

Schulz et al., 2023 conducted underway surface measurements of N₂O concentration in the Elbe estuary on 9 cruises across spring, summer and winter. They showed a large spatial variation of N₂O concentration and flux, and identified two hotspots of N₂O production including the Port of Hamburg and region near the estuarine turbidity maximum. They argued that there is not much seasonal variation in N₂O flux because high riverine N₂O concentration in winter may compensate for its low biological N₂O production compared to summer. This manuscript presents a valuable dataset of N₂O concentration and flux from a European estuary. Especially, the seasonal pattern of estuarine N₂O flux has been poorly observed and understood. However, there are some points needed to be considered/corrected such as the interpretation of the relationship between excess N₂O and AOU, evaluation of environmental controls on N₂O concentration/flux, calculation of annual N₂O flux and uncertainty estimate. See detailed comments below.

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 reviewers criticism. Reviewer comments are written in bold italics, our answers are kept in plain font.

Line 17-18: what do you mean by “compensated the effect of decreasing dissolved inorganic nitrogen (DIN) loads”?

This statement was supposed to summarize our findings of section 4.1, where we complemented observations done by Bräse et al. (2017) with our new data sets: Previous N₂O measurements done in 1980s and 1990s showed a significant reduction of N₂O saturation due to reduced riverine nitrogen loads and higher dissolved oxygen conditions. Water quality in the Elbe estuary improved significantly after the reunification and collapse of East German industries (e.g. Guhr et al., 2000). In the 1980s, high nitrogen loads and low oxygen conditions favored denitrification leading to high N₂O saturations (Hanke and Knauth, 1990). With improving water quality, the importance of denitrification decreased (Dähnke et al., 2008), which probably led to the decrease in N₂O saturations (Bräse et al., 2017). However, compared to the study in 1997 (Barnes and Upstill-Goddard, 2011) our measured N₂O saturations did not further decreased despite a continuous reduction of nitrogen loads entering the estuary from the upstream river (e.g. Figure 5, FGG Elbe, 2018; Radach and Pätsch, 2007). These findings suggesting that in-situ N₂O production along the estuary is important and compensates the overall effect of decreasing nitrogen loads in the last decades.

Also considering the comments of reviewer 2, we will revise the manuscript to highlight the relevance of our research for a broader audience. Thus, we will rephrase or remove this finding from the abstract, and will focus on a comparison of our results with other research in heavily managed estuaries, highlighting that we found seasonal varying drivers of N₂O emissions that did not scale with DIN loads and were directly linked to eutrophication phenomena.

Line 25: How does 0.24±0.06 Gg N₂O y-1 emission compare to global estuarine N₂O emission?

As mentioned above we will address the relation to other estuaries and global scale in the abstract of the revised version. Also, as we will change the emission estimation by using several parametrizations to calculate the k value (as suggested by the other reviewer) and use a different upscale approach as suggested by this reviewer in a comment below. Thus, the N₂O emission estimate will probably change.

Lines 40-42: Denitrification could also occur in anoxic water column contributing to N₂O production (Ji et al., 2018; Tang et al., 2022).

We will list water column denitrification as possible N₂O production pathways in this section of the text.

Line 44: specify Port Hamburg as the third largest port in Europe.

We will change “biggest” to “largest” port in Europe.

Line 70: how deep is the Elbe estuary? This gives an idea if sedimentary processes (e.g., N₂O production) may affect N₂O concentration in the surface water column.

We will add information about the depth of the Elbe estuary in our study site description. The Elbe estuary has a depth of 15 – 20 m, which is maintained by regular deepening and dredging operations. Upstream of the Port of Hamburg, the water depths is less than 10 m (Hein et al., 2021).

Figure 1: There are too many city names on the map, which is distractive. It may be clearer to label only the key cities like Cuxhaven or island Scharhorn where the Elbe River enters the North Sea or Oortkaten.

We will change the map and label only relevant cities as suggested.

Lines 85-87: Why transect sampling was performed after high tides? What's the effect of tides on N₂O concentration? Tidal cycles of N₂O concentration have been observed in other estuaries (Goncalves et al., 2015; Barnes et al., 2006).

