

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 reviewer #2:

The authors gratefully thank the reviewer for the constructive feedback and in-depth evaluation of our manuscript. We appreciate the detailed comments, which are very valuable, and will incorporate the various suggestions.

We wrote a general chapter at the beginning of the response letter, because the reviewers' comments (nitrite oxidation only, other potential nitrite sinks like nitrite assimilation, denitrification and dilution, box model, Rayleigh fractionation...) were partly similar.

Primary concern is that rivers are inherently dynamic... Impractical for the authors to isolate a single biogeochemical process within this physically complex and hydrologic system...

The authors agree with this concern and have refined the interpretation of nutrient concentrations and nitrite removal as mentioned in our general comments at the beginning of the response letter. The main change is that we, as this reviewer suggested, expanded our "back-of-the-envelope calculation" to assess isotope dynamics, now including other potential sinks and isotope effects. We will discuss this in detail in the manuscript.

In fact, the authors acknowledge that the increase in the nitrate and SPM concentrations on the rising limb and crest of the flood reflect changes in sources of watershed inputs (e.g., P6 L10-14).

Yes, but we assume this is mostly important for nitrate, which is known to be leached from the catchment in high amounts (Bergemann and Gaumert, 2008). The very smooth succession of nitrite concentration values, in comparison to a pretty dynamic nitrate plot, suggests that it is supplied, and later removed, at a constant rate, or at least at a constant ratio of rates. This is a strong indication for a biological source.

However, since many (most?) of the features being observed by the authors' measurements may indeed be related to changes in the hydrologic and geochemical inputs to this volume – it becomes virtually impossible to assign the biogeochemical changes observed to processes occurring within the box.

We agree. Unfortunately, this makes a box-model approach impossible, but we have extended our back-of-the-envelope calculation as explained in the general comments.

Can any other conservative tracers of flow ($\delta^{18}\text{O}$ water, bromide, chloride, major ions, etc.) be measured to help constrain water (and N) sources during the flood hydrograph?

Na^+ and Cl^- concentrations were indeed analyzed at the University of Hamburg. At the onset and after flood conditions, they measured concentrations of 2.18 and 2.23 mmol L^{-1} , respectively. These values are slightly higher than maximal 2 mmol L^{-1} expected for fresh water (Appelo and Postma, 2005). This is indicative for manure/fertilizer and road salt, which leaches from the catchment. In the course of the flood, the concentrations decrease and loads increase, which suggests water input from the catchment area. These parameters are not in the manuscript, because due to a lack of endmember and a detailed discussion of different sources, analogous to (Mengis et al., 1999) is not possible.

I wonder whether a simple box model could be constructed...

See general discussion above.

Another thought is that the overall discussion might benefit from a re-focusing...

We agree and as mentioned in our general comments, we have changed the discussion from nitrification only towards nitrite removal, which can be a combination of nitrification, nitrite assimilation and denitrification.

...they could make reasonably well-constrained estimates of the rates of multiple processes...

As written in the general comments, we have unpublished nitrification rates for this station from an annual cycle in 2012, which vary between 1 to 14 $\mu\text{mol L}^{-1}\text{d}^{-1}$, but due to the very different hydrological situation we carefully extrapolate them to the flood conditions. 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.

A Rayleigh model cannot be justified here.

We agree, it has been changed to an open system approach, see general discussion above.

Can it be demonstrated that the nitrite concentrations are not the product of low levels of NO_3^- reduction occurring in sediments/hyporheic exchange/groundwater? In fact, the nitrite concentrations vary in a smooth fashion (in contrast with the NH_4^+ concentrations, for example) – which to me might suggest more of a hydrologic control on their dynamics.

As suggested on page 8 line 26, an influence of sedimentary denitrification to nitrite concentrations is unlikely and groundwater residence time is low (~30 years, (Behrendt et al., 2002) and thus direct influence of groundwater inflow can be expected to be negligible (Montenegro et al., 2000). A hyporheic exchange or input from the catchment area is also unlikely in steady-state systems, because the toxic nitrite is usually immediately detoxified. However, due to the intense atmospheric deposition in the catchment area, we truly cannot fully exclude the possibility of nitrite input from hyporheic flow. We included this sink in our calculation of processes and isotope effects (to be included in the revised version, see general comments).

