

Comments to „Nitrification and Nitrite Isotope Fractionation as a Case Study in a major European River” by Juliane Jacob et al. (2016)

Referee comments in Times, author responses in Arial

The authors have provided the information in the revised manuscript as requested by the reviewer. We thank the anonymous reviewers for their suggestions and the valuable concerns. In parts, the reviewers address similar issues, we decided to address these points jointly in more detail.

One concern was that no independent rate measurements and clues on what processes are active/negligible were done and that our assumption that nitrite oxidation was the main nitrite sink might not be valid.

With regards to this subject, we reconsidered our data, and we agree that our focus might have been too narrow to account for potential nitrite sinks. Accordingly, we have rephrased “nitrite oxidation” to “nitrite removal”, and added a section about other potential sinks, like riparian denitrification, nitrite assimilation by phytoplankton, dilution and source-mixing.

Of these four potential processes, we assume that nitrite assimilation as a sink is of lesser importance. Even though the possibility of nitrite assimilation by phytoplankton is commonly accepted (Collos, 1998), it is energetically expensive because phytoplankton would have to reduce four electrons for every molecule of nitrite to assimilate nitrite. Furthermore, this reduction of nitrate to nitrite usually happens within the cell in the cytoplasm and the chloroplast, respectively. A direct assimilation of nitrite requires active transport through the chloroplast membrane and needs additional energy (Lomas and Lipschultz, 2006), making this process unfavorable in the presence of ample nitrate.

This process would not bias our isotope calculation, because nitrite assimilation in a pure culture was associated to a very small fractionation factor of -0.7 to +1.6‰ (Wada and Hattori, 1978), and thus would only have a minor influence on the isotope signature in the river.

Regarding denitrification, our initial assumption was that it would be negligible in the water column, because the oxygen concentration is above 6 mg L⁻¹, and that sedimentary denitrification, while potentially quantitatively important, has little to no impact on isotope values of the water column nitrate pool (Brandes and Devol, 1997; Mariotti et al., 1988; Mariotti et al., 1982). However, in the revised version, we consider riparian denitrification as a nitrite sink, which can have a notable apparent isotope effect (Mengis et al., 1999; Sebilo et al., 2003).

Dilution with water masses containing lower nitrite concentrations is unlikely because of the changing nitrite and nitrate isotope values. Source-mixing has also not been taken into account because nitrite is generally not abundant in the catchment and is immediately removed due to its toxicity (page 7, line 7).

We suggest the different shaped graphs of ammonium and nitrite concentrations and isotopes are not only influenced by hydrology, but more by biology. AOB and NOB have a different behavior/sensitivity to surface irradiance (Horrigan et al., 1981). NOB are more light sensitive (Olson, 1981) and poorly recover from photoinhibition (Guerrero and Jones, 1996). This could be a reason why nitrite can accumulate and the variations in concentrations and isotope values are less pronounced.

We did indeed not present rate measurements, however, we conducted incubation experiments to determine ammonium oxidation and nitrite oxidation rates over an annual cycle in 2012. We find nitrification rates of 1 to 14 $\mu\text{mol L}^{-1} \text{d}^{-1}$ in winter and summer, respectively. However, due to time constraints, these measurements were not done during the flood event. In any case, such rate measurements can only serve as a proof that nitrification is active, because our sampling scheme does not really contain a temporal component, and rates cannot be connected to the isotope changes we see.

Another concern was the calculation of the fractionation factor of nitrite removal, which was based on Rayleigh closed-system equations. In the original manuscript, we decided to use this assumption, because ammonium concentrations are below the detection limit and from this perspective nitrite is the substrate being consumed. However, we reconsidered this and agree with the reviewers that this assumption is not valid in our case, as we also discussed in the original submission when we evaluated ammonium production. Consequently, we replaced the Rayleigh calculation with an open-system assumption (Sigman et al., 2009) in the revised manuscript. Using this approach, we calculated an apparent isotope effect of $-10.0 \pm 0.1\text{‰}$, which is still conventional.

Both reviewers suggested that the use of a simple box model or simple reaction model should be constructed to assess rates and processes occurring in the river. We took this into account and intensively discussed modeling options with two colleagues experienced in isotope modeling and in nutrient modeling in the Elbe. Our idea was to include isotopes in a biogeochemical model previously published by Friedhelm Schröder, who intensively studied the Elbe River in the 1980s and 1990s (Schroeder, 1997). However, both colleagues agreed that a model is nearly impossible to build based on one sampling station only; because incoming and outgoing concentrations are basically unknown and no mass balance can be set up.

