Response to Reviewer 3

Summary

The manuscript titled "Dissolved Nitric Oxide in the Lower Elbe Estuary" by Ingeniero et al. quantified the fluxes of nitric oxide (NO) in relation to other nitrogen cycle parameters in the Elbe River Estuary and Hamburg Port Area. Using a clever chemiluminescent detection method and flowthrough sampling system, the authors measured dissolved NO concentrations in surface waters alongside temperature, salinity, pH, and dissolved oxygen (O₂). The authors made concurrent measurements of nitrate, nitrite, and ammonium with an autoanalyzer and nitrous oxide (N₂O) with laser spectroscopy. The authors found that NO was supersaturated in the surface layer of both study areas, so they were both a source of NO to the atmosphere. Based on the concurrent [O₂] and dissolved inorganic nitrogen measurements, the authors conclude that this NO is likely produced via biological processes (nitrification, denitrification, and nitrifier-denitrification), as opposed to the photolysis of nitrite.

General Appraisal

In this paper, the authors present the first-ever measurements of NO in the Elbe River system. NO measurements in the literature are scarce because its short lifetime makes analysis difficult, so this paper represents a substantial contribution to our understanding of NO in the marine environment. Furthermore, the authors measure significant NO supersaturation and fluxes in the surface waters of much of the Elbe River, which is important because NO is a contributor to smog, acid rain, and ozone.

The major strengths of this paper are the presentation of novel, high-resolution NO measurements and the clear relationships that emerge between NO and other inorganic nitrogen species, $[O_2]$, pH, and chlorophyll. The authors present a clean, concise interpretation of these results and the paper is generally straightforward and easy to read.

The major weakness of this paper is that the discussion of temporal variability (day/night and seasonal variations) is not linked to the clear boom-and-bust cycle seen in the Hamburg Port area. The authors have locations with peaks of chlorophyll and $[O_2]$, and other locations with oxygen and pH minima and N_2O and NO maxima. This implies to me that there are some locations where you captured net production and others where they captured net respiration, which draws down $[O_2]$ and creates an ideal environment for N_2O and NO production in sediments or particles. The authors allude to this in the conclusions, but how would day-night temporal variation at each site affects the data? Would blooms in some locations propagate downstream and create pockets of high respiration further downstream? The authors have a paragraph in the conclusions about potential temporal effects, and my suggestion would be to move this paragraph into the discussion and link it more clearly to their results.

The paper is generally well-written. There are only a few grammatical errors and clumsy sentences that I note in the technical corrections.

My primary concern is about the conclusion (and I believe this is only stated in the abstract) that nitrifier-denitrification is the primary source of NO in the Hamburg Port area. While I agree 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, I don't think you can rule out one or the other. In other words, all you can conclude from this data is that there is a process other than nitrite oxidation that is consuming nitrite. Likewise, if you invoke denitrification and/or nitrifierdenitrification in sediments or particles, I don't think you can rule out the presence of anammox. In fact, instead of ruling out anammox based on water column $[O_2]$, you should list it as a potential alternative source of NO. The strong correlations between NO, nitrite, and ammonium may indeed be a sign of anammox as a source of NO in the Hamburg Port area. Also, while denitrification and/or nitrifier-denitrification may be present in this zone, the water column $[O_2]$ suggests that the primary source of NO would still be nitrification, and this is supported by the strong correlations in this zone between NO, nitrite, and ammonium.

We appreciate your recognition of the novel contributions our work makes to the field – the first-ever measurements of NO in the Elbe River system and the identification of significant NO supersaturation and fluxes in surface waters. Your acknowledgment of the clarity and readability of the paper is encouraging.

Your critique concerning the discussion of temporal variability and its connection to the observed biogeochemical cycles within the Hamburg Port area is well-founded. We will incorporate your suggestion in our revised manuscript.

Regarding the primary sources of NO, we acknowledge the Reviewer's concerns about the conclusiveness of nitrifier-denitrification as the dominant process in the Hamburg Port area. We agree that the current data does not definitively rule out other processes, such as anammox, and that it is prudent to consider such alternative NO consumption processes. We will revise the manuscript to reflect a more balanced view of potential NO sinks or sources, acknowledging the strong correlations observed and how they may implicate various nitrogen-transforming processes, including anammox.

