

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; Sebiló 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 intensely 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 reviewer #1:

Reviewer #1: We would like to thank the anonymous reviewer for the detailed evaluation of our manuscript and for the suggestions for improvement. This reviewer had some general concerns regarding (1) the fact that we addressed nitrite oxidation as single nitrite sink, (2) the use of the Rayleigh closed-system equation to calculate the isotope effect of nitrite consumption and (3) suggests the use of a reaction-rate model to constrain relevant nitrite turnover pathways.

These issues are also brought up by Reviewer #2, and we addressed them jointly in a response addressing both reviewers (see above).

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).

As we outlined above, we agree that our focus on nitrite oxidation only might have been too narrow. While we do have (as yet unpublished) independent rate measurements that support the occurrence of nitrite oxidation, these cannot be extrapolated to the flood situation. Regarding the role of denitrification, we now consider it as a potential turnover pathway in a revised version of the manuscript. However, we would like to point out that the 25% estimate of denitrification in the Elbe was a maximum estimate by (Deutsch et al., 2009).

With regards to DNRA, we do not consider it separately in the manuscript for the following reasons: First, we now compute a joint fractionation factor of nitrite sinks (assuming that denitrification and nitrite oxidation are the most important ones). The insertion of yet another sink will not significantly change our conclusions. Second, surprisingly enough, there seem to be no assessments of the isotope effect of DNRA, making it difficult to include in our nitrite sink calculation. Third, most importantly, we are not aware of rate measurements of DNRA in the Elbe, but (Burgin and Hamilton, 2007) assume that its contribution to nitrate removal in rivers is relatively low. This hypothesis is supported by a recent doctoral thesis (In-stream nitrogen retention in a large nitrogen rich river: estimates from open-channel methods, S. Ritz) addressing nitrate removal in the Elbe.

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)?

The reviewer's suggestion is a good idea to improve the manuscript and have one additional isotope effect. We have calculated the isotope effect of nitrate using an open system approach (see above). The calculated isotope effect for nitrate decrease after discharge peak (7 samples) results in $^{15}\epsilon$ of $-4.0 \pm 0.1\text{‰}$ ($R^2 = 0.90$) and $^{18}\epsilon$ is $-5.3 \pm 0.1\text{‰}$, $R^2 = 0.93$. This is on the low end of isotope effects reported for nitrate assimilation (Granger et al., 2004; Needoba and Harrison, 2004; Waser et al., 1998),

but fractionation can be affected by residence times, such that the isotope effect is lower when residence times are low (Kendall, 1998):

Also, the ^{18}O to ^{15}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}O to ^{15}N enrichment ratio of 0.82 (page 6, line 32), which is not characteristic for nitrate assimilation.

This isotope effect reflects the ^{18}O to ^{15}N ratio of 0.82. While this ratio is below the 1:1 ratio from batch culture experiments ((Granger et al., 2004) found values between 0.9 and 1.1), it is typical of the Elbe River. (Deutsch et al., 2009) have calculated a comparable ^{18}O to ^{15}N enrichment ratio of 0.89, which is attributed to at least 75% nitrate assimilation. Our deviation could be a hint for nitrification in the water column and addition of depleted N. However, nitrite dynamics are rather complex indeed, and isotope changes are more subtle than for nitrite. We would like to point out that we cannot (and do not) exclude a certain role of denitrification – which will in the revised version also be discussed with reference to nitrite.

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”.

We agree that the title might not have been appropriate and will modify it to account for the fact that not only nitrite oxidation might have been active.

Page 1:

Line 8. Why do we care about a nitrate source from NH_4^+ ?

In our manuscript, the focus is set on nitrification as an important process producing nitrate from ammonium. Nitrification is one source of nitrate besides different N sources like urban waste water, surface runoff, and leachate from agriculture soils (Brion et al., 2004; Van Breemen et al., 2002), where nitrification of ammonium occurs. We care about nitrate because generally, ~ 97.0% of total DIN are present as nitrate, it is a monitoring parameter and a link to denitrification, an ultimate removal pathway of Nr that can disrupt the nitrogen cascade of assimilation-remineralization-assimilation.

From an environmental perspective the source of total fixed N is important to know.