The smooth variation of nitrite concentrations is more likely indicative for biologic controlled processes than for hydrological processes.

After much confusion - I think that the Figure 2B legend is wrong.

Indeed! We apologize for the mistake and the arising confusion, symbols have been changed.

The decrease in nitrate concentrations on the falling limb of the flood are explained by phytoplankton assimilation – why could this not also possibly explain the concomitant loss of nitrite and the positive isotope excursion?

To our knowledge, nitrite assimilation of phytoplankton is usually not measured, even if the possibility is commonly accepted (Collos, 1998). The assimilation of

nitrite is energetically expensive, because phytoplankton has to reduce four electrons for every molecule of nitrite. Usually, phytoplankton reduces nitrate to nitrite within the cell in the cytoplasm and the chloroplast, respectively. If phytoplankton would reduce nitrite, active transport of nitrite through the chloroplast membrane would need additional energy (Lomas and Lipschultz, 2006). Phytoplankton would need enough energy from light, which is limited during flood conditions. Assimilation of ammonium cannot fully be excluded, but is assumed to be of minor importance.

Specific Comments

P1 L9: 'bulk isotope effect of nitrification' is not clear and should be defined.

This has been deleted in course of the reworking. "bulk isotope effect" has meant the isotope effect of both nitrification steps (ammonia and nitrite oxidation) together.

P1 L11: 'divergent' is unclear

Has been clarified to "negative isotope effect of ammonia and the positive isotope effect of nitrite oxidation"

P1 L16: In concert with...

Done, as suggested.

P1 L19: from the catchment area

Done, as suggested.

P1 L22: I'm not convinced that you can conclude the changes in isotopes are the result of nitrite oxidation only. You should state that this is the 'apparent' isotope effect of nitrite consumption (although this may also not be valid as calculated – e.g., violation of Rayleigh model assumptions).

Done, as suggested, see general comments.

P1 L30: has increased 20-fold

Done, as suggested.

P2 L7: Or, the nitrate can be simply exported from the watershed.

The nitrate concentrations decrease after the peak of $280 \mu\text{mol L}^{-1}$ on 13 June. Taking only the decreasing concentration into account, nitrate could be exported, but isotope values change and indicate biological processes. In general, background concentration in winter is about $300 \mu\text{mol L}^{-1}$ (page 2, line 29). Furthermore, $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ of unprocessed nitrate are about 7.8 – 9.3 and 0.8 ‰, respectively (page 7, line 15; (Johannsen et al., 2008; Schlarbaum et al., 2011). During the flood and after peak discharge when biological activity re-started, especially, $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ increase (page 6, line 18 – 19, figure 2c), which would not be the case, if water parcels with nitrate just discharge.

P2: I think the imperative for understanding nitrification in riverine systems should be better justified – perhaps in terms of its frequent coupling to N loss processes (anammox and denitrification) and ecosystem services.

We agree and will stress this in a revised version. It is coupled to N-loss indeed, and is an oxygen sink as well.

P2 L13: Not just nitrogen uptake – but any enzymatically catalyzed nitrogen transformation process.

The reviewer is right and the sentence has been changed to “During enzymatically catalyzed nitrogen transformation processes...”

P2 L14: This is somewhat of a colloquial expression – and should be restated to reflect that enzymatically catalyzed processes occurring slightly faster for lighter isotopes than heavy isotopes.

It has been clarified as “lighter isotopes are catalyzed faster than heavier isotopes and this changes the isotope composition of the source and the reaction product (Kendall, 1998; Mariotti et al., 1981).”

P2 L15: The Rayleigh model explicitly requires the assumption of a unidirectional process and no replenishment of the reactant pool. It’s not clear that this can be assumed.

