

Reply to the anonymous comments by reviewer #1 and #2 and the editor's comments

We would like to thank both reviewers and editor for the constructive feedback on the manuscript. We now modified the manuscript according to the suggestions and hope that all issues that were raised could be solved.

A major concern of both reviewers was that the isotope mass balance model mentioned in the previous version of the manuscript was not well defined. As we outlined in the original response letter, referring to the calculations as a model was misleading. We actually performed mass balance calculations for the N-bearing substances, including their isotope composition, to track areas where the mass balance was violated. We now clearly refer to the calculations as a mass balance approach (e.g. lines 387, 414), and additionally added a supplement in which the underlying calculations are spelled out.

Both reviewers also noted that we refer to the hydrological situation in the Elbe during our study as so unusual, which indeed would raise the question of the study motivation. We modified the introduction to point out that oxygen minimum conditions, similar to the one we captured, occur regularly in summer, with the resulting ecological problems. However, these events are rarely captured in detail, and the spatial extent of nitrite and ammonium peaks in the harbor region gave us the opportunity to assess summer biogeochemical processes based on isotope effects, and with an isotope mass balance.

To make these points clear, we moderated the language use in the manuscript, and refer clearly to other studies that address oxygen conditions in the estuary (lines 35 – 38, 43 – 46, 70 – 74).

Following the suggestion of especially Reviewer #2 and the editor, we also revised the method section. We added methodological details throughout the section, and amended and largely simplified the description of rate calculations (lines 135 – 142). In detail, we added some text regarding the constraints of the method, referred more clearly to previous used of the method, and deleted the misleading section dealing with the separation of nitrite and ammonia oxidation.

Additionally, we made minor editorial edits to the discussion and conclusion section, and included a table in the supplementary material to show the changes in SPM content and quality. In the following, we now describe in detail which changes and modifications were made in response to the individual comments. Comments are in italics; our reply is in plain font.

Reviewer 1

Line: 62 The significance of evaluation of nitrogen transformation along Elbe estuary under “intense summer oxygen depletion” is a little vague. Why do sample the water column under unusual condition? Please revise them for clearer description.

→As outlined above (and in the original response letter), we now clearly contextualize the oxygen conditions in the Elbe, and rephrased the intention of our study to avoid confusion and to make clear that we could use this situation to unravel nitrogen cycling under summer conditions in the estuary (lines 35 – 38, 43 – 46, 70 – 74).

Line: 70 - In the explanation of study site, there is a lack of information where an agricultural catchment area and areas of nutrient discharge (Line 71-73) exist. Where is the input of N ? Those information helps us to understand the interpretation of geochemical data along Elbe Estuary.

→We added some information regarding diffuse and point sources of nitrogen (lines 78 – 81).

Line: 128 - As for nitrate isotope analysis, how was nitrite removed from the nitrate samples? Some samples contained enough nitrite for isotope measurements. Thus, the presence of nitrite interfered with nitrate isotope measurements.

→Nitrite was measured separately using *Stenotrophomonas nitritireducens*, as outlined in the methods section. In samples containing nitrite and nitrate, the nitrate isotope composition was determined by difference. We now mention this in the method section (lines 154 / 155)

Line: 150 - It seems that both of ammonium oxidation and nitrite oxidation occur between stream km 641 and 656. Isotope compositions of nitrite could be affected by both of ammonium oxidation and nitrite oxidation. Do you consider the influence of ammonium oxidation on nitrite isotopes?

→As we explain in the original response letter, we do not consider the effect of ammonium oxidation, because the ammonium concentration is already low in the section where both processes prevail, and should thus have little effect on the nitrite isotope composition. We now explain this briefly in the manuscript (lines 183 – 185).

Line:261 - How did you calculate and conclude that the drop of oxygen isotope values in the harbor region was due to nitrification? What is the value of oxygen composition of river water ($\delta^{18}O_{H_2O}$) ?