We disagree. Budget-wise, Nr might be the most relevant parameter, but from an environmental perspective, it does make a difference whether N is present as nitrate, nitrite, or ammonium.

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

Has been rephrased to “N-cycling.” Potentially important processes are explained in detail in the introduction (page 2, line 4 and following) and like mentioned above like assimilation-remineralization-nitrification-assimilation....

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.

Yes, did mean the isotope effects of ammonium oxidation (to nitrite) and nitrite oxidation (to nitrate). Ammonia oxidation is associated with conventional fractionation, whereas nitrite oxidation fractionates inverse and preferentially oxidizes ^{15}N . In the oceans, nitrite is an intermediate in some key biological processes and can accumulate to high concentration in the primary nitrite maximum (Lomas and Lipschultz, 2006), and in the secondary nitrite maximum in OMZs (e.g. (Lam et al., 2011). Isotope effects (i.e. bulk isotope effects) are investigated in these cases to unravel N-cycling, and isotopes can be indicative of oxidative versus reductive processes. The occurrence of inverse fractionation can indeed complicate the interpretation of bulk isotope effects. We found that the accumulation of nitrite in the Elbe on this rare occasion provided a unique opportunity to study isotope changes in nature.

However, we rephrased this sentence to [...] makes it difficult to study the apparent isotope effect of nitrite removal in natural systems.

Line 16. Delete “the” before concert.

Done.

Line 16. Define SPM.

Has been clarified to “suspended particulate matter (SPM)”.

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

Here, we refer to remineralization and nitrification. We rephrased this in the manuscript. As mentioned above, this is addressed in the introduction.

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.

Was rephrased to “We found that in the water column, ammonium and nitrite built up during the flood event, whereas nitrate was leached from catchment area and appeared to be subject to assimilation”.

Nitrate is 97.0 – 99.4% of total reactive nitrogen during the flood. However, nitrification IS important; it is the process generating nitrate from ammonium, which is present in fertilizer, making it indeed an important process. In summer nitrification takes place in the water column as well, as rate measurements (not part of this dataset) prove. Actually, the fact that nitrate is so abundant in itself is proof of nitrification – any ammonium that is produced from remineralization is usually oxidized.

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?

We apologize for this mistake. Unfortunately, the symbols were mixed up, this has been corrected.

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

This is discussed in detail in the manuscript (amongst others page 7, line 6, 16). Ammonium is tightly bound to clay particles due to its positive charge, such that an external source is unlikely. Nitrite is usually immediately detoxified by oxidation. The succession of ammonium and nitrite is a strong indication for nitrite stemming from ammonium. However, in the abstract, we feel that a detailed explanation is not appropriate – this is done in the manuscript itself.

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

We refrained from using this Rayleigh model – see detailed response above.

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).

It has been changed to “Our data show a unique co-occurrence of ammonium, nitrite and nitrate in a river system during an extreme event in summer. Based on this, we calculated an apparent isotope effect of $10.0\pm 0.1\%$ during nitrite removal and evaluate different scenarios that may cause this isotope effect.”

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

Done, as suggested.

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?

No, this is not the case. Nitrate input decreased by 48% (54000 tN a-1 in 1986 to 28000 tN a-1 in 2008) and ammonium input decreased by 93% (12000 tN a-1 in 1986 to 1400 tN a-1 in 2008), resulting in an overall decrease of about 56% (Bergemann and Gaumert, 2008).

Page 2:

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

Has been clarified and changed to “Today, the riverine DIN load consists mainly of nitrate, which stems from urban waste water, surface runoff, and leachate from agriculture soils (Brion et al., 2004; Van Breemen et al., 2002). However, nitrate regeneration in rivers can also modify DIN loads (Middelburg and Nieuwenhuize, 2001)...”

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

See discussion above.

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

“Individual uptake processes” refers to assimilation, denitrification etc., i.e. all enzymatically catalyzed nitrogen transformation processes that are relevant in N-cycling.

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

Has been rephrased to “actively nitrifying environments”. In “healthy” environments, the co-occurring processes interact and toxic nitrite does not accumulate.

Page 3:

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

Has been clarified to “from a quay wall at the shore”.

Line 21. Define SPM.

Has been clarified to “suspended particulate matter (SPM)”.

