

Response to Reviewer 1

The authors present a recent effort of NO measurement in the Lower Elbe Estuary and the Hamburg Port Area, filling research blanks of this trace gas in coastal areas and estuaries. This manuscript is well-organized, with nice figures. It does provide an important picture of estuarine NO, an active trace gas difficult to measure, showing the distribution, flux, and potential production mechanisms of NO in the study region.

However, I have two major concerns here (also see specific comments below):

- 1. This paper is a good case study, but, as a manuscript expected to be published in bg, the text is lacking in the laying out of the scientific issues as well as extrapolation. For example, in the introduction there is a lack of elicitation of the gaps for the current research, and in the discussion, there is a lack of implications of the conclusions for other research in the field (i.e., what is the new knowledge compared to other published NO studies).*
- 2. The whole discussion section and the present of implications is still weak, e.g., the main conclusions are mainly drawn through correlations, but without sufficient explanation and logic connection between correlation and their conclusion. This problem is particularly evident in section 4.4.*

In the present version, I think there are still some gaps away from the publication level, and a major revision would be recommended.

We thank the Reviewer for dedicating her/his/their time and effort to offer constructive feedback, which is instrumental in enhancing our manuscript. We acknowledge the reviewer's feedback to expound on the gaps in research on nitric oxide in the marine environment in our Introduction section. We will revise our manuscript to mention these gaps in our paper.

We acknowledge the reviewer's point that the discussion section requires strengthening, particularly in establishing a robust causal link between the observed correlations and our discussion/conclusions. Our approach was to interpret the available data in order to explain the patterns of NO distribution in the Elbe Estuary. In our revised manuscript, we will enhance the clarity of the manuscript, ensuring that the role of NO as an intermediate in the nitrogen cycle is comprehensively explained and clearly articulated. We will address points raised by the reviewers to enhance the discussion section.

The reviewer noted that we have an insufficient explanation of the correlation analysis on nitrogen nutrients, NO, N₂O, excess N₂O (Δ N₂O), and apparent oxygen utilization (AOU). We recognize that relying on correlation alone may not adequately illustrate the complexities of NO cycling. We will provide references to substantiate the use of the said ratios in our discussion. For example, it has been well-established that a linear relationship between Δ N₂O and AOU indicates the occurrence of N₂O production from nitrification (Yoshinari, 1976; Nevison et al., 2003; Schulz et al., 2023).

To assist readers unfamiliar with the complexities of the role of NO in the nitrogen cycle, we will include a schematic diagram. By providing these, the reviewer and readers will better appreciate the various correlations we reported between the different dissolved inorganic nitrogen substrates and NO and N₂O. Despite these limitations, we view this study as an initial step in laying the groundwork for future research.

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.

Specific comments:

Introduction

Lines 36-39 This is just a list of past study areas, and the authors should have devoted some space to specifying the major scientific conclusions and advances made by these studies in the marine environment NO.

Line 40 What is the research gap of NO? Where might the behavior of estuarine NO differ from that of the study areas described above, or what is the scientific importance of studying estuarine NO? These should be briefly described in the Intro section.

We appreciate the reviewer's constructive comments on the Introduction section of our manuscript. We recognize the importance of providing a clear scientific context and the specific research gaps our study addresses. Our paper indeed presents a novel case study on the measurement of dissolved NO concentration on the interface between the riverine environment and coastal seas in a well-studied estuarine system in Europe—the Elbe Estuary.

In the Introduction section, we briefly enumerated the areas where dissolved NO concentrations were already measured. It supports our argument that limited studies are done on NO in the marine environment. We will modify the paragraph from lines 34 to 39 and add a text that provides context on the importance of measuring NO concentration and estimating sea-to-air flux densities:

“The determination of dissolved NO concentration in surface water is challenging because of its reactivity, which results in a very short lifetime in (sea)water (Lancaster, 1997) ranging from 3 s to 100 s (Zafiriou and McFarland, 1981; Olasehinde et al., 2010). Nevertheless, measurements of dissolved NO in aquatic environments such as open and coastal oceans and rivers have received increasing attention during the last decade. Examples of recent NO measurement campaigns include those in the Kurose River in Japan (Anifowose et al., 2015), the Seto Inland Sea in Japan (Olasehinde et al., 2010), the tropical Northwestern Pacific Ocean (Tian et al., 2019b), the oxygen minimum zone off the coast of Peru (Lutterbeck and Bange, 2015; Lutterbeck et al., 2018), and the coastal seas off Qingdao (Tian et al., 2021, 2024). The majority of the studies performed at different time frames have indicated that both open and coastal seas are a source of atmospheric NO with fluxes ranging from 0.70 (Anifowose and Sakugawa, 2017) to as high as $45.00 \times 10^{-17} \text{ mol cm}^{-2} \text{ s}^{-1}$ (Tian et al., 2019a).

