

Response to Reviewer 2

This is an interesting paper providing new data on NO distribution and fluxes and with potential for improving our insight in the complex biogeochemistry of N-transformation in estuarine/riverine environments. The analytical procedures for data acquisition are explained in detail and the quality of the data seems very robust.

Next to presenting the estuarine profile of NO concentrations and fluxes together with other physico-chemical parameters (Temp, Sal, O₂, n₂, Chl ..) authors proceed with discussing possible processes steering the observed distributions. This is done exclusively based on regression analyses. I found this part of the paper based on a lot of speculation, forcibly as no other tools permitting process identification and process rate assessment were applied. This weakens somewhat the strength of the paper which therefore rests mostly on the quality of the analytical part. Especially N, O isotopic composition measurements of nutrients could possibly confirm/infirm occurrence of nitrification/denitrification and resolve impact of both processes. I can understand such an approach was not possible in the present context, but isotopic data for the Elbe have been published by others (Dähnke et al.), and some thoughts on how these fit with the present observations might have been a useful addition to the paper. Can authors comment on this?

We are grateful to the Reviewer for dedicating his/her/their time and effort to provide constructive feedback, which is instrumental in enhancing the depth and clarity of our manuscript. We are heartened by the positive evaluation of our analytical procedures and the robustness of our data.

Indeed, to explain the NO distribution observed during our campaign in the absence of additional data, we employed regression analysis to assess the relationship of NO with various dissolved nitrogen substrates. Regression analysis allows us to determine the degree to which NO concentrations vary with changes in the levels of these parameters, providing insights into potential underlying biogeochemical and microbial processes.

Our analysis revealed significant correlations between NO and other measured parameters. These significant correlations are suggestive of systemic relationships that may not be immediately apparent without statistical investigation. By employing regression analysis, we were able to quantify the strength and direction of these relationships, offering a foundation for hypothesizing about the interactions occurring between NO and these parameters. This method, while inferential, presents a valuable first step toward understanding complex environmental interactions, particularly when more direct methods of assessment are not available.

The significant findings from the regression analysis warrant further study. We acknowledge that our approach could be enriched by incorporating other biogeochemical tools such as measurements of nitrification/denitrification rates, assays for nitrogen marker genes, and analyses of stable isotopes of nitrogen and oxygen.

Nevertheless, as the initial measurement of NO in the area, our study could lay the groundwork for future research. To enhance the discussion, we will ensure that relevant previous publications are explored comprehensively in the revised manuscript, rather than merely cited in passing.

In earlier manuscript drafts prepared for submission to Biogeosciences, we explored including data on dual stable isotopes of nitrate. Our analysis indicated that mixing or dilution predominantly affects the coastal/brackish and limnic zones, with nitrogen cycling processes being more pronounced in the Hamburg Port Area. We ultimately decided against including this data to maintain the focus of our manuscript without

delving into the intricacies of dual stable isotopes of NO_3^- in a study not primarily focused on stable isotope biogeochemistry. We believe this decision helps to maintain clarity and focus in our discussion.

Specific comments:

Were any data obtained for the tributary rivers Oste, Meden, Stör ?

No data were obtained for the tributary rivers Oste, Meden, and Stör. However, we still added this on the Map and Figure legends since there are a few sentences in the manuscript that we referred to Oste and Meden. It might guide readers not familiar with the study site about the tributaries we mentioned in the manuscript.

- An increase in NO_2^- and NH_4^+ concentrations was also observed downstream of the maximum turbidity zone (Dähnke et al., 2022) at the confluence of River Oste and Meden.
- Concentrations started to increase slightly above the detection limit at the outflow of the River Meden near Otterndorf at Elbe-km 710 and 714.

Regarding Stör, we observed the following: A slight decrease in O_2 concentration and pH and a slight increase in chlorophyll a (Fig. 2) and nitrate (Fig. 3) concentrations at the confluence of River Stör. These are minor changes that noticeably deviate from the general mixing in the Estuary. We did not discuss this in the manuscript as it deviates from the main focus of the paper. However, future researchers working on rivers and estuaries might conduct further research on the influence of tributaries on NO dynamics in the Elbe Estuary. As suggested, we will ensure that the caption describes the tributaries to enhance clarity. We will also correct the typographic error Stör to Stör in the revised manuscript.

In section 4.3 photolysis is mentioned as a source of NO but this is very little discussed further. Can it be a significant process in a turbid estuary? Are there data for suspended matter load, vertical light profiles?

Previous research (Zafiriou and McFarland, 1981; Zafiriou and True, 1979; Gong et al., 2023) has established that the photolysis of nitrite (NO_2^-) constitutes a primary source of nitric oxide (NO) in marine environments. However, the significance of this process in turbid estuarine systems, such as the Elbe Estuary, remains an open question.

The literature presents conflicting evidence regarding the influence of nitrite concentrations on the levels of dissolved NO. Some studies suggest a direct correlation, while others do not find a significant relationship, indicating the complexity of the factors that control NO levels in dynamic environments. We did not find any direct relationship between NO and suspended particulate matter.

Lines 313-315: Can presence/absence of anamox activity be confirmed based solely on information of O_2 conc.? Could this process possibly proceed inside micro-environments such as aggregates, flocs with low internal O_2 ?