Thank you for the helpful and very detailed comments. The detailed suggestions will be thoroughly implemented to enhance the manuscript's technical quality. We have addressed the concerns highlighted by the reviewer and detailed the changes we intend to implement in the revised manuscript to address the reviewer's critiques. Reviewer comments are presented in bold italics, while our responses are in plain font. We look forward to submitting a comprehensive revised manuscript that addresses the points you've raised.

Specific comments

Line 14: Is the same chemiluminescent optode spot system often used for O_2 (Frey et al., 2023)?

No. The Luminescence Measuring Oxygen Sensors used by Frey et al. are different from our detection method: We used a chemiluminescent method for NOx which is typically used for atmospheric monitoring of NOx. Lutterbeck and Bange (2015) describe the method in detail. In our earlier drafts of the manuscript, we cited the method paper by Lutterbeck and Bange (2015) in the Abstract for clarity. However, adhering to standard writing practices, we omitted this citation from the Abstract in the final draft when we submitted the paper to Biogeosciences. This paper, if published, would be the first application of the method in a coastal and estuarine environment. We will edit the text as follows:

"The discrete surface water samples were analyzed using a chemiluminescence NO analyzer connected to a stripping unit."

Line 15: Why not write pM instead of 10^-12 mol/L? You do so later in the manuscript.

Thank you for your comment. For consistency, we will follow your suggestion to use pM instead of 10^{-12} mol L⁻¹.

Line 20: Based on your discussion, this could be nitrifier-denitrification or denitrification. I don't think you can rule out one or the other based on your data.

We agree with this comment. While we cannot rule out which exact nitrogen cycling processes could be present, we think that nitrifier-denitrification or denitrification influences the NO distribution in the Hamburg Port Area. We will be including denitrification in the text.

Line 34: What is the lifetime of NO in seawater/water?

The lifetime of nitric oxide (NO) in seawater or water is relatively short due to its high reactivity. In aquatic environments, NO can rapidly react with oxygen, metals, and organic compounds. The exact lifetime can vary depending on several factors, including temperature, pH, and the presence of reactants, but it is typically on the order of a few seconds to a few minutes (i.e. 3 - 100 s) (Zafiriou and McFarland, 1981; Olasehinde et al., 2010). We will provide these values in the revised manuscript.

Line 72: The way this equation is written is confusing. Are you multiplying the corrected O_2 by 1.12? Or the uncorrected? What are the units of the intercept? Also, does the intercept of 13.41 mean that the detection limit of the oxygen optodes is 13.41 (units?)?

We will edit the O_2 correction equation. The revised equation will be stated as: $[1.12 \times O_{2(optode measurement)}] + 13.41$ ($R^2 = 0.97$). The unit is μ M.

Line 83: Give us some numbers for what this lifetime is

Please see our response above (-> Line 34). We will add these values in the revised manuscript.

Line 84: So the calibrator is just an NO source, right?

Yes, this is right. It is a portable calibration source that operates using a compact nitrous oxide (N_2O) cartridge, producing gas output that is traceable to the US National Institute of Standards and Technology (NIST) standards, as detailed in the study by Birks et al. (2020).

Line 90: Why do you need the calibrator in addition to the aqueous NO standard solutions?

The calibrator is used to adjust the NO analyzer, ensuring its responses are accurate and reliable. This step is fundamental because it directly affects the instrument's precision and accuracy, ensuring that its readings are consistent with "true" NO concentrations. Calibration with the calibrator involves adjusting the instrument's response to known concentrations of NO gas. This process ensures that the instrument's detection and measurement systems are properly aligned with the actual concentrations, correcting for any drift, sensor degradation, or other factors that might affect accuracy over time. Meanwhile, the aqueous NO standard solution is used for method calibration.

Line 94: This calculation is to convert the mole fraction you measure in the headspace to the dissolved NO concentration, right? Is there a reason to assume that the headspace is at a pressure of 1 atm? I would assume it would be slightly over pressurized... how would that affect your measurements?