Global estimates for oceanic NO emissions and their temporal (i.e., diurnal, seasonal) and spatial variability are still lacking. To address these gaps, expanded measurements of NO distribution in the open ocean and coastal waters are essential to enhance our understanding and provide a more accurate assessment of sea-to-air flux densities.

This paper presents the first measurements of dissolved NO concentrations in the lower Elbe Estuary and Hamburg Port basins during a ship campaign in July 2021. The overarching objectives of our study were (i) to determine the distribution of dissolved NO along the salinity gradient, (ii) to estimate the flux density of NO across the water/atmosphere interface, and (iii) to identify the potential production pathways and controlling factors on NO distribution in the lower Elbe Estuary and Hamburg Port area.”

Method

Line 51 Define Elbe-km here.

The definition of Elbe-km will be moved from line 62 (Figure caption) to the main text. For better coherence, we will move the definition after the sentence “Originating from the Karkonosze Mountains in the northern region of the Czech Republic, the Elbe River basin is the fourth largest catchment area (148,268 km²) in Central Europe (Amann et al., 2012) with average long-term freshwater runoff of about 720 m³ s⁻¹ (Kerner, 2007).”

Line 79 Method uncertainty and detect limit should be presented here.

We will include the uncertainty (i.e., average standard error of 1.28%) and refer to the previous publications for the detection limit of the methods (Schulz et al., 2023; Brase et al., 2017).

Line 83 The text here says that triplicate NO samples are measured. But I don't see the error bars in the figures. Uncertainty of NO flux density estimate also needs to be added.

We will revise the Figures and add the error bars in the NO concentration distribution and estimated NO flux density.

Line 128-129 and Fig. S2. I was surprised by the range of data in the figure, which, given the error bars, can range from -5 to 15 µg/m³. I'm a bit curious whether this range of error is primarily from (a) limitations of the detection method, (b) spatial heterogeneity, or (c) temporal variability. If it's from (a), the authors' averaging method may be reasonable, and if it's from (b) and (c), how large are the potential calculation errors? It looks like it might have (up to) an order of magnitude impact on the flux calculations.

We acknowledge the Reviewer's concerns regarding the precision of our NO flux calculations. For the same reason, we have clearly stated and emphasized in the manuscript that the calculated NO flux represents a rough estimate. Ideally, measuring atmospheric NO concentrations directly onboard the research vessel would enhance accuracy, as *in situ* measurements reduce potential errors in calculating flux.

Nonetheless, due to the lack of necessary additional onboard instrumentation (i.e., NO analyzer dedicated to atmospheric measurement), we have followed a methodology similar to that used by Tian et al. (2019a), published in Biogeosciences. They also used the average atmospheric NO concentrations (2.13 ppb) in their study area for estimating flux density in the Bohai Sea. While their study just noted personal communication as the source of the average atmospheric NO concentration, we provided the source of our data (i.e., atmospheric NO measurement by the Hamburg Institute for Hygiene and Environment).

The atmospheric NO concentration was measured using the chemiluminescence method and follows the DIN (Deutsches Institut für Normung e.V.) EN 1411 standard. The DIN is the German national organization for standardization and is the German ISO member body. Calibration and quality assurance on measurement data are discussed on their website (<https://luft.hamburg.de/allgemeine-informationen/kalibrierung-und-qualitaetssicherung-598742>). In summary, they ensure the following:

- Use of Suitability-Tested Devices: Only devices that have passed suitability tests are employed.
- Regular Checks and Calibrations: Gas measuring devices are checked every 25 hours, and manual calibrations are performed quarterly or post-repair, using traceable standards to monitor and adjust for deviations and long-term drift.

