

## ***Interactive comment on “Biogeochemical evidence of anaerobic methane oxidation and anaerobic ammonium oxidation in a stratified lake using stable isotopes” by Florian Einsiedl et al.***

### **Anonymous Referee #1**

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Review of “Biogeochemical evidence of anaerobic methane oxidation and anaerobic ammonium oxidation in a stratified lake using stable isotopes” by Einsiedl et al.

#### Summary

The authors present a comprehensive dataset highlighting key redox transformations of carbon and nitrogen in a stratified lake in southern Germany. The study takes advantage of patterns of stable N, O and C isotope fractionation together with changes in concentrations to deduce activity of microbial catalyzed transformation pathways. Among the conclusions drawn from these data – the authors suggest the strong likelihood for biogeochemical linkages between and among denitrification, anaerobic methane oxi-

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dation and anaerobic ammonium oxidation (anammox). Such linkages have not been widely demonstrated or observed in field settings, making this study relevant for understanding the prevalence of such cryptic connections across a range of other environmental settings.

#### Major comments

Overall, I found the study to be well executed and the data to be of high quality. The inclusion of the microbial community analyses offers a great complementary perspective on the purported geochemical connections drawn from the isotopic and concentration analyses. The overlapping zonation of several key redox-active species in this vertical profile of a lacustrine system provides compelling evidence for their putative involvement with one another. However, compelling evidence is not always enough to draw definitive conclusions about the proposed interactions. In this respect, the authors seem well aware of the limitations of their data, and temper their conclusions with an appropriate amount of caution. In some cases, however, I still feel that there can be slight over-reach (e.g., L339), in concluding that direct coupling of denitrification and anaerobic methane oxidation was demonstrated, for example. In most cases, demonstrating the environmental coupling of reactants requires careful ex-situ experiments, with manipulations and controls. The authors are leading experts in the field of microbial and (isotope) biogeochemistry – so they understand these aspects very well. I would just recommend that they pay special attention throughout the manuscript to state (which they mostly do already) that their data provide compelling evidence for – or suggest the existence of these linkages (without needing to claim that their data prove it).

In general, there are some sentences that suffer from awkward wording. I made suggestions to help smooth these sections as outlined below. I recommend publication after the authors address my (mostly minor) comments as outlined below:

1. The quality of the figures seems sub-par and some effort should be given to improve

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on details such as text and symbol sizes and colors, axes labels and ticks, etc.

2. In general, I find the choice of units (e.g., mg/L) problematic (not knowing whether mg of N or mg of NO<sub>3</sub><sup>-</sup> are being presented, for example). I would strongly suggest expressing concentrations in the more widely understood values of molarity (e.g., micromolar). This also has the advantage of facilitating more direct comparisons (at molar stoichiometries) among N, C, O and S cycling, etc. – thereby helping readers better understand the arguments being presented.

3. L 137: What is the reasoning behind the two diffusion coefficients for methane? This is presented in an apparent attempt to bracket a range of acceptable flux estimates, but is not explained in the text.

4. L131: Something about Equation 1 seems incorrect. Are there meant to be two equalities here? Do the 'x' terms both refer to depth? In general I think 'z' is more frequently used for referencing to depths. Please confirm that the expression of this diffusion equation is properly written.

5. L310: I understand that in the presence of NO<sub>3</sub><sup>-</sup>, sulfate reduction is not thermodynamically predicted to proceed, however some arguments have been made for processes occurring in micro-zones inside of particles, for example. How much anticipated change in sulfate would be predicted – and would the IC measurements actually be sensitive enough to this? The increasing presence/abundance of Deltaproteobacteria also lend some credence to the idea that at least some level of SO<sub>4</sub><sup>2-</sup> reduction could be occurring. Changing units into molarity would help readers with this comparison as I noted above.

6. The  $\delta^{18}\text{O}$  values of nitrite are reported, but nowhere in the text is it explained how these values were determined or calibrated. Further, given the low pH of lake water, the  $\delta^{18}\text{O}$  values of nitrite are very likely to be in isotopic equilibrium with the water, yet appear to fall around -4 to -6‰ which would be much too low. Given a lake water  $\delta^{18}\text{O}$  value of  $\sim -10\text{‰}$  – the  $\delta^{18}\text{O}$  value for nitrite in equilibrium should fall closer to

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+4‰ (see Casciotti et al., 2007). Finally, the  $\delta^{18}\text{O}$  values of nitrite in this study are not mentioned or involved in aspect of the conclusions – and should probably be omitted for clarity (e.g., they aren't used to bring any new insight into the system as presented).