We use the stripping method detailed in Lutterbeck and Bange (2015). Furthermore, the NO analyzer operates with atmospheric pressure input and will display an error if it exceeds a certain pressure threshold. A needle valve was also installed to reduce pressure variations.

Line 97: Here you use pM. I would stick to this throughout the text.

Thank you for pointing out the inconsistency. We have revised the manuscript to ensure that 'pM' is consistently used throughout the manuscript.

Lines 102-103: In eqn. (2) you assume the barometric/atmospheric pressure is 1 atm. Is this a reasonable assumption at this time of year, in this part of the world?

The average air pressure in Hamburg during this time is at 1009 hPa, or when converted to atmosphere, is 0.9958 atm which is close to 1 atm.

See https://meteostat.net/en/place/de/hamburg?s=10147&t=2021-07-27/2021-07-29 (last accessed 1 March 2024), which uses weather data from NOAA.

Line 125: Same comment as above with setting atmospheric pressure to 1 atm.

Please see our response above for Lines 102-103.

Lines 129-130: How was this mean value calculated? Mean of all hourly measurements at all monitoring stations over the study period? Given the short lifetime of NO, doesn't it make sense to calculate a mean c_{EQ} on a day-by-day or even shorter basis - or do all of the stations look like figure S2, where the hourly concentrations are all within error of the average?

You are correct. This is the mean of the average hourly measurement at all monitoring stations over the study period. We excluded nighttime values as NO concentrations are rather low in the evening due to low emissions from vehicles. We think this is a conservative estimate of the NO concentration in the Elbe Estuary.

Lines 172-174: Is the variability of $[O_2]$ because of changes in productivity?

The variability in $[O_2]$ levels can indeed be a result of changes in productivity. Note that the measurements were taken during the daytime when net productivity should be higher. During photosynthesis, phytoplankton consume CO_2 and release O_2 , which increases the $[O_2]$ in the water. The higher the phytoplankton productivity, the more O_2 is produced. Additionally, photosynthesis affects pH levels. As phytoplankton consume CO_2 , they can reduce the amount of CO_2 in the water, which can cause the water to become less acidic (increase in pH level).

Lines 184-185: Report a number for the maximum concentration to give a sense of scale - 200 μ M is a lot!

To improve specificity, we will edit the sentence and provide the value of the concentration.

Lines 200-201: It looks like the peaks in N_2O correspond to the minima in $[O_2]$ - if that's the case, worth pointing out here.

Yes, this is correct. We will edit the sentence to emphasize N_2O production in minimum dissolved O_2 concentration (See also Fig. S6 f).

Line 225: You should also mention that the peaks in NO in the Hamburg Port area correspond to the peaks in N_2O , NO_2^- , and NH_4^+ ?

This is correct for two peaks in the Hamburg Port Area but not in the maximum NO concentration measured.

Line 232: I would recommend converting these flux values to fM: 0.31-55 fmol cm⁻² s⁻¹.

We agree that it would have been better to use the shorter name. However, for the sake of intercomparability with previous research, we decided to use scientific notation in reporting the flux values.

Line 238: How do your measurements compare to previous measurements in terms of saturation? If 147-274% saturated is at the low end of marine NO measurements, I'm curious what these higher concentrations correspond to. This would imply that the ocean could be a major source of NO to the atmosphere!

We will update the discussion to include saturation values of previous studies (if these are available).

Lines 251-269: I would avoid interpreting a relationship that is not statistically significant. This section is mostly literature review anyways.

While the relationship in our findings is not statistically significant in linear correlation analysis, the general trend remains that at lower salinity values (with higher DIN) NO values are also relatively higher. In this section, we want to emphasize the importance of DIN input from freshwater, particularly ammonium and nitrite on the NO distribution.

Lines 276-278: This is a really important finding: you have much higher NO_3^- , NO_2^- , and NH_4^+ than previous studies in other rivere and coastal areas, but not higher NO. What is unique to the Elbe river compared to the other rivers cited here?

The question of why the Elbe Estuary, with relatively higher nutrients, has a lower NO concentration is still unresolved and requires further investigation. Nevertheless, we think that this observation is an important finding of our study. This observation challenges the assumption that higher concentrations of nitrogen nutrients automatically lead to increased dissolved NO concentration. In our manuscript, subsequent to our discussion that highlighted the Elbe Estuary's relatively higher nutrient levels yet lower NO concentrations compared to other study sites, we delve into the conflicting findings concerning the relationship between NO distribution and nitrogen-containing nutrients (refer to lines 280-291).