- Traceability: Calibration standards are biennially compared with national and European reference laboratories to ensure alignment with European standards.
- Participation in Round Robin Tests: Annual nationwide and regional tests are conducted to synchronize standards and test instruments across federal states.
- Regular Maintenance: Comprehensive maintenance schedules are followed at all measuring stations in compliance with EN standards, with more extensive tests being less frequent but more intensive.
- Validation of Measurement Data: Data is manually reviewed daily, monthly, and annually to confirm its plausibility based on technical, meteorological, and empirical factors.

To improve the accuracy of our study, we selected all seven background monitoring stations located near the Hamburg Port Area. These designated monitoring stations measure background concentration levels of air pollutants and are typically far enough from emission point sources. We think that all the seven background stations near the Elbe Estuary reflect the ambient atmospheric NO concentrations over the Elbe Estuary. Moreover, to further minimize error, we specifically selected data from the period coinciding with our study. We did not include nighttime atmospheric NO measurements, typically lower due to reduced vehicular and industrial emissions at night. We used the average NO value at the seven background monitoring stations to provide a conservative estimate of the atmospheric NO concentration in the Elbe Estuary during the study period. If we look at the average values at each time point, it is near the average concentration of $4.3 \mu\text{g m}^{-3}$ that we used to calculate the flux density. Notably, measurements outside the typical rush hours are close to this average concentration value. Here is the statistic of the hourly NO measurement ($\mu\text{g m}^{-3}$):

Minimum: 2.00

Maximum: 8.25

Standard deviation: 1.76

Median: 3.86

We should have been clearer in the Figure S2 caption that the error bars or whiskers in the scatter plot represent the standard deviation of the values measured at the “background” monitoring stations for each time point and not the minimum and maximum NO concentration values typical for box and whisker plots. We will edit the Figure caption to indicate that the error bars represent the standard deviation.

Atmospheric NO concentration may vary spatially and temporally as NO_x can be emitted from vehicles and ships. You would notice that high variability at each time point is more pronounced from around 6:00 to 8:00 AM, which may be attributed to the morning rush hour.

Section 4.1

The discussion in this section was a bit weak. I really like the summary of NO in Figure 6, but there wasn't much discussion of it in the main text. For example, why is it that estuaries with higher nutrient instead have lower NO concentrations than open ocean/nearshore? This study' NO is already supersaturated but still on the lower end of all the studies, what is causing the high concentrations (potentially supersaturated several times over) on the other sites?

Will some of the correlation patterns in this work appear in whole compile data set? How important are estuarine/oceanic NO emissions relative to terrestrial/human systems based on currently available data? Etc... This may require more work to sort out, but I believe it may expand the scientific value of this paper to be more than just like a case study.

Thank you for your feedback. We acknowledge the need to improve the discussion and provide a more comprehensive analysis of our results. We will resolve this in our revised manuscript.

The question of why the Elbe Estuary, with relatively higher nutrients, has a lower NO concentration is still unresolved and requires further investigation. Nevertheless, 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 highlighting the Elbe Estuary's relatively higher nutrient levels yet lower NO concentrations than 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 NO_2^- 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 NO_2^- to form NO in areas with low NO_2^- ."

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). We are not certain whether reaction with transition metals is a sink in the marine environment, particularly in coastal and estuarine environment as this has not been explored. Additionally, organisms (algae, phytoplanktons) can both consume and produce NO. A recent paper by (Bange et al., 2024) noted that the consumption processes for NO in the sea(water) are still unresolved.

While current literature suggests that coastal areas could potentially act as significant sources of emission to the atmosphere, this may vary temporally and spatially across the studied sites. Up to now, the majority of the literature reports positive sea-to-air flux, indicating emissions as a major sink; however, regional exceptions, such as one measurement in the Shandong Peninsula (Gong et al., 2023), indicate that generalizations should be made cautiously.

Regarding the Reviewers' comment on the importance of estuarine/oceanic NO emissions relative to terrestrial/anthropogenic emissions, we will briefly discuss these in our revised manuscript. We will add a few examples from highly cited papers on NO emissions from the terrestrial environment, including but not limited to Bouwman et al. (2002a), Bouwman et al. (2002b), Stehfest and Bouwman (2006), Williams et al. (1992). As the reviewers pointed out, this would enhance the discussion on the importance of NO emission from coastal areas as a source to the atmosphere.

