

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

We thank the editor and the reviewers for the thorough review of the manuscript. We modified the manuscript, with particular focus on section 4.1 and 4.2, to clarify the connection of water column and sediment N processing to particulate matter concentration and quality. In line with this, we also replaced Figure 6 by a new version that hopefully makes it easier to follow the discussion on N sources and sinks derived from the budget calculation. In the following, we reply in detail to each individual comment by the editor / the reviewers.

- The main part of the abstract: Thanks for revising it in response to my suggestion, but I still find it difficult to figure out how you have derived your interpretations from the “actual findings”. For instance, what are the relevant results for “the estuarine nitrogen budget of the Elbe estuary is governed by settling, resuspension, and remineralization of particulate matter”? On which finding did you base your interpretation that “the reactivity and concentration of particulate matter in the estuary is the main control of nitrogen isotope dynamics and of remineralization”?

➔ As suggested, we have now revised the argumentation in the discussion to more strongly highlight the role of suspended matter in supplying substrates for nitrogen cycling (nitrification and denitrification). The main point is that organic matter remineralization fuels remineralization, so nitrification as well as oxygen consumption, and that settling and resuspension of particulate matter seem to be directly linked to these processes. We largely re-ordered the discussion, especially sections 4.1 and 4.2, to make these findings clearer.

- Section 3.1 & Fig. 2: You repeatedly refer to the “harbor (port) region” and its entrance in Fig. 2. I wondered if you could indicate the area or a key location on the figure, like by using some shaded area or vertical line(s).

➔ Done. Additionally, we now mention the harbor region in the Figure Caption of Figure 1, where it is also indicated in the figure itself.

- As I requested earlier, please avoid general (introductory) statements in Discussion and focus on key findings and their implications in line with your research questions. For instance, the new first sentence doesn't fit into the first paragraph. Rather you can start with the second paragraph, and put the first paragraph at the end of the second one to explain how your approach contrasts with previous studies. The third and fourth paragraphs, which repeat background information described in Introduction, can be removed or incorporated into the introductory sentences.

➔ We re-evaluated the discussion section and re-ordered large parts of the sections 4.1 and 4.2. In the introduction, we now included information on denitrification and its isotope effects to unburden the discussion section (e.g., lines 59 – 65).

- Another example of vague discussion point: “Based on C / N ratios and chlorophyll, we find that SPM is not equally reactive throughout the estuary. O<sub>2</sub> saturation, an indicator for heterotrophic metabolism, reaches its minimum in the harbor region, after chlorophyll fluorescence decreased, suggesting active decomposition of limnic algal material (Fig. 2a)”. I guess you meant “depletion of labile algal material” in the low-oxygen harbor region by “active decomposition of limnic algal material”. However, this and the following sentence are confusing in the sense of active decomposition zone.

➔ We refer to limnic material here, as it stems from the freshwater upstream (i.e., limnic) part of the estuary, upstream of our sampling stretch. We rephrased the section for clarity (lines 330

– 336). Algal material is decomposed, i.e., remineralized, in this section, which consumes oxygen. We avoided the term “Depletion”, because it can, in the context of this manuscript, easily be misinterpreted as “isotope depletion”, which we think would be confusing to the reader.

- As the first reviewer pointed out, I would also like to ask you to pay more attention to enhancing the cohesiveness of many short paragraphs (in contrast to the very long first paragraph of 4.4). And a thorough proofreading would reduce grammatical errors such as inconsistent use of tense (just to select one example: From 0.2  $\mu\text{mol L}^{-1}$  at stream km 618, concentrations “decreased: to  $< 2 \mu\text{mol L}^{-1}$  (1.7  $\pm$  0.6) and “remains” relatively stable along the estuary.), many present tenses used in Results and Discussion, and some awkward sentences like that appearing in Conclusions (“Ammonium assimilation competes with nitrification and produces reactive organic matter is produced that can in turn increase reactivity in the estuary.”)

➔ We have thoroughly proof-read the results and discussion section to spot remaining errors and corrected these.

### Reviewer 1

Firstly, I would like to thank the authors for the time and consideration they put into the revision and reviewer response, which I believe has resulted in an improved manuscript. However, while the overall message is clearer, clarity is still needed in some places.