We agree that the current data does not definitively rule out other processes, such as anammox, and that it is prudent to consider such as an alternative NO production source. We will revise the manuscript to reflect a more balanced view of potential NO sources, acknowledging the strong correlations observed and how they may also include anammox.

Lines 322-326: As written, the reader gets the impression AOU is solely set by O₂ consumption during nitrification. What about respiration?

In our discussion of the linear relationship between $\Delta\text{N}_2\text{O}$ and AOU, it appears the original text led to the misunderstanding of AOU being solely attributed to oxygen consumption during nitrification. We acknowledge this oversight and clarify that AOU is influenced by a variety of biological and chemical processes in the ocean, including both nitrification and aerobic respiration. We will improve the clarity of the text to present our intention to note that the significant linear relationship between $\Delta\text{N}_2\text{O}$ and AOU is usually associated with N_2O production through nitrification (Schulz et al., 2023a; Brase et al., 2017; Nevison et al., 2003; Walter et al., 2006).

Lines 335-336: This sentence leaves us asking so what ? Detail the meaning. How does it clarify the foregoing statement?

The sentence in Line 335-336 reads: “Furthermore, we observed that five sampling sites in the coastal-brackish zone with $\text{O}_2 > 200 \mu\text{M}$ had NO concentrations less than the detection limit (Fig. 6).”

This sentence discussed the observation that NO concentration appears to be very low in the coastal-brackish zone, probably due to the relatively higher oxygen concentration. It is known that NO is reactive with O_2 . In the nitrification reaction, NO can be oxidized further to NO_2^- and NO_3^- . We will elaborate on this sentence and provide substantial references to support our argument.

Lines 361-365: These statements are unclear and the rationale is difficult to follow. Try to clarify.

We will edit these statements and discuss in detail the nitrification process. Another Reviewer agreed that the lack of correlation between nitrate and apparent oxygen utilization (AOU) in the Hamburg Port area may indicate the presence of denitrification or nitrifier-denitrification. However, we cannot rule out one or another. What we can conclude from the data is that there is a process other than nitrite oxidation that consumes nitrate in the Hamburg Port.

Lines 366-368: The possible role of suspended particles with low internal O₂ is mentioned for the port area. How does this look in the downstream maximum turbidity zone ?

We observed that the NO concentration started to increase in the downstream maximum turbidity zone near the confluence of River Meden and Stör.

Lines 400 and further (Conclusions): Will a higher temporal resolution and improved sampling strategy be sufficient to get insight into the dynamic interplay of controlling factors? Would adding stable N, O isotopic methodologies be helpful ?

Increasing the temporal resolution of our sampling would indeed yield helpful information on whether seasonality affects NO concentration and sea(water) to air fluxes. To date, no study has done this; doing so will enhance our understanding of the nitrogen dynamics and the processes of NO production and consumption within the estuary. Such detailed temporal data could reveal patterns that are not discernible at lower sampling frequencies including diurnal cycles and episodic events.

Incorporating dual stable isotope techniques and the measurement of nitrification rates across all sampling sites would significantly strengthen our study. This methodology would allow us to trace the pathways of nitrogen transformations more precisely and could provide definitive evidence of nitrification and other nitrogen-related processes. Additionally, the use of molecular or genetic tools to detect marker genes

specific to nitrogen-cycling microbes would offer insights into the microbial contributions to observed nitrogen transformations. These genetic markers could help us pinpoint the active microbial communities and link them to the biogeochemical processes we are studying. Overall, integrating these advanced methods in future studies will deepen our analysis and provide a more comprehensive interpretation of the results.

Technical issues:

Figure 1: legend should mention tributaries Oste, Meden, Stör.

We will revise the Figure caption to mention the tributaries.

Line 336: reference to Fig. 6.. is this correct, or should it be Fig. 5 ?

Thank you for your attention to detail. We will ensure that the revised manuscript will properly reference the final Figures in the text.

Line 361: Fig 7g should be Fig S7g

Thank you for your attention to detail. Similar to our response above, we will thoroughly check that the revised manuscript will properly reference the final Figures in the text.

AOU is given without unit

We apologize for the oversight. In our revised manuscript, we will provide the unit ($\mu\text{mol L}^{-1}$) of AOU.

References:

Gong, J.-C., Jin, H., Li, B.-H., Tian, Y., Liu, C.-Y., Li, P.-F., Liu, Q., Ingeniero, R. C. O., and Yang, G.-P.: Emissions of Nitric Oxide from Photochemical and Microbial Processes in Coastal Waters of the Yellow and East China Seas, *Environmental Science & Technology*, 57, 4039–4049, <https://doi.org/10.1021/acs.est.2c08978>, 2023.

Zafiriou, O. C. and McFarland, M.: Nitric oxide from nitrite photolysis in the central equatorial Pacific, *J Geophys Res*, 86, 3173, <https://doi.org/10.1029/jc086ic04p03173>, 1981.

Zafiriou, O. C. and True, M. B.: Nitrite photolysis in seawater by sunlight, *Mar Chem*, 8, 9–32, [https://doi.org/10.1016/0304-4203\(79\)90029-x](https://doi.org/10.1016/0304-4203(79)90029-x), 1979.