We chose our sampling strategy (upstream against the outgoing tide) to prevent interference of tidal effects on our measurements. Aim was to have comparable data for each cruise at similar tidal states with comparable current and mixing conditions. We started after high-tide and travelled against the outgoing tide to make sure that we did not move with the same water masses while travelling upstream. We will explain our chosen sampling strategy in a revised manuscript version.

Tidal effects will very likely affect nitrous oxide concentrations in the Elbe estuary, but this is not the focus of the present manuscript. We will briefly address possible tidal effects in a revision.

Line 116 in Equation 1: is N₂O_{cw} the partial pressure of N₂O in water? Otherwise, the saturation should be calculated as the N₂O_{cw}/N₂O_{eq}*100 where N₂O_{eq} is the equilibrated N₂O concentration with atmosphere. Similarly in Equation 3. N₂O_{air} should be N₂O_{eq}.

For our calculations, we used the average atmospheric N₂O concentrations measured on each specific day of the cruise to calculate expected atmospheric equilibrium concentration considering the solubility function of Weiss and Price (1980) and atmospheric pressure. We will change N₂O_{air} to N₂O_{eq} to prevent confusion and add a short description about the calculation in the method section.

Line 143: Why nitrate concentration increased at 700 km? Are there tributaries or point sources?

We regard this as a result of nitrification, rather than a point source. Dähnke et al. (2008) identified the Elbe estuary along its salinity gradient as a significant source of nitrate with high nitrate production in the maximum turbidity zone (MTZ). Sanders et al. (2018) measured highest nitrification in the Hamburg port region, which was not covered in the research done by Dähnke et al. (2008). Both studies highlighted the importance of nitrification along the Elbe estuary. Further, our results in section 4.2 showed ongoing nitrification fueled by marine organic matter along the mesohaline estuary and indicated that nitrate is produced by coupled remineralization with nitrification from both the riverine and marine site of the estuary.

In general, point sources play a subordinate role in the nitrogen input of the Elbe estuary (Hofmann et al., 2005; IKSE, 2018) with dominating agricultural sources in the upper and middle Elbe River. We will add this information to the study site description. We believe the offset between region of highest nitrification rates (Port of Hamburg) and nitrate peak in the estuary (stream kilometer) is a result of different spatiotemporal scales, as suggested by Sanders et al. (2018). They found that the position of

the nitrate maximum and nitrate gain over the entire estuary depended on the processing rates and was thus coupled to discharge conditions. We will briefly address this in a revised version of our manuscript.

Lines 148-149: Why ammonium and nitrite concentration increased near Hamburg Port? Is it due to internal organic matter remineralization or point sources or sedimentary flux?

Ammonium and nitrite concentration increases in the Hamburg port due to remineralization of organic material coming from upstream regions of the estuary. The sudden increase of water depth in the Port leads to a light limitation and decomposition of riverine organic material. As a result, ammonium and nitrite can accumulate leading to measurable peaks of both nitrogen forms in the Hamburg port region with the ammonium peak usually occurring upstream of the nitrite peak. At the same time, the decomposition of phytoplankton leads to increasing C/N values. Respiration processes predominate causing along with nitrification intense oxygen depletion in the Port of Hamburg. This succession of biogeochemical turnover has been addressed previously (e.g. Schroeder, 1997; Kerner and Spitz, 2001; Schlarbaum et al., 2010; Schöl et al., 2014; Sanders et al., 2018; Dähnke et al., 2022). We addressed the succession of nitrogen turnover in the Port of Hamburg in section 4.3 of the discussion (L324 – 329). We will revise this section to clarify the state of research.

This also led us to one of our main conclusion, as riverine organic material is not only lead to remineralization/nitrification but also to intense oxygen consumption and therefore possible denitrification in the Hamburg port region fueling N₂O production in this area of the Elbe estuary. Further, we will discuss possible nitrogen turnover processes and benthic-pelagic coupling in more detail as also suggested by reviewer 2.

Figure 2: It is hard to tell the difference among each cruise with so many colored lines. How about presenting data from the same season using the same color to illustrate the seasonality as a supplementary figure?

Thanks for this suggestion, we will add a figure like suggested by the reviewer to the supplements and will refer to it in the text.

Lines 211-218 and lines 232-234: Figure 4 a and b are both from June, summer. The linear positive relationship between AOU and excess N₂O suggests N₂O production from nitrification (e.g., Nevison et al., 2003). The increase in the slope should be interpreted as an increase in the N₂O production yield or external N₂O input (e.g., point source).

