Response to Referee #2

We thank the referee for the constructive and elaborate comments on the manuscript. Below, we address each issue individually, and explain the changes we will make to the revised manuscript to meet the reviewers criticism.

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.

The most important difference between the cruises that are regarded in Sanders et al (2018) is that oxygen saturation is even lower (32% saturation in comparison to ~40% in the lowest depletion situation in Sanders et al. 2018). This leads to further elevated nitrite and ammonium concentration. However, we admit that the exceptionality of biogeochemical conditions during our cruise may be a bit overemphasized. Accumulation of ammonium and nitrite, and concurrent oxygen depletion, occurs regularly in the Elbe – only rarely so that the number of sampling points within the regions of ammonium and nitrite accumulation allows for computation of isotope effects, which probably tempted us to refer to the situation as a bit too exceptional. We will moderate the language use in the manuscript and insert a short paragraph in section 4.1 where we compare the data to Sander et al. (2018). This may also alleviate some issues regarding the nitrification rate assessment, as discussed below.

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

Nitrification rate measurements (and the intentional use of the long incubations, including potential caveats) are discussed in more detail in Sanders et al., 2018. Below, we will address the individual issues that were raised in more detail.

- *Nitrite and nitrate concentrations were stable, meaning all ammonium had been utilized?*

  Yes, that is the underlying assumption. Ammonium is utilized, and remineralization has apparently ended. Based on this approach, we aim to evaluate the nitrification potential of a given water sample, without addition of any extra substrate. We will refer to this briefly in the method description.

- *room temperature, was this close to insitu conditions?*

  Yes, under the given conditions in the Elbe, room temperature was indeed comparable to the water temperature in situ. The average water temperature in the sampling stretch was close to 24°C, and temperature in the labs is held at 22 +/- 2 degrees. We will add this to the method section.

- *14 days seems a long time and that bottle effects would be likely, was there any sign of this in the data? Exponential behavior for example? It would be beneficial to show some of this data, maybe in a supplement. How representative are these rates of insitu, as there seem to be a number of caveats, none of which are mentioned and there is also no comparison of the rates determined to those in the literature to put them in context, it is only mentioned that they are high.*

Indeed, nitrification rates are not addressed in detail in our study, as this was not the most important focus area. The incubation method is based on DINXXX, and is described in Sanders et al., 2018. We reckon that 14 days appear long, especially in comparison to frequently used techniques that are based on addition of labeled ammonium. However, the caveat of these methods is that ammonium is usually rapidly converted (or taken up) by any kind of micro-organisms, and our approach was precisely to address nitrification based on the material that was present in the water at the time of sampling.

Any rate determination in the lab will have its advantages and disadvantages, and a long-term incubation may overestimate remineralization and thus in-situ rates (see (Sanders and Laanbroek, 2018). We will, as mentioned above, compare the situation during our cruise to the data from Sanders et al. 2018, and will use this opportunity to briefly compare the nitrification rates. The measured rates are comparable, but slightly higher than average in Sanders et al., hence referred to later as "high". We will address this, but will refrain from a more detailed comparison to nitrification rates measured with other methods, because this is not the focus of our study and has been done previously by Sanders et al. (2018)).

- *you mention in the methods that ammonia and nitrite oxidation rates were determined but this is not mentioned in the results/discussion.*

In the manuscript, we refer to incubation rates as the average of all 4 incubations. Nitrite and ammonium oxidation rates were very similar, which is why we decided to present the overall nitrification rate as an average of all assessments. We will more clearly write this in the revised version of the manuscript to avoid confusion.

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. The way it is currently phrased out in the manuscript, the calculations are not as clear as they could be. We address this topic as well in the response to reviewer #1. We actually used an isotope mass balance approach rather that an actual box model, so there are no model parameters we could spell out.

In the mass balance approach, we considered the four pools of N (PON, NO3-, NO2-, NH4+), and calculate the total N (TN) pool as:

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

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

$$\delta^{15}N_{TN} = [\delta^{15}N_{NO3} * [NO_3^-] + \delta^{15}N_{NH4} * [NH_4^+] + \delta^{15}N_{NO2} * [NO_2^-] + \delta^{15}N_{PN} * [PN]]/$$

$$[[NO_3^-\text{-}] + [NH_4^+] + [NO_2^-] + [PN]] \qquad\qquad \text{Eq. S2}$$

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. To make this point clear, we will add a short supplement with some details on the mass balance and with the above equation.