The authors agree with the reviewers’ criticism. As the definition of a closed Rayleigh distillation model, it is not applicable in the case of the flood. Therefore we have changed this assumption in the whole manuscript towards an open steady-state model (Sigman et al., 2009).

P2 L18: obstacle to what?

The first step of nitrification (oxidation of ammonia to nitrite) is associated with wide ranging fractionation factors, which hampers the use of model assumptions, in combination with the inverse fractionation of nitrite (second step of nitrification), especially. This is the reason, why we have not investigated a box-model – one can generate a variety of results, not knowing, which is confidential.

P2 L21: and the remaining nitrite

Has been changed.

P2 L26: what is meant here by the term ‘healthy?’

The authors mean the Elbe River under actively nitrifying conditions, when toxic nitrite is immediately removed by organisms and do not accumulates. The term “healthy” might have been not quite correct for the Elbe anyway, and is not relevant for our manuscript anyway. We changed this to “actively nitrifying”.

P2 L37: Phytoplankton are light dependent

Done, as suggested.

P3 L8: the second largest river discharging

Done, as suggested.

P3 L25: Isotope analyses

Done, as suggested.

P5 L4: Previous studies have found

Done, as suggested.

P5 L8: Either present data as singular or plural – not both. : : : nitrate concentrations were: : : Nitrite concentrations were <1.2: : : and ammonium concentrations were below the detection limit

Corrected throughout the manuscript, as suggested.

P5 L28: Not sure I would say that the nitrite concentrations rose ‘quickly’ – they seem to evolve more gradually in fact.

True – we changed this.

P5 L30: For reasons discussed above, I think it should be stressed here that this is an ‘apparent’ fractionation factor.

Has been changed.

P6 L2: Remove “it is interesting, however” – opinions don’t generally belong in a results section.

Done, as suggested.

P6 L 20: Although as noted later – the relatively large nitrate pool is far more resistant to isotopic perturbations by biogeochemical processes.

The authors agree and have clarified this.

P6 L25: While this may be true – the watershed flooding potentially may have also introduced a nitrate source having a slightly different isotopic composition.

Has been changed to clarify different flow regimes: “In our study, before the SPM peak, $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ are not correlated with $[\text{NO}_3^-]$, probably because of water with slightly different isotope signatures from the watershed flooding. Only after the SPM peak $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ are negatively correlated with $[\text{NO}_3^-]$ (R^2 of 0.897 and 0.816, respectively), which pinpoints...”.

The main source of nitrate under normal flow is agriculture in the watershed (cf. Johannsen et al. 2008), so a new, relevant source appears unlikely.

P6 L29: As the phytoplankton are recovering – couldn’t they be assimilating nitrite?

As explained in our general comments, phytoplankton theoretically can assimilate nitrite. To our knowledge, this has not been observed in situ and would be energetically much more inefficient than assimilating nitrate, especially, when the surface irradiance has decreased due to high turbidity and energy is limited. Even if it occurred, its isotope effect would be negligible (Wada and Hattori 1978).

P6 L32: This $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}$ slope is actually much lower than that observed by Granger and colleagues. Could this be indicative of nitrification?

Yes, this can indeed be a hint for nitrification and has been clarified in the manuscript.

P7 L3: Why can't phytoplankton be playing role in assimilation of nitrite and/or ammonium?

We address this in the general comments and our answer above (to P6 L29). We have added a statement in our manuscript.

P7 L23: But phytoplankton activity was specifically invoked as explaining an increase in N and O isotopes of nitrate and contributing to a drawdown of $\sim 100\mu\text{M}$ nitrate. Thus, it seems hard to discount phytoplankton activity for drawdown of $\sim 1\mu\text{M}$ nitrite and $\sim 3\mu\text{M}$ NH_4^+ .

We agree that the focus on nitrite oxidation might have been too narrow. We will discuss the role of nitrite assimilation in the revised manuscript, but still assume it to be less important than other sinks like oxidation or denitrification, for the reasons outlined above, in response to reviewers 1 and 2.

P8 L10: : : : suggesting conventional normal (as opposed to inverse) fractionation during: : :

Has been changed as suggested.

