

**“Nitrification and Nitrite Isotope Fractionation as a Case Study in a major European River”,  
by Juliane Jacob, Tina Sanders, and Kirstin Dähnke**

In this submission, the authors present concentrations and N (and O) isotope compositions of N-species during a flood event in the Elbe River in summer 2013. The dataset includes nitrate, nitrite, ammonium and SPM (concentrations and isotope compositions) data, which were analyzed simultaneously and in high resolution (up to twice a day), although unfortunately only for a short period of time (14 days). The presentation of this dataset in the main Fig. 2 is good, however, I have some major concerns regarding the discussion and main conclusions (see also general comments below). The biggest problem, in my opinion, is that neither independent rate measurements nor convincing clues on what processes are active/negligible exist. This makes data interpretation difficult. Instead of trying to deduce isotope fractionation factors with a Rayleigh approach (which I think is not justified in this system), I would suggest to use a simple reaction-model with reaction rate constants as tunable parameters (and assuming literature fractionation factors). What processes need to be active and at what rates in order to reproduce all of your concentration and isotope data? Do these predicted rates make sense? Or do you see any reason why your underlying assumptions (e.g., literature fractionation factors) could be different in your system?

Datasets like the one presented here are scarce but interesting. I therefore see potential for its publication in *Biogeosciences*, but only after addressing the major issues raised here.

**General comments**

I have a few general concerns with the data interpretation. First of all, it is not clear to me why the authors conclude that all nitrite must be oxidized to nitrate. Why can we exclude nitrite assimilation into biomass (while nitrate assimilation was the main argument for nitrate removal...)? And why was dissimilatory nitrite reduction disregarded? I understand that the water column is oxic, and denitrification unlikely, but what about sedimentary denitrification and nitrite reduction? Apparently 25% (which is not negligible!) of nitrate loads to the Elbe River is lost through denitrification (page 8, line 30).

Second, I don't think a Rayleigh model is of much use here, where there is simultaneous nitrite consumption and production, mixing and dilution. If the authors decide to keep such a calculation of apparent fractionation factors in the manuscript, the results should be discussed much more carefully and adequately. To conclude that in this environment we observe a normal isotope effect associated with nitrite oxidation is wrong: First, the calculation of the isotope effect is biased, second we don't know if nitrite oxidation to nitrate is the only nitrite removing process (see comment above). On the other hand, if an apparent fractionation factor is calculated for nitrite consumption, why not for nitrate removal as well (which the authors claim is through assimilation only)? Also, the  $^{18}\text{O}$  to  $^{15}\text{N}$  enrichment during nitrate reduction (assimilatory or dissimilatory) should be independent of mixing/dilution and could be more diagnostic of the actual process affecting nitrate dynamics. The authors calculate an  $^{18}\text{O}$  to  $^{15}\text{N}$  enrichment ratio of 0.82 (page 6, line 32), which is not characteristic for nitrate assimilation.

## Specific questions and comments

The title is rather weak. What does “nitrification as a case study” mean? I would change the title to something like “Nitrification and nitrite isotope composition after an exceptional flood in the Elbe River”. You could also change “a major European River” to “the Elbe River”.

### Page 1:

Line 8. Why do we care about a nitrate source from  $\text{NH}_4^+$ ? From an environmental perspective the source of total fixed N is important to know.

Line 9. What do you mean with recycling processes? DNRA?

Lines 10-12. Do you mean the isotope effects of ammonium oxidation to nitrite and nitrite oxidation to nitrate? Why do we care about isotope effects associated with nitrite reduction/oxidation if no nitrite accumulates? The isotope effect of ammonium oxidation should be easier to predict if no nitrite accumulates, not more difficult.

Line 16. Delete “the” before concert.

Line 16. Define SPM.

Line 18. What do you mean by “internal recycling processes”? DNRA?

Line 18. State that xy% of reactive N in the water column was present as nitrate, which leached from the catchment area. This also contradicts the first sentence of the abstract, which says that nitrification is “an important nitrate source” in the water column.

Line 19. The maximum ammonium concentration stated here does not agree with Fig. 2b. After reading all the paper I think there is a mistake in the legend in Figure 2b. The symbols for ammonium and nitrate concentrations are mixed up?

Line 20. Write here on what basis you conclude that the increase of ammonium and nitrite concentrations is due to remineralization and nitrification.

Line 25. What is the justification then to use a Rayleigh model?

Line 26. I think this statement is not justified. The data are not a first approximation of the isotope effect of nitrite oxidation in a natural environment. First, as stated one line above, we have co-occurring nitrite production, and second, we have not seen a proof that nitrite is indeed oxidized (and not assimilated/reduced).

Line 33. Write “dissolved inorganic nitrogen (DIN)”.

Line 34. What about Nr inputs? Ammonium input has been reduced, so now the same amount of Nr is introduced to the system as nitrate?

### Page 2:

Line 4. Sentence starting with “nevertheless” makes no sense. Nitrate is part of DIN.