Thanks to the reviewer for this comment. We will include figures from all cruises in the revised version of the manuscript.

We indeed identified nitrification as responsible production processes both in the mesohaline estuary (section 4.2) and the Hamburg port region (section 4.3). In a revised manuscript version, we will clarify that our data suggest that N₂O yield varies due to changes in nitrification dynamics, rather than point sources. As stated above, we will also address the role of point sources in the study site description.

Further we will also correct the statement in L232-234:

"As shown in Fig. 4, the relationship between N₂O_{xs} and AOU were influenced by either initial riverine N₂O production, or in-situ production along the estuary."

Figure 4: It would be interesting to systematically/statistically assess the relations between excess N₂O and environmental factors like salinity (non-conservative behavior of N₂O) or dissolved inorganic nitrogen (infer N₂O production pathways), PN, PC, and SPM. There seems to be a good relation between N₂O and ammonium/nitrite concentration shown in Figure 2.

We understand that a systematically and statistical assessment of the relations would help the reader to follow our discussion. We did assess the statistical relations in sections of our discussions (e.g. L:283, L:309, L:314-316, L319), but for clarity, we will add a section regarding the statically analysis in the results chapter in the revised manuscript.

During data interpretation, we tested diverse presentation and analysis methods and found correlations not necessarily the best suited outlet to describe and analyze our data. Correlations were distorted by the spatial offset between the ammonium, nitrite and N₂O peaks, which we attribute to a succession of nitrogen bearing substances during turnover processes like nitrification (Sanders et al., 2018 and section 4.2). Thus, we chose another way to visualize the data by inserting the Figure 6 (L268) and S3 of the supplementary material. Further, we addressed the relations of N₂O and various forms of nitrogen to identify N₂O production processes and their controls in the section 4.2 (e.g. L258-259, L283-285, L310-317). For the Hamburg Port region, we decided against a detailed derivation of N₂O production processes as this was already done by Bräse et al. (2017). Therefore, we added the statement in L303-305.

We hope that a section on statistics in the results section, as mentioned above, and an earlier reference to the supplementary material in *Section 3.1* is sufficient to meet this criticism.

Lines 242-243 and Figure 5: What about the variations of the N₂O%, oxygen and total nitrogen concentration? The riverine N concentration is decreasing, what about the changes in other point sources of N input along the estuary (e.g., from wastewater treatment plants) or concentration in the estuary?

We agree with the reviewer that a more detailed analysis of a long-term trend of N₂O concentrations and reasons for changes would be very interesting. However, for our study we focus on seasonal variations rather than a long-term trend analysis. With section 4.1 we aimed to compare our results with a broader spatial and temporal scale by including a short comparison to other estuaries as well as with previous measurements from the Elbe estuary. This gives a hint towards temporal trends, but seasonal variability and data coverage make the long-term data difficult to interpret.

Briefly, the biogeochemical processes occurring in the Elbe estuary have drastically changed over the last 50 years: (1) the reunification of Germany and the collapse of East-German industry had led to significant improvements of water quality (e.g. Guhr et al., 2000), (2) decision to combat eutrophication in the North Sea in the 1980s and (3) improved waste water management resulted in a significant reduction of riverine nutrient loads (de Jong, 2007; Van Beusekom et al., 2019; Bergemann and Gaumert, 2010). Dähnke et al. (2008) showed that this led to a change of dominating denitrification towards significant nitrification in the Elbe estuary. Thus, a profound long-term analysis would be in need of its own paper, for which we think our data coverage is insufficient given the seasonal variability. As an example, measurements from only one cruise are available for the 1990s.

However, due to this comment as well as from other comments of the reviewer, we see the need to address the influence of point sources on nitrous oxide concentrations and nitrogen turnover in the Elbe estuary, which we already elaborated in a reply of an early comment above and which we will include in a reviewed version of our manuscript.

Line 272: "this suggests"

We will add the "s".

Line 273: how is MTZ defined? What threshold of suspended particle material is used to define the MTZ?

Generally, the occurrence of an MTZ is unique to each estuary and is generated by the balance between river-induced flushing and upstream transport of marine SPM as well as a function of estuarine geomorphology, gravitational circulation and tidal flow, trapping the particles in the MTZ (Bianchi, 2007; Sommerfield and Wong, 2011; Winterwerp and Wang, 2013). Thus, the MTZ is usually located in the onset of the salinity gradient of an estuary (Burchard et al., 2018).