P8 L29-30: The contribution of 25% sedimentary denitrification actually seems substantial – and therefore hard to rule out. Also – can the same conclusions of Deutsch et al., 2009 be drawn for the extreme flood conditions of this study? Couldn't flooding act to increase hyporheic exchange?

Sedimentary denitrification has little to no impact on isotope values (Mariotti et al., 1988; Mariotti et al., 1982). Regarding hyporheic exchange, a model study showed that it can indeed increase during the flood, but this would also lead to a reduced residence time (Boano et al., 2007), which in turn may decrease denitrification rates. However, this interaction is quite speculative, and we will thus not include it in the manuscript.

P8 L35: As conceptualized by the authors, since the $\delta^{15}\text{N}$ of the NH_4^+ is $\sim +2\text{permil}$ to begin with ($\sim 4\text{permil}$ lower than the SPM $\delta^{15}\text{N}$) – the $\delta^{15}\text{N}$ of the NO_2^- produced (in a closed system) would follow the accumulated product equation – and under conditions where NH_4^+ was being completely oxidized to NO_2^- – the newly produced NO_2^- would have a $\delta^{15}\text{N}$ of $+2\text{permil}$. In developing the argument about the contribution of heavy nitrite from ammonia oxidation, the authors should be careful to explain how this new nitrite composition will evolve in step with the degree of NH_4^+ consumption. Initially the new nitrite will have a $\delta^{15}\text{N}$ even lower than the existing nitrite, while as NH_4^+ is consumed – the $\delta^{15}\text{N}$ of the newly produced nitrite will approach the original $\delta^{15}\text{N}$ of the NH_4^+ ($\sim +2\text{‰}$). This value of $+2\text{‰}$ is actually not the 'isotopically enriched' nitrite that seems to be invoked here by the authors. Later on P9 L18, the authors explain how the complete consumption of NH_4^+ would quantitatively transfer the $\delta^{15}\text{N}$ value of the NH_4^+ pool into the nitrite pool – yet it is not clear whether the authors are using the evolving NH_4^+ pool as a closed system – or simply invoking the instantaneous product equation at each step. Notably – these values and mass balance estimates will play importantly into their 'back-of-the-envelope' calculations.

The reviewer here suggests to carefully check the effects of partial or complete consumption of substrates and their effect on the product isotope pool. We agree that this needed to be developed further in the manuscript. Accordingly, we expanded our 'back-of-the-envelope' calculation (and explanations thereof) to account for this. We aimed to develop scenarios that may explain the nitrite isotope and concentration trends we see and will discuss this in detail in a revised version, see also general comments above.

P9 L21: I think the discussion could be clarified if these calculations were explained in more detail.

As we outline in our detailed response at the beginning, we expanded this calculation and the interpretation of the results.

P9 L36: I don't think there is any sort of cryptic ammonium cycle occurring here. More likely, it seems that the authors are just witnessing more 'conventional' N cycling processes (e.g., remineralization, nitrification, assimilation, etc.) from the perspective of nitrite isotopes for the first time in a river.

Even if ammonium is not abundant, we assumed a very rapid remineralization of ammonium and its immediate consumption (nitrification – assimilation). We rephrased this, basically not referring to a cryptic cycle. However, we assume that the N-cycle is in so far not conventional, as normal links between processes are disrupted, resulting in accumulation of ammonium and nitrite. Of course, the reviewer is right that these processes occur (likely at different rates) in a river also under normal conditions. We now highlight that the flood disrupted normal N-turnover.

Figure 2b: Caption is wrong?

It indeed was - we apologize for this mistake, it has been corrected.

Figure 4b: this should be labeled as 'apparent isotope effect for nitrite consumption' (not nitrite oxidation). As articulated by the authors in the discussion, I don't think you can tie these isotope changes to a single process.

The authors agree with the reviewer and have changed the to an open system steady-state assumption with a fractionation factor $^{15}\epsilon$ of $-10.0\pm 0.1\%$. We also now do not exclusively refer to nitrite oxidation.

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