

Response to Referee #1

We thank the referee for the helpful and constructive comments on our manuscript. Below, we address how the individual issues that were raised will be addressed in the revised manuscript version.

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.

We were indeed somewhat carried away describing the situation as so unusual and exceptional. It is indeed so that low oxygen conditions occur in summer in the Elbe /Port region, and that they can lead to fish kills and overall a bad ecological status for the river/ estuary. Our intention was two-fold: (1), to use this disruption of nitrogen cycling (as visible in nitrite accumulation) to assess isotope effects of nitrogen recycling processes in situ, and (2), to determine driving factors for biological turnover – which will hold regardless of low oxygen concentrations.

We will rephrase the intention of the study in the introduction to make this clearer.

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.

This is true. The catchment is largely agricultural, especially in the Czech republic and the middle reaches of the River in Germany, which creates a high N input to the estuary. Diffuse input of N into the stream occurs mainly upstream of our sampling stretch via input from upstream tributaries. These receive N from agricultural (diffuse) sources. We will explain this in more detail in the revised version of the manuscript. As for the area of N-discharge, we were here referring to the mouth of the Estuary. We will clarify this in the revision.

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 these samples, the nitrate (or rather, nitrite + nitrate) isotope composition, as determined with *Ps. aureofaciens*, was corrected for the measured nitrite isotope value. We will add an explanation to the method section.

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?

It is true that both processes may occur in parallel between stream km 641 and 656. However, in this section, ammonium concentration is already low (3.5 μM at stream km 641, then 1.5 μM or less). Hence, we did not consider the isotope effect that ammonium

oxidation may have on nitrite isotopes. We actually assume that in this river section, ammonium assimilation gains in importance, because chlorophyll fluorescence increases. In this case, little ammonium would be oxidized to nitrite. We will insert a short explanation regarding low ammonium concentration, and will briefly assess this matter when discussing alternative ammonium sinks.

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 ($\delta_{18O_{H2O}}$) ?

In this sampling, we did not measure the isotope value of Elbe water. However, as a large river, the Elbe has relatively little seasonal fluctuations in d_{18O_H2O} , and outside the salinity gradient, the isotope value should be $\sim -8\text{‰}$ (Johannsen et al., 2008). We intentionally kept this statement qualitative, because the effects of nitrification on oxygen isotopes in nitrate are addressed elsewhere (Sanders et al., 2018). There, the authors find that the newly added nitrate has isotope values that are between -2.8 and -9.5‰ , which is in the range of ambient water in the Elbe, and thus supports the relevance of nitrification. We will more clearly refer to this study in the revised version.

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.

Going through the text again, we agree that the description of the calculation may appear a bit too brief. However, the initial description of a isotope box model probably was misleading. The calculations are actually rather based on a mass balance approach: Each point of measurements along the river is conceptualized as representing the homogenous composition of a parcel of water, with the sampling location as the center of the parcel. We consider four pools of N (PON, NO_3^- , NO_2^- , NH_4^+), and calculate the total N (TN) pool as:

$$[TN] = [PON] + [NO_3^-] + [NO_2^-] + [NH_4^+] \quad \text{Eq S1}$$

The mass weighted isotopic composition of total nitrogen (TN) is computed as:

$$\delta^{15}N_{TN} = \frac{[\delta^{15}N_{NO_3^-} * [NO_3^-] + \delta^{15}N_{NH_4^+} * [NH_4^+] + \delta^{15}N_{NO_2^-} * [NO_2^-] + \delta^{15}N_{PN} * [PN]]}{[[NO_3^-] + [NH_4^+] + [NO_2^-] + [PN]]} \quad \text{Eq. S2}$$

We use this isotope mass balance model to examine downstream changes to total N and explore biogeochemical explanations for regions where observations are in violation of isotope mass balance. The description of the mass balance as such is already incorporated in the text in section 4.3.

Beyond this mass balance, and isotope mass balance, approach, we did not construct a box model. So, to avoid confusion, we will clearly refer to a mass balance approach (instead of referring to a box model) in the revised version. We will also add a supplement in which the above equations and assumptions are spelled out.

References

Johannsen, A., Dähnke, K., and Emeis, K.: Isotopic composition of nitrate in five German rivers discharging into the North Sea, *Organic Geochemistry*, 39, 1678-1689, 2008.

Sanders, T., Schöl, A., and Dähnke, K.: Hot Spots of Nitrification in the Elbe Estuary and Their Impact on Nitrate Regeneration, *Estuaries and Coasts*, 41, 128-138, 10.1007/s12237-017-0264-8, 2018.