We also reported the hypothesis of Ayeni et al. (2021) regarding these conflicting relationships: "...Likewise, Ayeni et al. (2021) also noted that some rivers in Japan with higher NO2– concentrations had lower rates of photoproduction of NO and vice versa, attributing these imbalances to nitrogen cycling processes (nitrification, denitrification, and anammox), which could produce or consume NO, or the photochemical transformation of organic nitrogen from dissolved organic matter producing NO₂– to form NO in areas with low NO2–."

The high reactivity of nitric oxide (NO) as a radical initiates various consumption mechanisms which may influence its concentration in the Elbe Estuary. Zafiriou et al. (1979) reported that there is no evidence of interaction between NO and metals under marine conditions, though NO is known to react with metals yielding nitrosyl (M-NO) or iso-nitrosyl (M-ON) metal complexes (Ford and Lorkovic, 2002; Richter-Addo et al., 2002). Additionally, biological productivity can both consume and, produce NO, further

contributing to its dynamic cycle in the environment. A recent paper by (Bange et al., 2024) noted that the consumption processes for NO in the sea(water) are still unresolved.

Lines 291-292: What about the DN_2O/NO_3^- ratio?

Thank you for your helpful comment. There is a strong correlation between NO and DN_2O/NO_3^- ratio in the Hamburg Port area (R²=0.95, p<0.001) and limnic zone (R²=0.72, p<0.001). We will provide the DN_2O/NO_3^- ratio plot in our revised Figure.

Lines 299-302: So the overall trend (which is positive) is driven by the Hamburg Port area, and the overall trend masks the negative relationships in the limnic and coastal-brackish zones. This is a good example of an ecological fallacy.

Indeed, this is accurate, which underlines the rationale for incorporating this information into the discussion. It is often essential to focus on finer details as opposed to the broader context, as this approach can reveal features influenced by biogeochemical processes within particular ecological zones that might otherwise be overlooked.

Lines 307-308: Add citation: Burlacot et al. (2020).

We will add this important paper discussing algal photosynthesis utilizing NO and producing $\mathrm{N}_2\mathrm{O}$ in our citation.

Lines 308-310: It's worth pointing out that the Chl. peaks occurred right before the NO peaks.

We will revise the manuscript to note this relevant observation.

Lines 313-315: What about the anaerobic process (anammox, denitrification) in the river sediments?

We understand that anammox and denitrification processes could occur in the river sediments. We will briefly provide references regarding these anaerobic processes in the sediments (Schroeder et al., 1991; Deek et al., 2013). There are also previous studies that measured NO in sediments (Sørensen, 1978; Schreiber et al., 2014).

However, we are not certain whether NO released from sedimentary processes significantly impacts NO in the sampled water in the Hamburg Port Area. The overall water depth in the Hamburg Port Area was >15 m, so NO released from the sediments is unlikely to make it to the surface layer where we took the samples (because it has a short lifetime in seawater).

Line 323: You could also look at the relationship of DN_2O/NO_3^- vs. [O2] or DN_2O/AOU vs. O2 (Nevison et al., 2003).

Thank you for your helpful comment. In a revised manuscript we will look at this relationship to support our discussion on the nitrification process in the Elbe Estuary. Shown is the result of our regression analysis. We noted a significant positive correlation between DN_2O/NO_3^{-1} vs. [O2] in the limnic zone but a negative correlation (not significant) in the Hamburg Port Area. Meanwhile, the DN_2O/AOU ratio vs [O₂] is negatively correlated in the overall plot (R²=-0.4031, p=0.02) but there is no significant linear relationship in the coastal brackish zone and limnic zone. There is a significant negative correlation between DN_2O/AOU vs $[O_2]$ ratio in the Hamburg Port Area (R^2 =-0.61928, p=0.03).