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

Ammonia analysis has a detection limit of 0.5 $\mu\text{mol L}^{-1}$ and an analytic precision of 0.1 $\mu\text{mol L}^{-1}$. Nitrite analysis has a detection limit of 0.1 $\mu\text{mol L}^{-1}$ and an analytic precision of 0.1 $\mu\text{mol L}^{-1}$. Nitrate analysis has a detection limit of 1.0 $\mu\text{mol L}^{-1}$ and an analytic precision of 0.1 $\mu\text{mol L}^{-1}$.

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

Done, as suggested.

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 N₂O with the bacterial method as stated above, but with sodium azide instead?

The cited reference includes detailed descriptions of the method. It has been clarified to “For analysis of the ammonium isotopic composition, nitrite is first removed by addition of sulfamic acid (Granger and Sigman, 2009). Afterwards, ammonium was chemically converted to nitrite using basic hypobromite oxidation and then reduced to N₂O using sodium azide (Zhang et al., 2007).” The denitrifier method is prohibited by using these toxic chemicals.

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

Done, as suggested.

Line 18. What are the “additional standards”?

We now specified this in the manuscript (we used commercially available KNO₃, KNO₂, and NaNO₂ salts). $\delta^{15}\text{N}$ -values are determined using our EA-IRMS.

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.

As specified in the general comments, we agree with the concern of using the Rayleigh assumption. This conventional normal fractionation factor indicates a removal of light ¹⁴N from the nitrite pool resulting in an increasing pool signature or this could be additionally coupled with heavy ¹⁵N-nitrite from ammonia oxidation.

Page 5:

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

We apologize for this typo, corrected in the revised version (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?

The discrepancy was due to a rounding error, has been changed in the abstract to 4.4 $\mu\text{mol L}^{-1}$. The precision of the nitrite analysis is 0.1 $\mu\text{mol L}^{-1}$. We have clarified this following your suggestions for page 3 lines 22-24.

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

The succession has been changed for clarity.

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

The number was included merely for precision, this is no specified detection limit is 0.1 $\mu\text{mol L}^{-1}$. It has been clarified in the “Materials and Methods” chapter as your suggestions for page 3 lines 22 – 24. Sentence has been changed (see below).

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.

The sentence has been changed to “The further elevation of nitrite concentration coincides with a decrease in oxygen (<7.6 mg L^{-1}).”

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

We defined “flood conditions” on page 4 line 26 as discharge >3000 m^3s^{-1} . Sections like “before the flood” should make it easier for the reader to follow.

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

Has been clarified to “from 1.6 to 1.8 $\mu\text{mol L}^{-1}$ ”

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

We assume that the nitrite maximum and isotope minimum do not entirely match because of the influence of the isotope value of the substrate ammonium, which is 2‰ shortly before the lowest nitrite isotope value and increases afterwards.

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.)

We have changed the mode into an open-system approach and rephrased to “apparent isotope effect”.

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

We apologize for this mistake. Unfortunately, the symbols were mixed up. This has been corrected.

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.

SPM is the source for remineralization to ammonium and therefore ammonium isotope values change with SPM concentration. We will briefly explain this in the revised manuscript version.

Page 6:

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

Indeed, we refer to the sample from 11 of June, where ammonium concentration was firstly high enough for isotope analysis.

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

We apologize for the mistake. It has been changed to “With increasing discharge, nitrate concentrations decreased from 270 to 228 $\mu\text{mol L}^{-1}$...”

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

Has been clarified to “On 9 June, lowest nitrate concentration coincides with a SPM peak of 70 mg L^{-1} . After 10 June, nitrate concentrations increased with discharge.”

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

Has been changed to “Phenomena like this can be attributed...”

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?

This is a characteristic succession for a flood (Baborowski et al., 2004; Pepelnik et al., 2005). SPM is pushed in front of the crest of the flood, whereas nutrients concentrations increase later, because they are dissolved in the water, and also, depending on the substance investigated, because they may be drained from the catchment area.

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?