The MTZ is – also in literature – often assessed based on relative changes in SPM or turbidity, and is mostly located between stream km 670 and 710 (e.g. Bergemann, 2004) in the Elbe estuary. During our cruises, SPM and turbidity were not always measured consistently, depending on instrument and personnel availability – during some cruises suspended particulate matter concentrations were measured using filtration techniques, during some cruises we obtained the data from turbidity sensors, which are not entirely intercomparable. Therefore, we did not define a threshold of suspended particulate matter to define the MTZ, but also used relative change during each cruise to define it.

We will add colorbars indicating the relative change for SPM concentrations to the figure 6 and supplement material S3.

Line 287 and 296-297: clarify the reference: Kappenberg and Fanger, 2007 (German?) and source of organic matter from the North Sea into the Elbe estuary.

Kappenberg and Fanger (2007) is a German report from the former research center GKSS studying the sediment transport events in the tidally influenced Elbe estuary, the German Bight and the North Sea. The report (including a short summary in English) is available at https://www.hereon.de/imperia/md/content/hzg/zentrale_einrichtungen/bibliothek/berichte/gkss_berichte_2007/gkss_2007_20.pdf, last accessed: 21.03.2023.

We will include a peer-review reference that shows an upstream transport of suspended matter into the Elbe estuary due to tidal transports in the Elbe estuary (Schoer, 1990). For the Ems estuary, Schulz and Umlauf (2016) showed an upslope transport of suspended matter due to tidal pumping is possible.

Lines 311-313: How about showing the relations between ammonium, nitrite and N₂O in figures?

We decided to show the relation between ammonium, nitrite and N₂O plotted against stream kilometers in Figure 6b and supplementary material S3. We found that the spatial progression of nitrogen containing substances were more illustrative than scatter plots or correlations for each substance, cruise and production areas, which would lead to a lot of figures added in the supplements. These relations were distorted by the spatial offset between the occurring ammonium, nitrite and N₂O peaks, which we explained by a succession of nitrogen bearing substances during turnover processes like nitrification (e.g. section 4.2). Therefore, we found our choice of presentation better suited. We will add a section about the statistical analysis and relations of individual parameters in the results section. We will further rephrase the statement (L311-313) and add a reference to Figure 6 and the supplementary material.

Line 315: What are R values? R is positive for nitrite concentration.

R is the Pearson correlation coefficient. We will clarify this in the text by adding a short section about our statistical analysis in the *Methods* section of our manuscript. Nitrite concentrations correlated positive with N₂O leading to a positive correlation coefficient.

Line 320-321: Is nitrification responsible for the remaining oxygen consumption?

Yes, nitrification is responsible for the remaining oxygen consumption. Sanders et al. (2018) assessed 25 % of the oxygen consumption in the Hamburg Port region were caused by nitrification, which was in line with results from Schöl et al. (2014). We will clarify this in the text.

Line 326 and Figure S1: why C/N ratio was so high in 2021 March?

Thanks for this good observation from the reviewer. We double checked our measurements, and the data appear correct and sound. We have no easy explanation at hand, but speculate that a calcareous algae bloom in the North Sea might be a potential cause. However, we have no further evidence for this hypothesis. We will not address this further, as the C/N ratios are not a crucial parameter for our discussion. However, we would like to keep the data in the manuscript as they might be insightful for later research and other researchers.

Line 345-347: "Ammonium and N₂O concentrations are high in the pore water of underlying sediments". Reference or example of the concentration. What about the timing of deepening and dredging works in the Hamburg Port compared to the cruise periods?

We will add references for this statement (Zander et al., 2020) and rephrase the sentence as we only assumed elevated N₂O concentrations in pore waters, eg.: "Ammonium concentrations are high in the pore water of underlying sediments (Zander et al., 2020), which we assume lead also to elevated N₂O concentrations due to occurring nitrifier-denitrification in the sediments that was found by Deek et al. (2013)."