Line 15. The fractionation factor can be calculated differently, not only approximated using a Rayleigh distillation model.

Line 16. (Half-)sentence makes no sense. What are the “individual uptake processes”?

Line 26. What do you mean by “relatively healthy”?

#### Page 3:

Line 16. How were samples taken? From the shore? From a boat?

Line 21. Define SPM.

Line 22-24. Please give a detection limit and analytic precision for nitrate, nitrite, and ammonium concentration measurements.

Line 26. “Dual nitrate plus nitrite...”

#### Page 4:

Line 1. How was ammonium converted to nitrite? Does this mean that the isotope measurements include the naturally abundant nitrite? Why was the produced nitrite not reduced to N<sub>2</sub>O with the bacterial method as stated above, but with sodium azide instead?

Line 9. Delete “, which both by definition have a delta value of 0‰”. This is obvious.

Line 18. What are the “additional standards”?

Lines 19-23. See my comments above. State here under what conditions a Rayleigh model can be used. Why do you think these conditions are met in your system? What could such an apparent fractionation factor tell us, what certainly not. What are the limitations. It is too simplistic (even wrong) to just say that a fractionation can be calculated this way in your system.

#### Page 5:

Line 6. I guess the cited 2015 is a mistake, if the here presented data are from 2013?

Line 12. In the abstract it was a maximum of 4.5  $\mu\text{mol L}^{-1}$ . Overall, there is only a 1.1  $\mu\text{mol L}^{-1}$  decrease over 6 days. What is the precision of your measurements?

Line 15. Logic is not clear. First nitrite is discussed, then ammonium, then nitrite again.

Line 16. What is this limit of 2.7  $\mu\text{mol L}^{-1}$ ?

Line 16. We see an increase in nitrite concentrations and we also see a decrease in oxygen concentrations. The nitrite concentration increase is not necessarily due to an oxygen concentration decrease.

Line 27. Here and throughout the manuscript: define “flood” and use it consistently. Or what does “before the flood” mean here?

Line 27. I don't see the nitrite concentration increase in the figure. Can you give numbers?

Lines 28-29. The isotope minimum does not correspond to the concentration maximum. Why?

Line 30. See comment above and avoid saying “calculated” fractionation factor, as if this would be a real/robust value. With a Rayleigh model you can at best approximate a fractionation between a substrate and a product, and only under certain assumptions (no co-occurring production, product continuously removed from a well-mixed system, etc.)

Line 35. Is the legend in Fig. 2b mixed up? Otherwise this statement makes no sense.

Line 36. Explain why you compare  $\delta^{15}\text{N-NH}_4^+$  to [SPM]. Also, I do see some zic-zac correlation between the two. The second half of the sentence “, but not so much for  $\delta^{15}\text{N-SPM}$ ” makes no sense.

#### Page 6:

Line 2. What means “initial”? Your first measurement?

Line 8. Legend in Fig. 2b is mixed up. Also give here the actual nitrate concentrations.

Line 10. The sentence makes no sense. Or what does “an increase in SPM peak” mean?

Line 10. Sentence starting with “both...” needs to be rephrased.

Line 13. How do you explain the time shift between the [SPM] and  $[\text{NO}_3^-]$  peak? If the same mechanism is causing the peak (leaking from agricultural soils) I would expect a simultaneous peak?

Lines 13-14. I don't understand the point made here. Can you elaborate more on why you expect a  $\delta^{15}\text{N-SPM}$  decrease from 7.8 to 6.2‰? And why would along this argumentation  $\delta^{15}\text{N-SPM}$  increase after 13 June and then decrease again after 16 June?

Line 21. “reduced biological activity” relative to what? To summer, when we observe average  $\delta^{15}\text{N-NO}_3^-$  values of ... ‰?

Line 22. What “then”? in summer?

Line 23. Add a reference to where it was shown that nitrate concentrations decrease due to assimilation only (and no denitrification).

Line 29. Rising oxygen concentrations to above saturation? Otherwise it would be more interesting to know what caused the  $[\text{O}_2]$  decrease before the system is ventilated again.

Line 30. Can you show here this correlation?

Line 32. Can you add a plot showing the  $\delta^{18}\text{O-}$  to  $\delta^{15}\text{N-NO}_3^-$  increase?

Line 33. No! Exactly the opposite is the case. 0.82 is significantly different from  $1.00 \pm 0.01$ , which could have been indicative of assimilation (Granger et al. 2004). The lower value of 0.82 is interesting though. Can you elaborate on the reason for the observed  $^{18}\text{O}$  to  $^{15}\text{N}$  enrichment ratio?

#### Page 7:

Line 2. I am not convinced at all. You did not prove that nitrate concentrations decreased due to assimilation only. In contrary, the  $^{18}\text{O}$  to  $^{15}\text{N}$  enrichment ratio seems to be indicative of other processes.

Line 2. What do you mean by “explained by hydrographic properties”?

Line 3. What is “not quite the case”? Why can nitrite and ammonium concentrations not be explained by assimilation and “hydrographic properties”?