→The oxygen isotopes are not discussed in detail in our study, we refer here to previous study by Sanders et al (2018). We modified this section to point this out (lines 288 – 293). We did not include water isotope values, because we do not evaluate isotope changes of oxygen in detail in this present manuscript.

Line:320 - The authors constructed a total isotope mass balance by modeling. I understood the assumption of the model. However, the equation, parameters and calculation method were not presented in this text. Therefore, it is difficult to understand the conclusion that Total N fluctuations are driven by PN fluctuations. I'm not so familiar with this box model, but it seems better that the authors briefly explain equations and parameters in the box model and a modeling software in the method section or supplemental information.

→As outlined above, we now refer to our calculations as a mass balance approach rather than a model in section 4.3. We also added a supplement for further reference.

Reviewer 2

Throughout, language such as unusual, exceptional, and unique are used to describe the conditions encountered, so how applicable are your results, just to these conditions? How often do these conditions occur? Are your findings applicable to the conditions seen in Sanders et al, 2018? It would be nice to see more comparisons drawn to this dataset.

→As outlined above, we now present some more background information regarding the oxygen conditions, and moderated the language use. We also rephrased the study intention for clarity (lines 35 – 38, 43 – 46, 70 – 74). Later in the manuscript, we now compare our findings in some more detail to those by Sanders et al (2018), lines 288-291; 299 – 303.

Nitrification rates: more details are needed both in the methods and data interpretation.

→We revised this section in accordance with the reviewer's comments, including all bullet points that are mentioned separately. We specify implications of the used method now, clearly refer the reader to (Sanders and Laanbroek, 2018) for a method description, and included more details regarding the incubation. We also shortened and simplified the description of the rate calculation for clarity (lines 130-133; 135 – 143).

Isotope mass balance box model: it is difficult to assess the outcomes of the model as no details are provided, equations, parameters etc, please provide this in the methods or supplement.

→We agree. As outlined above, we now clarify that we use an isotope mass balance calculation rather than a model, and also included a supplement with mass balance calculations that the reader can refer to.

Line 34 to 36: it is not clear how the second half of the sentence links to the first

→We revised this section so that the linkage of oxygen and nitrification now is clear (lines 36 – 39).

Line 106: How was chlorophyll analyzed

→We now specify that chlorophyll fluorescence data are used as a quality indicator (117/118).

Line 126 / Nitrate Isotopes: There is no mention of a nitrite removal step, so are these actually N+N and not nitrate only? Please note the implications of this.

→We now mention that nitrate isotopes are determined by difference in cases where nitrite is present (lines 154/155)

Line 137: You note here that high concentrations were needed for isotope analysis of nitrite and ammonium, please include what concentrations needed to be greater than for isotopic analysis

→Done (lines 158; 168).

Line 225: For ammonium you use ϵ_{amm} to represent the isotope effect for ammonium removal and then go on to discuss uptake and oxidation, which is great, but why not the same for nitrite? Here you assume it is just nitrite oxidation (ϵ_{nitox}), but highlight later in the manuscript a potential role for denitrification in this system (e.g. Line 372), which would also consume nitrite, what would be the implications of this for your calculated isotope effect?

→In the initial response letter, we explained that the main motivation to evaluate alternative ammonium sinks was the mismatch between $\Delta\delta(\delta^{15}\text{N}_{\text{NH}_4} - \delta^{15}\text{N}_{\text{NO}_2})$ and $\delta^{15}\text{N}_{\text{ammox}}$ (line 274 - 289). We now explain that the isotope effect for nitrite removal meets our expectations, making alternative sinks unlikely (lines 308 - 310).

Figure 4 and associated text: it would be nice to see some errors on the calculated isotope effects.

→We included the slope uncertainty in figure 4 and in the text

Line 294 to 298: Across these lines, you discuss how nitrification scales / correlates with N content (%) and indicators of OM quality, where do I see this, you refer to Figure 4, but this is your isotope effects figure. These relationships need to be clearly evident to support your conclusions.

