supplementary information are "accepted" in word/latex/etc. for final publication.

Response: We have followed the Reviewer's suggestion to ensure that revisions or changes are accepted.

# **Reviewer 2:**

### General appraisal

In their revised manuscript and author comments, Ingeniero et al. addressed my major concerns about the paper (namely, the assumption that nitrifier-denitrification was the only reductive process that may be occurring in their study site). While the authors do not spend a lot of time on the implications of their study for global biogeochemical cycles, the measurements are novel and provide another piece of the puzzle of marine NO cycling.

My main criticism of the revised manuscript is that it should be streamlined and revised for clarity. As it is, the discussion is a bit convoluted and difficult to read — especially section 4.3 (see below).

Also, at this stage of publication, the data should be deposited in a repository with an associated DOI. Not enough to say it "will be made available."

*Response: Thank you for your valuable input and for helping to make our manuscript ready for publication. We appreciate the effort and time you have dedicated to reviewing our paper.* 

We acknowledge that our discussion on the implications for the global biogeochemical cycle is not extensive. We tried to avoid overstating or exaggerating the implications of our findings. Nevertheless, we think that our work serves as a solid foundation and will support future research on nitric oxide measurements in estuarine and coastal systems.

We have revised the data availability section. The FerryBox data are readily available at the Coastal Observing System for Northern and Arctic Seas (COSYNA) data portal (https://codm.hzg.de/codm/). We have also uploaded our data to another open-access data repository Zenodo: https://doi.org/10.5281/zenodo.11548798.

### **Specific comments**

Lines 130-131: Why not just use the GSW MATLAB toolbox to calculate density?

Response: We acknowledge that there are multiple methods to calculate seawater density from temperature and salinity. Unfortunately, the main author was not familiar with the GSW MATLAB toolbox at the time of writing the manuscript. Additionally, the seawater density MATLAB function is straightforward and convenient for our purpose. Other papers have used the same tool in calculating seawater density:

Barker, S., & Knorr, G. (2023). A systematic role for extreme ocean-atmosphere oscillations in the development of glacial conditions since the Mid Pleistocene Transition. *Paleoceanography and Paleoclimatology*, 38, e2023PA004690. <u>https://doi.org/10.1029/2023PA004690</u>

292-303: I would drop these two paragraphs and just say, "Nonetheless, salinity alone is insufficient to explain the uneven distribution of NO at our study site, indicating that other parameters influence NO concentrations along the Elbe estuary." The salinity gradient tells you about mixing but not about the sources of NO, so I think it's sufficient in this section simply to point out that the weak negative correlation between NO and salinity indicates that higher NO concentrations in the Hamburg Port area mix out as you move towards the North Sea.

*Response: We followed the Reviewers' comments to remove the two paragraphs and edited the last concluding sentence.* 

Line 304/Section 4.3: This section still needs to be streamlined and clarified. Is the main point just that high DIN doesn't necessarily lead to high NO? Or that there isn't much evidence for NO photoproduction in your study area?

Response: We began this section by providing context on the primary sources of NO in marine environments, particularly in the open ocean—NO photoproduction and biological production. Previous research (Zafiriou and McFarland, 1981; Zafiriou and True, 1979; Gong et al., 2023) established that the photolysis of nitrite  $(NO_2^{-})$  is a primary source of NO in marine environments.

The main point in Section 4.3 is to highlight that high dissolved inorganic nitrogen (DIN) concentrations do not necessarily lead to high NO concentrations. Our observations in the Elbe Estuary demonstrated that despite high DIN levels, NO concentrations were not correspondingly elevated compared to other study sites. We have decided to remove the sentences about turbidity and suspended matter, which were added in response to another Reviewer's comment, as this part was highly speculative.

We hypothesize that microbial nitrogen cycling processes might have a greater influence on the NO concentrations observed in the Elbe Estuary than NO photoproduction. This section provides a good transition to the subsequent sections, where we discuss the role of microbial nitrogen cycling processes in detail, which we aim to highlight in this study.

Line 336/Table 1: Here, is N2O just the concentration or  $\Delta$ N2O? Figure 7 is  $\Delta$ N2O...

Response: Table 1 and Figure 7 are referring to different results. Table 1 discusses the correlation analysis between NO and different nitrogen components, while Figure 7 presents AOU vs  $\Delta N2O$ , which is helpful in our discussion of the nitrification process.

Line 360: Specify that this reaction is for ammonia-oxidizing bacteria; the exact pathway and enzymology for archaeal nitrification is still a matter of debate. Also, use the commonly accepted abbreviations for each enzyme to make this figure easier to read. E.g., amo instead of ammonium monooxygenase.

Response: We followed the Reviewer's comment that the reaction shown is for ammonia-oxidizing bacteria. Initially, we used the full names of the enzymes to aid readers who may not be specialists in the nitrogen cycle. However, we have now revised the text accordingly and used commonly accepted abbreviations for each enzyme.

Lines 460-462: Wait, I thought you had a whole section on how your study challenges the assumption that higher concentrations of nitrogen nutrients automatically lead to increased dissolved NO concentration?

Response: Yes, the study does challenge that assumption. It highlights that site-specific conditions, such as microbial nitrogen cycling, should also be taken into account. We noted that despite the high nutrient concentration in the Elbe Estuary compared to other study sites, the dissolved NO concentration did not correspond to a higher concentration in the Elbe Estuary. It is crucial to consider these site-specific conditions rather than assuming a direct correlation between nitrogen nutrient concentration and dissolved NO concentration. For clarity, we have removed the sentence.

Technical corrections

Line 46: global estimates OF oceanic NO emissions Response: We have edited the text as suggested by the Reviewer.

Line 423: should be "these sampling locations" *Response: We have edited the text as suggested by the Reviewer.*