In addition to this I urge the authors to think about the readability of their manuscript, currently the majority of the manuscript is short paragraphs of 2 to 3 sentences, resulting in the text lacking flow and cohesiveness.

➔ We have now thoroughly revised the discussion section according to the reviewer’s and the editor’s suggestions. Additionally, while we believe that 1 paragraph should represent one thought, we sought to adjust paragraph length accordingly.

Line 45: some additional explanation / references are needed here, why does low oxygen result in the accumulation of ammonium and nitrite?

➔ Nitrifiers are sensitive to environmental changes including availability of oxygen, which interrupts the usually smooth coupling of ammonium and nitrite oxidation (Heiss and Fulweiler, 2016). A negative correlation of nitrite and ammonium concentration with oxygen saturation has been observed previously (e.g.,(Jacob et al., 2016)). We have now reordered the paragraphs in the introduction and generally aimed to focus and disentangle it.

Line 67: The opening sentence of this paragraph where you refer to a key finding of Sanders et al, 2018 is totally disconnected from the following sentences where you introduce your study, I suggest you delete it or reword to incorporate it into the rest of the section.

➔ We agree that a deletion would have been an easy fix, but the sentence is needed to explain our study motivation. We added a connecting sentence to make this connection clearer to the reader while restructuring the introduction (now lines 37-39).

Figure 1 (same is true for Figures 2 and 3): throughout you use the term harbour region and I suggest you use this language here in figure 1 instead of or in addition to Hamburg port. For clarity it would also be beneficial to mark the harbour region onto Figures 2 and 3 (and maybe also in Figure 3 the region you use to determine ammonium and nitrite isotope effects)

- Done. We have replaced “port” with “harbor” throughout and shaded the area of interest in Figures 2 and 3.

Line 142: the fact that you saw no significant differences between treatments, needs to be commented upon. Was sufficient inhibitor used? 0.5 $\mu$ M seems very low when looking at Belser and Mays, 1980.

- True. The  $\mu$ M escaped all proof-readings, and we’re glad this was finally spotted. We changed the concentration to mM. We also clarified the explanation. Nitrite oxidation was indeed inhibited in the amended incubations, but due to the fact that the rates are governed by ammonium oxidation, the slopes for increasing nitrite concentration (addition of chlorate) and increasing nitrate concentration (unamended incubations) are statistically indistinguishable. We chose to mention both treatments, because we were under the assumption that the use of 4 incubations would decrease the uncertainty associated with the rate measurements. We hope that the extended explanation helps to solve this issue. Otherwise, we can easily remove the reference to amended incubations from the manuscript, the calculated rates will not significantly change, and all conclusions will remain unaffected.

Line 236: I find this text misleading and confusing regarding where you are defining the harbour region, as currently written here it sounds like the harbour region is from 618 to 656km, which I don’t think is the case. Please clarify.

- We have now rephrased this section (lines 234 – 236), the harbor region is indeed defined as 615 – 635, following previous studies of this region (Amann et al., 2015;Brase et al., 2017;Sanders et al., 2018). Oxygen consumption starts in the harbor region, but continues further downstream.

Line 273 and 276: If you want to use the words rare and unusual as you do in this paragraph you need to provide some context and compare / contrast to the literature to demonstrate this is the case.

- It is true (as also noted by reviewer 2) that summer oxygen depletion is not so rare. However, the magnitude and spatial extent is unusual compared to previous measurements. As such, we were uniquely able to determine isotope compositions in a sufficient number of samples and thereby derive isotope effects. This is usually not the case. We have now rephrased this section to put this into context and refer to Sanders et al. 2018 for comparison.

Line 308/309: See comment below on section 4.4 as well, this only makes sense once I get to the end of the manuscript. Here you need to comment directly on a potential role for sedimentary and water column denitrification.

- We have now included a paragraph on denitrification in the introduction section. (lines 59 – 65).

Line 338: ‘where organic matter is fresh’ this statement comes too early as you don’t provide evidence for this until later in this section where you introduce the C/N results.

- We have re-ordered the entire section 4.2 and in parts rephrased it for clarification.