In response to the reviewers' suggestion, we decided to try what we considered the next best option. As nitrite trends during the flood are smooth and isotope changes follow a linear pattern, we assume that the ratio of nitrite processing pathways is constant, even though we cannot quantify rates. The source signal is the isotope value of the maximum nitrite concentration, and then calculated different scenarios with varying rates of nitrite oxidation, ammonium oxidation and denitrification to reproduce our measured data, assuming isotope effects from the literature.

These fractionation factors vary depending on involved microorganisms and environment (Buchwald and Casciotti, 2010; Casciotti, 2009; Casciotti et al., 2003; Delwiche and Steyn, 1970; Mariotti et al., 1981; Santoro and Casciotti, 2011; Yoshida, 1988), but within a range that appeared plausible, we varied these effects and corresponding rates. One plausible scenario is that we see a mixed signal of riparian denitrification and nitrite oxidation, with a constant replenishment of the ammonium pool from suspended matter. We will discuss these calculations in a revised version.

Anonymous Referee #3

We would like to thank reviewer for the evaluation of our manuscript, and we will implement the suggestions.

In the beginning of the response letter, the authors have answered general concerns of all reviewers (nitrite oxidation only, other potential nitrite sinks like nitrite assimilation, denitrification and dilution, box model, Rayleigh fractionation).

Could the ammonium and nitrite not be imported from the catchment, from internal cycling therein (in soil)? Which aspects of the isotope data enable partitioning of processes that happened in situ vs. the catchment? Does it even matter?

As mentioned in the manuscript (page 7, line 16 – 18 and references therein), an ammonium and nitrite input from the catchment is unlikely, because of the positive charge of ammonium molecules in combination with adhesion to clay. Nitrite is toxic and not abundant in steady-state systems, because it is rapidly nitrified (page 7, line 7 and references therein). To our knowledge, these mechanisms are active during the flood in the catchment area and ammonium and nitrite in the Elbe River derive from internal processes (remineralization and nitrification). Theoretically, phytoplankton can release nitrite in stress situation (Lomas and Lipschultz, 2006), but an amount cannot be estimated. Therefore, isotope changes of ammonium and nitrite happens in situ. However, ammonium derives from remineralization of suspended particulate matter and the origin cannot be specified. The $\delta^{15}\text{N}$ -SPM values vary between 8.1 and 6.2‰ (page 6, line 1) and this show a much smaller variability then $\delta^{15}\text{N}$ - NH_4^+ values. Furthermore, the authors would like to point out that the origin of substrate (ammonium, nitrite) is of minor importance for the isotope effect coupled to nitrite removal. In any case, though, we now included other source and sink processes, but point out that nitrification is an important ammonium sink (and thus nitrite source). This is supported by rate measurements done in the river, where we find nitrification rates of up to $14 \mu\text{mol L}^{-1} \text{d}^{-1}$. We mention these (yet unpublished) data in the manuscript now, but would like to point out here that they can unfortunately not be directly linked to the isotope values we measure at this site, because the measurement at one site has no temporal component to it.

Could the authors not generate plausible scenarios of nitrite production/oxidation and associated isotope effects that could constrain the relative fluxes, given the measured isotope composition of ammonium and nitrite? I realize the range of solutions may be too broad, but perhaps some scenarios could be ruled out with such an exercise.

The authors have extended their back-of-the-envelope calculation (page 9, line 21) as mentioned in the general comments.

The isotope composition of nitrite in the environment is implicitly the result of multiple co-incident reactions, each of which is associated with an isotope effect. It's self-evident that a single Rayleigh fit to NO_2 consumption will not describe a single uni-directional reaction on said NO_2 , which does not mean that culture results cannot be extrapolated to the environment. What an odd conclusion! I urge the authors to refine this conclusion so as to appear less incongruous.

We agree with the concern of using a Rayleigh model and have changed this as mentioned in the general comments.

In large parts, nitrate derives from the catchment area (page 6, line 10 – 13) and isotope changes are within a narrow range because of reduced phytoplankton assimilation (page 6, line 26).

However, after revision of the manuscript, the authors would attenuate the statement of “no inverse isotope fractionation during nitrite oxidation” to something along the line of “conventional isotope fractionation during nitrite concentration removal”.

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