Isotope values are source specific and change with turnover processes due to fractionation. Fresh, unprocessed material has lower isotope values, because no less fractionation occurred. Furthermore, decreasing $\delta^{15}\text{N-SPM}$ values reflect another source, if suspended particulate material from the catchment enters the river. Terrestrial organic matter has a $\delta^{15}\text{N}$ -value of about 3.5‰, which is significantly lower than riverine SPM with $\delta^{15}\text{N}$ of about 9‰ (Middelburg and Nieuwenhuize, 1998) and references therein). Further, we have analyzed C/N ratios, which have high values of up to 10 with simultaneous SPM concentration peak of 70.5 mg L^{-1} on 9 June. This indicates a decrease of fresh organic material like phytoplankton and an increase of more refracted terrestrial organic matter. Terrestrial organic matter has a $\delta^{15}\text{N}$ -value

of about 3.5‰, which is significantly lower than riverine SPM with $\delta^{15}\text{N}$ of about 9‰ ((Middelburg and Nieuwenhuize, 1998) and references therein).

The increase and decrease of $\delta^{15}\text{N}$ -SPM can be explained with an increase of C/N ratio to 10 on 16 of June. Afterwards, the C/N ratio again decreases, coupled with a decreasing $\delta^{15}\text{N}$ -SPM. This indicates newly build phytoplankton with lower $\delta^{15}\text{N}$ -values, because they fractionate the nitrate source. Vice versa, nitrate $\delta^{15}\text{N}$ -values increase from about 8.1 to 8.8‰, which also show the increasing importance of nitrate assimilation by phytoplankton.

Line 21. “reduced biological activity” relative to what? To summer, when we observe average $\delta^{15}\text{N}$ -NO₃⁻ values of ... ‰?

Relative to summer conditions indeed. Has been clarified to “In the Elbe River, $\delta^{15}\text{N}$ -NO₃⁻ and $\delta^{18}\text{O}$ -NO₃⁻ values are up to 16.5 and 7.6‰ in June 2006, respectively, and in January 2006 $\delta^{15}\text{N}$ -NO₃⁻ and $\delta^{18}\text{O}$ -NO₃⁻ values are 9.3 and 0.2‰, respectively (Johannsen et al., 2008). Another reference sited mean summer $\delta^{15}\text{N}$ -NO₃⁻ and $\delta^{18}\text{O}$ -NO₃⁻ values of 18.0 ± 2.7 and 7.6 ± 2.7 ‰, respectively, and winter $\delta^{15}\text{N}$ -NO₃⁻ and $\delta^{18}\text{O}$ -NO₃⁻ values are 9.3 ± 0.7 and 0.8 ± 0.6 ‰, respectively (June 2005–December 2007, (Schlarbaum et al., 2011). During the flood in June, we see $\delta^{15}\text{N}$ -NO₃⁻ ranges between 7.4 – 9‰ and $\delta^{18}\text{O}$ -NO₃⁻ between 2.1 – 3.9‰, which is close to winter values (Fig. 2c).”

Line 22. What “then”? in summer?

Has been changed to “In summer and under normal flow conditions, nitrate concentration decreases due to assimilation and biomass production (Fig. 1).”

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

Johannsen et al. (2008) showed that assimilation is the main reason for the decrease, as did Deutsch et al. (2009). References will be included.

Line 29. Rising oxygen concentrations to above saturation? Otherwise it would be more interesting to know what caused the [O₂] decrease before the system is ventilated again.

Oxygen concentration increases from 6.0 mg L⁻¹ (corresponding to 63% saturation) to an intermediate maximum of 7.6 mg L⁻¹. The dissolved oxygen concentration is clearly correlated to discharge, because initial oxygen concentration is about 10 mg L⁻¹, corresponding to an oxygen saturation of about 100% and more, depending on the time of day of sampling. Furthermore, increasing discharge increase turbidity and decrease light irradiation, which weaken phytoplankton activity and their oxygen production. After the 7.6 mg L⁻¹ peak, [O₂] decreases, accompanied by a strong increase in water temperature (Fig. 2a).

Line 30. Can you show here this correlation?

As illustrated in figure 1 of the response letter, [NO₃⁻] vs. $\delta^{15}\text{N}$ -NO₃⁻ correlate on a slope of -0.014 with R² of 0.88 and [NO₃⁻] vs. $\delta^{18}\text{O}$ -NO₃⁻ with a slope of -0.019 with R² of 0.92. This figure will not be added to the manuscript.