Specific comments

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

True. We will revise this. The line of thought was that oxygen conditions have improved, but this has in turn fueled nitrification, so that the nitrate load today remains high (despite reduction measures) and is at times even doubled in the estuary. We will make this clear in a revised version.

Line 106: How was chlorophyll analyzed

Chorophyll is measured by fluorescence with an on-line sensor that was part of the Ferrybox system (SCUFA Fluorometer, Turner Designs, San Jose, CA, USA). This is mentioned in line 82/83. We will insert a short reference to the sensor data later in the text.

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.

The nitrate isotope values were measured with Pseudomonas aureofaciens, but they were corrected for the contribution of nitrite isotopes, which were measured independently with Stenotrophomonas nitritireducens. We will clarify this in a revision.

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

We usually measured samples that contained 2 µmol L-1 nitrite or ammonium, or more. We will add this to the text in the revised version.

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?

This is a good point. The main motivation to evaluate alternative ammonium sinks was the mismatch between $\Delta\delta$ ($\delta15N\_NH4 - \delta15N\_NO2$) and $\varepsilon15_{ammox}$ (line 274 - 289). We do not see indications for an unusual nitrite oxidation isotope effect in our study.

Additionally, throughout most of the sampling stretch, denitrification will most likely occur in sediments, where it will not affect d15N_NO2. The section of possible water column denitrification is stream km 618 – 635, where d15N-NO2 is relatively stable. The isotope effect of nitrite was calculated at decreasing nitrite concentration, from stream km 641 to 656. We have no indication for an additional sink process for nitrite in this stretch (in contrast to ammonium), so we did not evaluate the role of water column denitrification in this case. For clarity, we will insert a brief reference to this near the discussion of ammonium isotope effects.

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

We will add measures of uncertainty of the slope in the figures and text in the revised version of the manuscript.

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 clearly evident to support your conclusions.

We thank the reviewer for notifying us on this mismatch. This was probably a remnant of a more lengthy discussion in a previous draft; we apologize for the mistake. Nitrification rates and N%(SPM) are correlated, see figure below. However, this is indeed not shown in Figure 4. We will correct this in a revision and refer to the actual correlation.
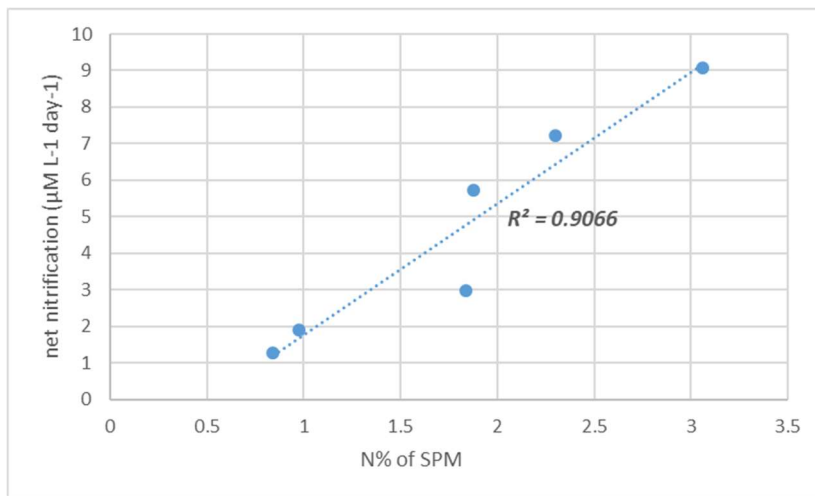

Figure 1: Correlation of net nitrification rates and N% in suspended particulate matter.

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 will revise this paragraph and back it up with references to better guide the reader and support our conclusions. For fresh organic matter, we refer to the Redfield ratio, for which a C : N ratio of 6 -7 has been calculated (e.g., 6.6 in (Martiny et al., 2014)). With increasing remineralization, easily accessible N is used, and the C/N ratio increases (e.g., (Islam et al., 2019). We will add a short paragraph on OM reactivity in this section.

References:

Islam, M. J., Jang, C., Eum, J., Jung, S.-m., Shin, M.-S., Lee, Y., Choi, Y., and Kim, B.: C:N:P stoichiometry of particulate and dissolved organic matter in river waters and changes during decomposition, Journal of Ecology and Environment, 43, 4, 10.1186/s41610-018-0101-4, 2019.

Martiny, A. C., Vrugt, J. A., and Lomas, M. W.: Concentrations and ratios of particulate organic carbon, nitrogen, and phosphorus in the global ocean, Scientific data, 1, 140048, 10.1038/sdata.2014.48, 2014.

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.

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.