Lines 4-5. There are lots of conclusions in this sentence without any argumentation, underlying data, or proofs. Ammonium and nitrite are also substrates for assimilation and anammox, and nitrite can be reduced to  $\text{N}_2\text{O}$ ,  $\text{N}_2$ , or ammonium. A decrease in ammonium and nitrite concentrations is hence not a proof for nitrification.

Line 6. Start here with a short discussion of where the  $\text{NH}_4^+$  can come from, instead of just claiming that all of it is originating from SPM remineralization.

Line 8. With “initially” you mean your first measurement?

Lines 14-15. Sentence not needed. What do you mean by “this also explains”?

Line 14. “...between  $\delta^{15}\text{N}$  of dissolved...”

Lines 16-18. Move this discussion up. (see comment above, line 6). Also, SPM is leaching, so  $\text{NH}_4^+$  could be attached to it and thus leaching as well?

Line 22. Why are  $\text{O}_2$  concentrations “low”?

Lines 22-23. But phytoplankton activity is not 0, right? Based on what are you so convinced that nitrification is the ammonium sink?

Line 23. Why do you need to say that AOB are active under low  $[\text{O}_2]$ ? What do you mean by low  $[\text{O}_2]$ ? 6 mg/l is not really low.

Line 24. Why can nitrification “obviously” not keep up with remineralization? Why is this obvious?

Line 27. Define “low oxygen concentration”.

Line 28. Not really. Only the last five data points (out of 21) show a negative correlation.

Lines 29-30. Where do you see the “newly produced nitrate”? If nitrate is produced it contradicts the first sentence of this paragraph, which says that nitrate dynamics are controlled by assimilation and “hydrographic properties”. And again, why do you completely rule out any ammonium assimilation? Why would only nitrate be assimilated and not ammonium?

Line 30. What causes the lowered  $[\text{O}_2]$ ?

Line 31. Within 7 days of or after what?

Lines 31-32. Again, this is an overstatement. You have not proven that all ammonium is oxidized to nitrate and even less so by AOB.

Line 33. Where is that  $0.5 \mu\text{mol L}^{-1} \text{day}^{-1}$  coming from? I see a sharp decrease in  $[\text{NH}_4^+]$  of about  $3 \mu\text{mol L}^{-1} \text{day}^{-1}$  on 15 June 2013.

Line 35. Where is that drop in nitrite concentrations after the flood? In Fig. 2b  $[\text{NO}_2^-]$  remains at  $>3 \mu\text{mol L}^{-1}$ .

Page 8:

Line 1. Title is misleading (wrong) and contradicts your conclusion from the last paragraph on page 9.

Lines 3-4. No input of new nitrite? This directly contradicts your conclusions e.g., on page 9, line3.

Line 5. How to you consider the surface water of a river a “closed system”?

Line 8. What was in steady-state during the flood?

Line 10-11. Again, I don't believe that all nitrite is oxidized to nitrate in the water column. What about dilution of the signal with other water masses? Nitrite assimilation? Sedimentary denitrification? (not to mention other processes such as anammox, DNRA in the sediments).

Lines 15-25. This is pretty obvious and could be deleted.

Line 27. Lehmann et al. (2004) do not show that denitrification does not take place in the Elbe River, nor that O<sub>2</sub> concentrations were >6mg/L.

Lines 26-29. Sentence makes no sense and needs to be rephrased.

Lines 27-28. Sedimentary denitrification can be the reason for low apparent isotope effects in the water column.

Lines 29-30. 25% of what? If 25% of nitrate is reduced to N<sub>2</sub> (or N<sub>2</sub>O) it is not negligible!

Line 32. I don't agree. (see comments above).

Page 9:

Lines 21-27. Generally, it is very difficult to interpret the isotope signatures presented in this study without any idea about reaction rates. Could you, instead of assuming a Rayleigh model, develop a simple reaction model, which allows you to reproduce all of your measured data (nitrate, nitrite, ammonium, and SPM concentration and isotope data (at least after 14 June)), based on reaction rate constants as tunable parameters? This would give you very important information on what processes could have been active during the flood. I feel this paper could be improved significantly with such a model.

Page 10:

Line 8. What do you mean by “not expressed”. It is expressed but overprinted by other effects?

Figures:

Figure 1. Caption. Define “flood”. I thought you argued that assimilation was the one and only nitrate sink?

Figure 2b. Legend/symbols got mixed up (see comments above). How do you explain the sharp drop in ammonium concentration on 15 June 2013?

2c. What is the dashed line? Why are there gaps in the  $\delta^{15}\text{N}$ -SPM profile?

Figure 3. I don't think this figure is of much use. Or explain in the caption (and main text) what we are supposed to learn from it.

Figure 4. Where is  $\Delta\delta^{15}\text{N-NO}_2^-$  defined? Write in caption what  $[\text{NO}_2^-]_{\text{initial}}$  is.

Generally: check for typos and text formatting throughout the manuscript and specifically on pages 4-7.