The Elbe estuary is constantly deepened and dredged along the entire transect to grant access for big container ships. However, we did not compare operation locations with the measured N₂O concentrations except for our March cruises as we did not see big differences in the spatial variation of the N₂O profiles, which were not explainable by in-situ production, nor found a strong correlations between N₂O and suspended particulate matter concentrations. However, the effect of dredging and deepening operations as well as the effect of higher suspended particulate matter concentrations in the water column are a very interesting research question that should be worked on in the future. Ideally, in cooperation with the Hamburg Port Authority, who is responsible for maintain the water depth in the Elbe estuary. We will address this in a bit more detail in section 4.3 while discussion possible effects on N₂O production in the Hamburg Port region as also suggested by the second reviewer.

Lines 360-361: Has there been any N₂O measurement from this wastewater treatment plant (WWTP) Köhlbrandhöft? The ammonium concentration in 2021/03 is not exceptionally high compared to previous cruises (e.g., 2020/06). What about the direct N₂O output from the wastewater treatment plant?

The operators of the WWTP measured N₂O concentrations during our cruise, but did not detect elevated N₂O concentrations. However, direct N₂O output from the WWTP was not measured and the increased ammonium loads leaving the WWTP corresponded to the increased N₂O concentration measured. We assume that the N₂O is not produced within WWTP itself, but from elevated ammonium concentrations in the Elbe in warmer waste water.

We assume that we did not see an extraordinary ammonium peak due to the distance the WWTP outflow and our measurement transect, ~ 2 km. The outflow of the WWTP is located in the Southern Elbe, which joins the sample stretch at stream kilometer 626. We assumed that ammonium were rapidly converted to N₂O as the warmer and biological active waste water entered the Elbe estuary before it reached the Northern Elbe.

We were admittedly surprised by the extraordinary N₂O concentrations in March 2021, and even more so when the results were not reproduced in the following year. Consequently, we concluded that an extraordinary event must have caused the N₂O peak. Since we could not detect any relation to the deepening and dredging work in the Port area and our measurements fitted to extraordinary operation condition in the WWTP, we assumed this might be the source, especially as our hypothesis was confirmed by the WWTP operators. Additional measurements confirmed that N₂O concentration was not elevated near the WWTP outlet under normal conditions.

We will carefully revise this discussion section for clarity.

Figure 7. Use month or season as the x axis instead of cruise number? Add description of the boxplot. Why not adding error bars for emissions?

We will add error bars to the emissions plot Fig. 7b.

Figure 7 already shows flux densities and emissions plotted against months not cruise numbers. We will adapt this plot by separating the data into seasons (winter: March), spring (April and May), summer (June and July) and late summer (August and September). We will change the color scheme to better distinguish the winter season.

Table 3. How is annual N₂O emission calculated? Since there is a seasonal variation in the N₂O flux, monthly or seasonal N₂O emission may be more representative. Because N₂O flux was measured at a high spatial resolution, it may be useful to calculate the N₂O flux across the whole estuary by integrating the flux and area section by section (e.g., River section, Hamburg port, Oligohaline section) instead of multiplying the average N₂O flux by the whole area of Elbe estuary.

We described our annual emission calculations in the method section 2.4 (L124-126). We used this annual average for comparability with other studies, but will revise this calculation, to individually address sections and seasons. We found respective areas for sections from stream kilometer 610 - 632, 632 - 704 and 704 – 750 (Geerts et al., 2012), which fit reasonably well with our described sections. We will probably use these to calculate the fluxes and emissions as suggested by the reviewer. Further, we will differentiate into more seasons between winter (March), spring (April and May), summer (June to July) and last summer (August and September) to better reflect seasonality.

Line 406: Why do you think there is no seasonality in N₂O emission? N₂O flux is different comparing spring, summer and winter shown in Table 3.

We were carried away by comparing summer and winter emissions, which were (for us surprisingly) similar and missed to discuss the effects of spring and late summer on N₂O emissions. As a starting hypothesis we considered winter emissions significant lower than summer emissions due to missing in-situ production. However, we found that high nitrogen loads coming from the river leading to high emissions in winter comparable to emissions in summer, which were driven by production at two production sites.

For the revised manuscript, we calculated emissions as suggested by the reviewer in a comment above by separating our data into more seasons (winter: March, spring: April to May, summer: June to July, late summer: August to September). With the new upscaling technique we found highest emissions in

winter. Thus, we will restructure our last section of the discussion in line with suggestions of the other reviewer focusing on the relevance of our research for a broader audience.

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