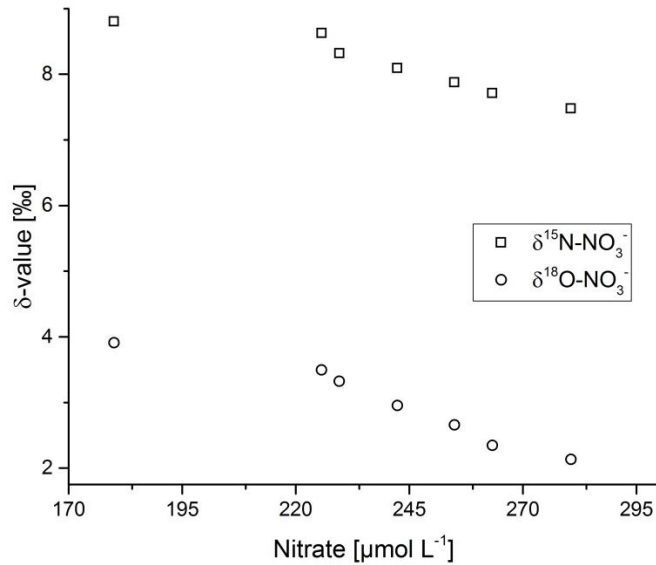


Fig. 1 Nitrate concentration versus $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ of water samples from 13 to 20 June 2013.

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

Figure 2 of the response letter illustrates the last 9 sample values and will be added to the manuscript as your suggestion to a “figure 3” for page 6, line 32.

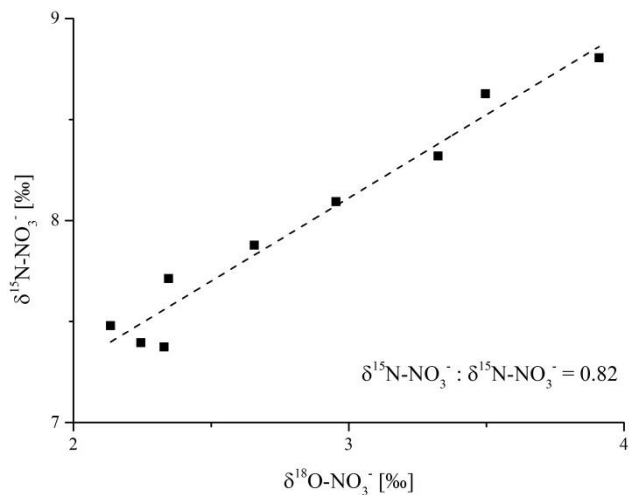


Fig. 2 $\delta^{15}\text{N-NO}_3^-$ versus $\delta^{18}\text{O-NO}_3^-$ of water samples from 13 to 27 June 2013.

Line 33. No! Exactly the opposite is the case. 0.82 is significantly different from 1.00 ± 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}O to ^{15}N enrichment ratio?

We agree 0.82 ± 0.06 is different from the 1:1 enrichment suggested by (Granger et al., 2004) for nitrate assimilation. We see a larger enrichment in the oxygen isotopes than in the nitrogen isotopes. This may be a sign of addition of depleted N from nitrification, or some riparian denitrification.

However, we disagree that this rules out that assimilation is the dominant uptake pathway for nitrate (see also response to this issue above).

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}O to ^{15}N enrichment ratio seems to be indicative of other processes.

The isotope values would not change, if the water parcel with higher nitrate concentrations discharges and water with the same source but lower concentrations follows. Therefore, the isotope signature of the nitrate from the catchment or in the river has to change due to fractionation. The possible process, where dual isotope values increase is assimilation on a slope on 1, and nitrification would lower this ratio towards 0.82 as mentioned above.

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

Has been clarified to “hydrographic properties associate with increasing discharge”

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

In our opinion, nitrite and ammonium changes cannot be explained with discharge and assimilation (only), and especially for ammonium, there is no clear link to discharge. In the revised manuscript, different reasons for varying concentrations are given. Ammonium and nitrite do not derive from the catchment, where nitrate, in contrast, always is present, but appear to be built during the flood event (cf. page 6, line 17). We suggested that ammonium stems from mineralization and nitrite stems from nitrification. We will now also address an additional nitrite source; nitrite may be linked to the breakdown of phytoplankton activity, analogous to the primary nitrite maximum in oceans. However, the isotope difference to ammonium is in the range expected for the isotope effect of ammonium oxidation, this suggests that ammonium is a relevant nitrite source. In either case, it stems from a disruption of normal biogeochemical cycles due to the flood. We will clarify this in the manuscript. The authors suggest little nitrite assimilation of phytoplankton, because nitrate is available and preferentially consumed.