Line 343: What is your evidence for the statement remineralization is prominent, im actually not even sure what that means – this section is about determining an isotope effect and comparing it to the literature.

- This section was rewritten, the isotope bit was moved to 4.1, and the role of remineralization is now hopefully addressed more clearly in the revised section 4.2

Line 359: potentially even denitrifying, where is the evidence for this prior to this statement? Or at this point of the manuscript are you relying on the literature, if so a reference is needed.

- We have rewritten this section, removing the statement. It originally related also to the reactivity of SM, which now is discussed later in the discussion (lines 339 – 343).

Section 4.4 : it is only here that things finally fall into place for the reader, you need to introduce the ideas of water column and sedimentary denitrification earlier and their impacts on N isotopes (maybe in the introduction along with where in the estuary you would expect to find each based on the literature?), as prior to this you just use the term denitrification. This is confusing for the reader as you conclude that your isotope effect is representative of only nitrite oxidation as it is unaffected by additional nitrite sinks or sources (line 308), but then mention a number of times in the text a role for denitrification in the Elbe and it is not 100% clear in all cases where in the estuary you are referring to (e.g. line 359). Would a cross plot of  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of nitrate not be informative with respect to picking these processes apart – this seems typical for nitrification / denitrification in other studies so would be good to highlight why this method is not relevant here?

- We now mention denitrification and the respective isotope effects in the introduction section. We also re-ordered the section 4.1, in which isotope effects are discussed, and added reference to denitrification here.

The use of a  $\delta^{18}\text{O}/\delta^{15}\text{N}$  cross plot is typically more informative under circumstances exhibiting net  $\text{NO}_3^-$  loss, and specifically highlighting how singular loss mechanisms may be overprinted by simultaneously occurring production. In the case presented here, there is an accumulation of  $\text{NO}_3^-$  over time (so net production), and the  $\delta^{18}\text{O}/\delta^{15}\text{N}$  cross plot, although strongly correlated, instead reflects a strong pattern of mixing between upstream sources (high values) and in situ  $\text{NO}_3^-$  production by nitrification (lower values).

Line 435: a reference is needed to demonstrate that denitrification inside particles is feasible

- With this statement, we postulate the hypothetical role of water column denitrification. References for the plausibility are given in the following lines. However, we do not have direct evidence for water column denitrification. We have rephrased this section for clarity (lines 420 – 427) and have included a reference for anoxic processing in flocculate material (Klawonn et al., 2015).

Line 445 to 448: more explanation is needed here for the non-expert, and references are also needed, for example it should be highlighted how these ideas do / don't differ from km 640 to 655 where you also postulate a role for sedimentary denitrification.

- The main difference with respect to sediment denitrification further upstream is that the isotope mass balance requirements are not met in this section. We changed Figure 6 to better illustrate our reasoning in this section and rewrote parts of this discussion section. Furthermore, we also added some more detail to our explanation to make our assumptions clearer non-isotope-specialists (434 – 441).

Line 457: language, produce used twice

- Changed.

Line 462: 'alter the balance of nitrification and denitrification' it is not clear to me where this was directly discussed in the manuscript with respect to OM.

- We have now reworded the conclusion section, to emphasize that organic matter reactivity (via remineralization) directly affects denitrification and nitrification, and thereby the relative activity of the two processes (lines 452 – 454).

## Reviewer #2

We thank the reviewer for the positive evaluation of the manuscript. We addressed the specific comment regarding “rare events” by rephrasing the respective sentence in the course of the revision. (Formerly line 273).

## References

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- Heiss, E. M., and Fulweiler, R. W.: Coastal water column ammonium and nitrite oxidation are decoupled in summer, *Estuarine, Coastal and Shelf Science*, 178, 110-119, <https://doi.org/10.1016/j.ecss.2016.06.002>, 2016.
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- Klawonn, I., Bonaglia, S., Brüchert, V., and Ploug, H.: Aerobic and anaerobic nitrogen transformation processes in N<sub>2</sub>-fixing cyanobacterial aggregates, *The ISME Journal*, 9, 1456-1466, 10.1038/ismej.2014.232, 2015.
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