Minor comments

L15: "Nitrate dependent anaerobic methane oxidation and anaerobic oxidation of ammonium (anammox) have the potential. . ."

L20: anammox does not require italics.

L24: Perhaps consider using "is the most parsimonious explanation"

L32: . . . I recommend: ". . . contain significantly different microbial communities that include bacteria known to be involved in. . ."

L47: . . .coupled to nitrate. . .

L54: . . . ANME-2d lineage promotes/conducts the reduction of nitrate. . .

L57: Besides

L59: . . .related to Crenothrix also have the. . .

L60: Crenothrix is misspelled.

L61: . . . may act as a driver. . .

L74: references do not need to be italicized

L86: . . .sequencing of 16S rRNA genes that provides evidence for the. . .

L115: . . . sterile filter, which was then kept frozen. . .

L149: . . . which represents the lower bound. . .

L153 – 157 – this should be moved to introduction or discussion.

L168: . . .(2007), respectively.

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L168: Nitrite was converted to N<sub>2</sub>O using acetic acid buffer sodium azide.

L169: ... mixture of both nitrate and nitrite was reduced to N<sub>2</sub>O via azide.

L171: Can you provide some estimate of error propagation for this inverse mixing calculation?

L173: ... by buffered azide solution for subsequent analysis.

L181: How were the  $\delta^{15}\text{N}$  values of the nitrite standards determined – and to what level of precision? There is no mention of calibrated  $\delta^{18}\text{O}$  standards for nitrite. Yet  $\delta^{18}\text{O}$  of nitrite data are reported (albeit not discussed). Please clarify or omit.

L192: Here it is unclear if the methane isotope analyses were conducted on the same bottles? Via manual injection? Was this a full bottle purge and trap approach? Was this automated? Were there standards included in this approach? How were the analyses standardized (e.g., extractions of methane of known composition from water)?

L236: Aerobic conditions...

L237: The average concentration of nitrate...

L239: ...nitrate concentrations decreased...

L261: ... were too low for stable isotope analyses.

L298-300 – Rephrase. Awkward wording.

L301: ... water depth below 16m, suggesting that only a very small fraction of methane can be oxidized with such trace amounts of oxygen.

L314: A stable isotope technique was used...

L327: Awkward sentence... perhaps reconsider phrasing “We also present several lines of qualitative and quantitative evidence for the co-occurrence of anammox together with denitrification coupled to AOM towards the bottom of the NMTZ.”

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L339: Here the language reads as though AOM coupled to denitrification has been unequivocally demonstrated, which isn't exactly the case. I think here it is best to qualify this a bit more. In fact denitrification could also easily be coupled to more canonical heterotrophy at the same time.

L344: Again – I would recommend softening and rephrasing: “This is consistent with the additional isotopic difference in  $\delta^{15}\text{N}$  values between nitrate and nitrite of around +15‰ arising as the result of production of highly <sup>15</sup>N enriched nitrate deriving from anammox.”

L350: ...superimposed on ‘normal’ isotope effects...

L356: As written this statement is incorrect. I would recommend restating: “During anammox, when nitrite is reduced with ammonium as electron donor and nitrate is produced, one oxygen atom from water having a  $\delta^{18}\text{O}$  value of around -10‰ is incorporated into the newly formed nitrate.” This incorporation of a new O atom is also most likely associated with a kinetic isotope effect – as has been demonstrated in nitrite oxidizing bacteria (see Buchwald and Casciotti, 2010).

L359: ... the anammox process leads to  $\delta^{18}\text{O}$  values of nitrate remaining low, while...

L361: ...by an inverse isotope effect and values continue to increase.

L368: Although not mentioned, I am curious whether any nitrite oxidizing bacteria were detected in the genomic analyses? I assume from their omission that they were not. This could be a useful fact to mention if so.

L373: ... environmental conditions, helping any nitrate reducing ANME-2d (with lower doubling times) in the denitrification zone...

L380: ... the meaning behind this sentence is unclear...

L382: ... potion? portion?

L398: ... as shown...

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L400: . . . This makes it highly likely. . .

Figure 1: There is a mismatch between the ticks and y-axis labels on both panels A and B. Perhaps an indication of where the bottom of the lake is on this figure would be useful for context. In panel B, the choice of symbols for the  $\delta D$  and  $\delta^{18}O$  of water are essentially indistinguishable. In general, the axis labels are too small and much of the text/labels are difficult to read.

Figure 2: All of the text, labels, and axes are too small to be read.

Figure 4: Caption is missing a parenthesis ('filled triangles').

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