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 N_2O , N_2 , or ammonium. A decrease in ammonium and nitrite concentrations is hence not a proof for nitrification.

The main conclusion is that nitrification is responsible. We widened the focus in the revised version to include other sinks like denitrification. However, anammox is likely

not important in an organic-rich shallow environment like the Elbe, and we addressed above that DNRA also appears to be of little importance (Burgin and Hamilton 2007, S. Ritz pers. comm.)

Line 6. Start here with a short discussion of where the NH_4^+ can come from, instead of just claiming that all of it is originating from SPM remineralization.

Page 7 line 16 – 18 is moved up, like suggested.

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

“Initially” means the first sample on 11 June, where ammonium concentration is high enough for isotope analysis.

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

It will be clarified in the revised version. $\delta^{15}\text{N}$ -SPM is 6.6‰ and simultaneously $\delta^{15}\text{N}$ - NH_4^+ is 2.0‰. We assume that the difference of 4.6‰ is due to fractionation during remineralization.

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

Done, as suggested.

Lines 16-18. Move this discussion up. (see comment above, line 6). Also, SPM is leaching, so NH_4^+ could be attached to it and thus leaching as well?

As mentioned in (Mancino, 1983; Mancino and Troll, 1990) ammonium loss by leachate is probably negligible, because in the catchment it is either consumed or nitrified. We will stress this in a revised version of the manuscript.

Line 22. Why are O_2 concentrations “low”?

This has been changed to clarify the correlation between $[\text{O}_2]$ vs. ammonium and nitrite concentrations. Further above, the sentence “The reduced phytoplankton activity is also indicated by decreasing oxygen concentrations.” is added to clarify the origin of decreasing oxygen concentrations.

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

We cannot rule out assimilation of ammonium by phytoplankton, but we have explained why we regard this as unlikely. Also, the initial difference in isotope values of ammonium and nitrite is in the range expected for ammonia oxidation. The succession of peaks also suggests a significant role of nitrification.

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.

The decreasing oxygen concentration is a hint for reduced phytoplankton activity and we merely wanted to highlight that nitrifiers are active during flood conditions.

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

Because ammonium accumulates, and the remineralization rate thus is higher than the oxidation rate. Rates and their differences will be discussed in a revised version when we assess nitrite isotope changes and calculate possible sinks (the approach we refer to is in the detailed response above addressing general issues).

Line 27. Define “low oxygen concentration”.

This has been clarified with an absolute value of “below 7.5 mg L⁻¹”.

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

Has been clarified to “From 14 June on...”

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?

The amount of nitrate from remineralization and nitrification is of course small in comparison to the nitrate pool, which is above 97.0%...Therefore, nitrate concentrations are controlled by assimilation and hydrology. It is, as we say above, true that we cannot entirely rule out ammonium assimilation. We will focus the manuscript to stress that normal conditions are disrupted – this is obvious – and that we assume nitrification is a sink for ammonium. It may not be the only one, but certainly is active – as we evaluate in the new isotope calculation mentioned at the beginning.

Line 30. What causes the lowered [O₂]?

Further above, the sentence “The reduced phytoplankton activity is also indicated by decreasing oxygen concentrations.” is added to clarify the origin of decreasing oxygen concentrations.

Line 31. Within 7 days of or after what?

Has been clarified to “From 9 June to 15 June”.

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.

We agree, and this is rephrased in the manuscript.

Line 33. Where is that 0.5 μmol L⁻¹ day⁻¹ coming from? I see a sharp decrease in [NH₄⁺] of about 3 μmol L⁻¹ day⁻¹ on 15 June 2013.

Right, this is a mistake, which has been changed to 3.8 μmol L⁻¹ d⁻¹: 14 June at about 5pm 3.4 μmol L⁻¹ → 15 June at about 2pm 0.0 μmol L⁻¹.

Line 35. Where is that drop in nitrite concentrations after the flood? In Fig. 2b [NO₂⁻] remains at >3 μmol L⁻¹.