Section 4.2

Because salinity is also an indicator of mixing, the negative correlation with salinity noted here is likely to represent "mixing" for NO (i.e., mixing affects both NO and salinity), not "salinity and freshwater input influencing NO concentrations" (i.e., salinity/freshwater itself influences NO).

We appreciate the reviewer's comment regarding the role of mixing in the observed negative correlation between salinity and nitric oxide (NO) concentrations. We recognize that mixing indeed plays an important role in the distribution of biogeochemical parameters in the Elbe Estuary. Indeed, Dähnke et al. (2008) noted that conservative mixing behavior could be observed in the Elbe Estuary irrespective of the season.

However, our intention in this section is to emphasize the significance of riverine/freshwater inputs as a primary source of higher NO concentrations. We supported our argument with two studies: one documenting relatively higher surface dissolved NO in the southern Bohai Sea due to the Yellow River's outflow ascribing it to high DIN input (Gong et al., 2023), and another study (Ayeni et al., 2021) noting a NO concentration gradient in the Kurose River, with downstream sections influenced by anthropogenic activities.

Section 4.4

The source/sink of NO is so complex that I would suggest that the authors include a suitable concept fig in an attachment or in the main text to allow more readers to easy follow the processes you describe.

We understand the importance of ensuring clarity for readers and a broader audience. We will provide a schematic diagram or illustration providing known sources and sink processes of NO, particularly as an intermediate in the nitrogen cycle. We will revise our manuscript so readers can easily follow the complex nitrogen cycle processes discussed and other known sources and sinks.

Section 4.4.1

Lines 322-323 Why this statement make sense? Nitrification only contribute minor part of AOU. Some explanations or references are needed.

We thank the reviewer for this comment and the opportunity to clarify this statement. Indeed, nitrification only contributes a minor part to the AOU. We understand the previous text could be enhanced for clarity, and as such, we will edit the text to ensure that readers understand the text better (edit this to be more precise).

However, it is established that a significant linear correlation between excess N₂O (Δ N₂O) and AOU indicates the occurrence of nitrification. We will revise the text and provide references to support this argument.

Previous: In this study, we noted that nitrification occurs in the entire stretch of the study site based on the plots of AOU and excess N₂O (Fig. 7a), as AOU correlates significantly with the Δ N₂O in all three salinity zones.

Edited: Based on the plot of AOU and excess N₂O (Δ N₂O), N₂O production can be observed in the entire stretch of the study site (Fig. 7a). We also observed that AOU has a significant positive linear relationship with Δ N₂O in the entire stretch of the Elbe Estuary— in the brackish coastal zone (R=0.95, p < 0.001), limnic zone (R=0.65, p < 0.05), and in the Hamburg Port area (R=0.75, p < 0.01). Previous reports (Schulz et al., 2023; Brase et al., 2017; Nevison et al., 2003; Walter et al., 2006) indicated that a significant positive linear relationship between AOU and Δ N₂O usually indicates N₂O production from nitrification.

Lines 324-325 I can't follow these sentences. Many ratios (e.g., N₂O/NH₄⁺, NO₂⁻/O₂ ...) appear in the correlation diagram. What do these ratios represent? Some background should be provided.

We understand the need to enhance clarity for readers. We will revise the text for readers to understand these ratios. We will provide a diagram of the nitrogen cycle for readers to understand how these ratios might be related to the different nitrogen cycling processes. For instance, by providing a diagram similar to that of Figure 1 in Caranto and Lancaster (2017) showing the nitrification process, it would be easier to pinpoint that N₂O is a product, NH₄⁺ is a reactant, and NO₂⁻ and NO₃⁻ can be oxidized from NO.

Lines 326-327 How “a significant positive linear relationship exists between N_2O and NO_3^- ” is linked to “These findings point to NO production via nitrification”? I can’t find the logic connection.

We will revise the text to clarify the link between N_2O and NO_3^- in nitrification. To establish a logical connection between these statements, it’s important to understand the following:

- Nitrous oxide (N_2O) is a known byproduct of nitrification and an intermediate of the denitrification processes.
- Nitrification is a microbial process where ammonia (NH_3) is oxidized to nitrate (NO_3^-), and it can also lead to the production of nitrite (NO_2^-).
- During nitrification, obligatory intermediates (Caranto and Lancaster, 2017), nitric oxide (NO) and hydroxylamine (NH_2OH) can be produced. NO can further yield N_2O .