{'Overall'	}	{'dN20NitrateRatio'}	0.23955	0.19431
{'Coastal-Brackish Zone	e'}	{'dN20NitrateRatio'}	0.23418	0.65515
{'Limnic Zone'	}	{'dN20NitrateRatio'}	0.66273	0.013565
{'Hamburg Port Area'	}	{'dN20NitrateRatio'}	-0.47012	0.12301
{'Overall'	}	{'dN20A0URatio' }	-0.40311	0.018083
{'Coastal-Brackish Zone	e'}	{'dN20A0URatio' }	-0.27444	0.44287
{'Limnic Zone'	}	{'dN20A0URatio' }	-0.16077	0.61768
{'Hamburg Port Area'	}	{'dN20A0URatio' }	-0.61928	0.031764
	-			

*Line 325: In this context, I would actually call NO*² *a product of nitrification, not a precursor, because* NH_4^+ *oxidation to NO*² *produces* N_2O *and NO as a byproduct; NO*² *oxidation does not.*

You are correct, NO_2^- is a product of nitrification. We will amend the text to accurately reflect nitrite as a product in the nitrification process. Thank you for bringing this to our attention, ensuring the precision of the scientific content of our manuscript.

Line 329: The limnic zone correlations in Figure S7 look like they're being driven by two points at either extreme of NO, while the rest of the points cluster in the middle. I would avoid over-interpreting these plots.

Thank you for your pointing this out. We agree that we have to avoid overinterpretation of the result. However, it is also important to note the significant correlation that exists at p < 0.001.

Lines 351-352: Elaborate here upon why the lack of a significant relationship between NO_3^- and AOU indicates the presence of denitrification or nitrifier-denitrification.

We will revise the text to note that there could be other processes aside from nitrification (not just denitrification/nitrifier denitrification) that affected the NO_3^- and AOU relationship in the Hamburg Port Area. This may also include high respiration/remineralization rates and mixing with water from the port basins, which might impact the correlation.

Line 363-365: If you imply that denitrification could be occurring in the sediments even though the water column oxygen concentrations are too high, I don't think you can rule out anammox based on water column oxygen concentrations.

We agree to this, and we will not exclude the possibility of anammox process without other evidence to rule it out. Genetic analysis of nitrogen cycle marker genes could have been helpful in our data analysis.

Line 367: ... or anoxic microsites within particles.

Thank you for your helpful suggestion. We will add this phrase to the revised manuscript and cite relevant publications (Liu et al., 2013; Xia et al., 2017).

Lines 370-371: I'm really interested in this apparent boom and bust cycle in your data. You have locations with peaks of chlorophyll and oxygen, and other locations with oxygen and pH minima and N_2O maxima. This implies to me that there are some locations where you captured net production and others where you captured net respiration, which draws down O_2 and creates an ideal environment for

N_2O and NO production in sediments or particles. You allude to this in the conclusions, but how do you think day-night temporal variation in each of your sites affects your data? Would blooms in some locations propagate downstream and create pockets of high respiration further downstream?

Thank you for highlighting these aspects of our dataset. The observed fluctuations in chlorophyll and oxygen concentrations, along with the corresponding variations in pH and N_2O levels across different locations, indeed suggest episodic events of net production and respiration. These biological processes are fundamental in shaping the biogeochemical profile of the estuary, with photosynthesis contributing to peaks in oxygen and chlorophyll during daylight hours, and respiration leading to oxygen depletion and potentially creating favorable conditions for N_2O and NO production during nighttime or in less oxygenated microenvironments such as suspended sediments or particulate matter (Schulz et al., 2022).

In line with your comment, we recognize the necessity for a more comprehensive analysis that accounts for temporal variations, including diurnal shifts, in the study of nitric oxide dynamics in estuaries. Additional research, potentially involving continuous monitoring at various sites, would be invaluable in deciphering these complex interactions and understanding how they might influence the distribution and nitrogen cycling in the estuary. We will discuss temporal effects and elaborate on the need for further research on this in our revised discussion

Lines 383-384: You talk very little about photolysis in your discussion so I would remove it here.

We agree to the Reviewer's comment. We will remove photolysis in our conclusion as this is not a major finding in our study.

Lines 385-397: I would move this paragraph on potential temporal effects into your discussion section (see my previous comment). Then summarize it in your conclusions.