We here show the flood situation only. The nitrite decrease after the flood is not shown, which we clarify in the manuscript (by adding “not shown in plots”).

Page 8:

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

Has been clarified to “isotope effect during nitrite removal”

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

In this paragraph, we explain why we have chosen a “Rayleigh” model, which is associated with no input of new nitrite. This assumption is indeed violated, and the manuscript has been changed to an open-system approach - therefore this paragraph has been rewritten.

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

Of course, rivers are not a “closed system”, but that was assumed to calculate the fractionation factor and compare this with laboratory results (which use closed systems). The isotope effect was also calculated using an open system approach. Both calculations have conventional normal fractionation factors. We deleted all closed-system assumptions from the manuscript.

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

We refer to the model name “steady-state” (Sigman et al., 2009). In this assumption, new substrate is continuously supplied and partially consumed. This is in contrast to the closed-system “Rayleigh” assumption. Rates are in steady state at each of the linear sections (increase and decrease) of the nitrite concentration plot.

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).

See general discussion at the beginning.

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

We have shortened this paragraph, but still want to discuss “objections”.

Line 27. Lehmann et al. (2004) do not show that denitrification does not take place in the Elbe River, nor that O₂ concentrations were >6mg/L.

(Lehmann et al., 2004) has of course not investigated the Elbe River, but the Santa Monica Bay. This has been rephrased; the reference was in the wrong place. We apologize for this mistake.

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

This has been rephrased.

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

Indeed. This is what we refer to – not sure what the reviewer’s point is here. Sentence has been rephrased for clarity anyway.

Lines 29-30. 25% of what? If 25% of nitrate is reduced to N₂ (or N₂O) it is not negligible!

As stated above, sedimentary denitrification is not associated with a pronounced isotope effect and thus has no or little influence on isotopes and can be neglected with regards to its isotope effect.

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

As stated, we have discussed this more carefully.

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.

We have investigated incubation experiments to determine ammonium oxidation and nitrite oxidation rates in 2012 and can estimate rates of up to 14 $\mu\text{mol L}^{-1} \text{d}^{-1}$. However, this does not apply accordingly during flood conditions. Furthermore, fractionation factors differ in wide ranges and a model using so many assumptions would misrepresent the processes and their isotope effects. However, we have investigated a back-of-the-envelope calculation (page 9, line 21), which we have extended in the revised version. Therein, we have tested our hypothesis assuming comparable ammonium and nitrite oxidation rates, the isotope value of ammonium increase with an isotope effect for ammonium oxidation of -35‰, which “dilutes” the nitrite pool with isotopically heavy ammonium, an inverse isotope effect of +10‰ during nitrite oxidation. This results in a conventionally negative isotope effect of about -8‰, which is comparable with the calculated isotope effect during the flood. This will be discussed in more detail in the revision.

Page 10:

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

Yes, it is. We have rephrased the sentence.

Figures:

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

Flood conditions were defined as “discharge $>3000 \text{ m}^3 \text{ s}^{-1}$ ” (page 4, line 26).

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

We apologize for the confusion, the symbols has been changed. 3.4 $\mu\text{mol L}^{-1}$ ammonium can be oxidized by ammonia oxidizing bacteria.

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

The lines in figure 2c have been changed into solid once to clarify the progress of values. $\delta^{15}\text{N}$ -SPM could (due to low N content) not be analyzed in all samples.

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.

The x-axis has been changed in "oxygen saturation". The figure illustrates that oxygen concentration decreases, which indicates reduced phytoplankton activity, and ammonium and nitrite, which are products and substrate of remineralization and nitrification, increase. We now refer to it in more detail in the manuscript.

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

We have added a definition for $\Delta\delta^{15}\text{N-NO}_2^-$ and $[\text{NO}_2^-]_{\text{initial}}$.

$\Delta\delta^{15}\text{N-NO}_2^- = \delta^{15}\text{N-NO}_2^- - \delta^{15}\text{N-NO}_2^-_{\text{initial}}$, which is the first nitrite isotope value of the calculation.

$[\text{NO}_2^-]_{\text{initial}}$ is the first nitrite concentration value of the calculation.

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

We have checked and changed typos and text formatting throughout the manuscript one more time.

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