→We corrected this now and revised this paragraph (lines 333-339). We now explain that SPM quality is linked to nutrient turnover, and refer the reader to Fig. 2. To allow a more detailed evaluation of SPM quality, we also included a Table S1 in the supplementary material that shows the quality parameters we refer to.

Line 305 to 307 (and throughout this section): more explanation is needed for SPM reactivity, use the literature, for example, why does low C/N suggest its fresh and labile, references and details are needed for the reader to keep up with your line of thinking and confirm your conclusions.

→We inserted a paragraph that addresses OM reactivity and C/N ratios to better guide the reader, including the corresponding references (lines 345 – 350).

Comments from the editor:

N sources: In your response to the first reviewer's comment (line 70), you focused on upstream "diffuse sources" (nonpoint sources). I wondered if you could also add some quantitative information about point sources such as wastewater effluents from big cities near and within the estuary.

→We modified this paragraph and now mention diffuse as well as point sources (lines 79 – 81). Point sources usually do not play a significant role. An exception may be extreme rain events, in which a local input from the waste water treatment plant in the port of Hamburg may occur. However, this was not the case during our study.

O₂ level: In terms of, again, providing quantitative information, I thought you could better respond to the first comment of the second review on the extent of O₂ depletion by providing the overall range of DO in the Elbe River (better citing papers that addressed the issue of O₂ depletion in the same river system), or temporal variations of DO in the estuary if data are available.

→We now provide more detail regarding the oxygen availability in the estuary to better introduce the study site and our study motivation (lines 36-38 and 43 – 46)

Lines 8-9: Do you mean "biogeochemical reactors that act to modify the loads and composition of nutrients transported to the coastal zone."?

→Indeed. We changed this sentence (line 8/9).

Lines 14-18: Please remove the very general background information (e.g., "estuarine biogeochemistry is governed by settling, resuspension, and remineralization of particulate matter"); instead provide "actual findings" that you want to highlight in the abstract. It should be noted that the abstract requires a substantial revision to provide the key findings and their implications.

→We revised this abstract to make clear that these general pieces of information are actually true and relevant specifically for the Elbe estuary. Moreover, we modified the abstract in accordance with our findings (lines 15 – 18).

Sampling and sample analysis: Please pay more attention to details during the revision. For example, "surface water samples" at what depth?; uniform descriptions of instruments (brand, company, country); QA/QC for water analyses (like blanks, replicates, reference materials,,)

→More detail was added to the methods section (e.g. lines 90; 110/111; 114; 120-127).

Discussion also requires a substantial revision, because many general (background-like) descriptions are provided without clear linkages to the findings. Please consider a more focused discussion to highlight your points in a clearer way; for instance, when you begin a discussion section, you could articulate key findings in the context of your research questions.

→We double-checked the discussion section. To streamline the discussion, we either removed some background statements (e.g. line 284, see also track change version of the manuscript), or, more frequently, linked them more clearly to our findings to highlight our points (e.g. lines 273; 276; 293; 304; 333-335). Additionally we added introductory sentences at the beginning of the discussion section to guide the reader (lines 268/269; 328/329; 408-410). . We paid attention to guide the reader to our findings throughout the discussion, and added more detailed background information (on SPM, oxygen, nitrification in the Elbe) whenever it was requested (see also response to the reviewers). If these revisions are not in accordance with the editor's expectations, we would be grateful for specific suggestions regarding changes. However, we do hope our changes are sufficient and in line with the editor's recommendations. We refrained from a more extensive restructuring because this would entirely change the manuscript and require resubmission.

- *Figs. 2-6: Please pay attention to details about axis titles and legends (e.g start the titles with upper-case letters; The initial "f" in Fig. 4 can be noted in the caption.*

→All axis titles in the respective figures were modified. We added a reference to "f" in the caption for Figure 4.

References

Sanders, T., and Laanbroek, H. J.: The distribution of sediment and water column nitrification potential in the hyper-turbid Ems estuary, *Aquatic Sciences*, 80, 10.1007/s00027-018-0584-1, 2018.