We will follow the Reviewer's suggestion to move the paragraph into the discussion session and summarize it in our conclusion.

Technical corrections

Line 24: Faulty parallelism: replace "and affecting" with "and affects"

We will edit the text to improve parallel structure.

Line 48: replace "Its estuarine part stretches" with "Its estuaries stretch"

We will edit the text for conciseness. We will replace it to "Its estuary".

Line 83: change to "within 20 minutes OF sampling"

We will edit the grammatical error.

Line 93/eqn. (1): It's confusing to have the letter "x" as the multiplication sign here because you also have an x variable. Use the mathematical symbol you use below or just take them out.

For consistency, we will use the multiplier symbol \times all throughout the manuscript.

Line 120/eqn. (10): Write e^{0.0447T} not exp.

We will edit the text to reflect the correction pointed out by the Reviewer.

Line 124/eqn. (12): p_{NO} and K_H are quantity symbols - italicize here as you did above.

Thank you for your attention to detail. We will italicize the quantity symbols.

Figures 2, 3, and 5: I would put the y axis labels (salinity, temperature, etc.) on the left side with the y axis ticks - it's confusing to have them on the opposite side of the plot. You can move the subplot labels ("a", "b") to the upper left corner.

Thank you for the comment. We understand the importance of clarity in presenting scientific data. We will edit the Figures to reflect the comment of the Reviewer.

Line 158: Add salinity units.

I initially added the practical salinity unit (psu) as a unit for reporting the salinity from sensors, but I learned that this is a common mistake and is strongly discouraged:

"It is important to emphasize that Practical Salinities do not have units. This fact, confusing to nonspecialists, is related to technical issues that prevented an absolute definition when PSS-78 was constructed. Sometimes this lack of units is awkwardly handled by appending the acronym PSU (Practical Salinity Units) to the numerical value, although doing so is formally incorrect and strongly discouraged."

See Pawlowicz, R. (2013) Key Physical Variables in the Ocean: Temperature, Salinity, and Density. *Nature Education Knowledge* 4(4):13 Available at https://www.nature.com/scitable/knowledge/library/key-physical-variables-in-the-ocean-temperature-102805293/

Therefore in our revised manuscript, we will retain the text excluding units for salinity.

Figure 6: This is a really nice compilation plot to put your measurements in context. Instead of saying the NO fluxes are x10⁻¹⁷, just report in units of fmol cm⁻² s⁻¹.

Similar to my response in Line 232: We agree that it would have been better to use the shorter name. However, for the sake of intercomparability with previous research, we decided that we will use scientific notation in reporting the flux values.

Table S3: Table S3: instead of superscripts "a", "b" and "c" corresponding to different significance levels, use *, **, and ***, which is the convention.

We will edit the superscripts and use *,**,and *** to signify different significant levels.

Figure 7: Use *, **, and *** instead of a, b, c superscripts.

We will edit the superscripts and use *,**, and *** to signify different significant levels.

Lines 332-333: I agree with the ammonium limitation idea but rephrase this and the following sentences to improve clarity and flow.

Line 344: Here and elsewhere: "were" not "are", since most of your results are reported in past tense.

We will ensure to check again the grammar and results are reported in the past tense. However we will use the present tense to report trivial information.

Line 348: Remove clause "when the nitrification proceeds" – unnecessary.

We will remove the unnecessary clause.

Line 350: Remove "therefore" - the support for this statement comes later in this paragraph, not from the preceding one.

We will remove "therefore" in the revised manuscript

Line 355: "correlations" should be plural.

We will correct the grammar errors in the revised manuscript.

References:

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Schroeder, F., Klages, D., and Knauth, H.-D.: Contributions of sediments to the nitrogen budget of the Elbe estuary, Int. Ver. für Theor. Angew. Limnol.: Verhandlungen, 24, 3063–3066, https://doi.org/10.1080/03680770.1989.11899231, 1991.

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Sørensen, J.: Occurrence of nitric and nitrous oxides in a coastal marine sediment., Appl. Environ. Microbiol., 36, 809–13, <u>https://doi.org/10.1128/aem.36.6.809-813.1978</u>, 1978.

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