The significant positive linear relationship between N_2O and NO_3^- , may suggest that as the concentration of nitrate increases, so does the concentration of N_2O . If this relationship is found to be significant within the context of the study, it is possible that the processes leading to the production of NO_3^- (like nitrification) are also associated with the production of N_2O (see Schulz et al., 2023). Hence, if N_2O levels are rising with NO_3^- levels, it could be indicative of active nitrification, during which NO is produced as an intermediate. The logic is that if N_2O is increasing with NO_3^- and we know that N_2O can be a byproduct of nitrification (which also produces NO_3^-) then an increase in both could point to nitrification as the source process, and thus, the production of NO as part of that process.

Line 331 What “observed trends” refer to?

We will revise the manuscript to enhance clarity for the reader.

Line 334 Authors discuss here that nitrification is the SINK of NO. I am a little confused because the whole section discusses about nitrification as SOURCE of NO.

Note that while NO can be produced in the nitrification process as an obligatory intermediate (Caranto and Lancaster, 2017), it can also be consumed in further oxidation steps. Shown below is **Figure 1** from Caranto et al. (2017):

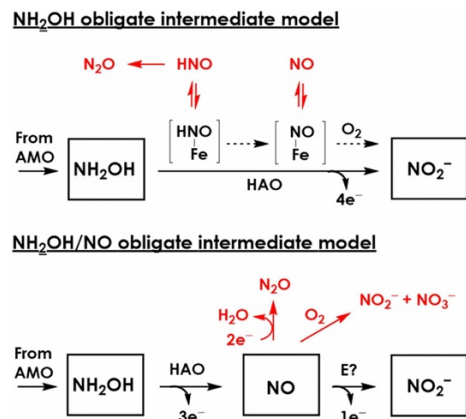


Figure 1. Schematic diagram comparing the prevailing view on the nitrification process and the model proposed by Caranto and Lancaster (2017) that shows nitric oxide is an additional obligate intermediate in the nitrification process (From “Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase,” by J.D. Caranto and K.M. Lancaster, 2017).

In the nitrification process, ammonia (NH_4^+) undergoes oxidation to form hydroxylamine (NH_2OH), which can further yield NO and then yield N_2O , NO_2^- , or NO_3^- . Another Reviewer agreed with the idea of the NH_4^+ limitation in the coastal/brackish and limnic zones leading to the observed significant inverse relationship between NO and NO_2^- and NO vs NO_2^-/O_2 ratio. If NH_4^+ is not limited or has a continuous supply in the reaction, one would see a direct relationship between NO and NO_2^- . When NH_4^+ is limited, NO will be consumed in the process, decreasing its concentration while increasing the product NO_2^- , NO_3^- , or N_2O .

Section 4.4.2

This entire section suffers from a problem like that of section 4.4.1, in that a large amount of the text simply suggests the correlation without explaining it, making the logical chain of support for the author's argument incomplete. For example, almost all of the text in lines 350-365.

We understand the need to enhance the manuscript by providing a thorough explanation of the nitrogen cycle processes. We will revise the manuscript accordingly for clarity.

Other notes:

Table S2: Why NO flux density ($\text{mol m}^{-2} \text{s}^{-1}$) have a different unit with N_2O flux density ($\mu\text{mol L}^{-1} \text{d}^{-1}$)? It also differs from unit in the main text and figure 5 and 6.

Thank you for the attention to detail. We apologize for the oversight. We have corrected the unit of NO flux density to $\text{mol cm}^{-2} \text{s}^{-1}$ in Table S2, and the unit of N_2O flux density to $\mu\text{mol m}^{-2} \text{d}^{-1}$. These are standard flux density units established in prior publications. For easier comparability and consistency with previous publications, we have used the units $\mu\text{mol m}^{-2} \text{d}^{-1}$ for N_2O flux density and $\text{mol cm}^{-2} \text{s}^{-1}$ for NO flux density.

Why don't you add NO to the correlation plots of the main text and attachments? I don't see NO in Figure 7 and Figures S4-S6? And if space permits, I suggest you place Fig. S4 (after adding NO) and Fig. S7 into main text.

We will follow the suggestion of the Reviewer to add correlation plots of NO to the main text. We removed NO in Figure 7 and Figures S4-S6 because we have made separate correlation plots of NO vs other parameters. However, we can add this to the Figures if it would enhance clarity.

References:

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