

Mazzoli and colleagues present a model of benthic N cycling that includes a description of isotopic signatures. They apply it to a dataset obtained in Lake Lucerne, discuss the dominant processes in the nitrogen cycle, and make the case that the model is broadly applicable.

The combination of measured porewater profiles of N₂, NH₄ and NO₃, together with their isotopic signatures and rates of denitrification, DNRA and anammox with the model is novel. What also sets this paper apart from previous work and many other early diagenetic modeling efforts is that it uses a strong parameter estimation component. They use Bayesian inference to connect prior knowledge with the data, and carry out sensitivity analyses on parameters for which the marginal posterior distributions differed substantially from the prior.

This is a well-written paper, providing a good overview on biogeochemical N models in the introduction. Overall, I really like this paper. The work seems to be technically sound, the analysis related to the model parameters is excellent (see e.g. the first paragraph of the conclusions). I also appreciate the clear demonstration that model fit to the measured profiles is not a strong indicator that the underlying processes are necessarily captured correctly (conclusion line 762ff).

We thank the reviewer for the kind and insightful feedback. We took the time to assess the implications of each comment. We plan to consider all of them and will adapt the manuscript accordingly. Please refer to the points below for a detailed plan on how we intend to revise the text.

The point that I struggled with most is the very low isotope effect reported for denitrification, which I am a bit skeptical about given this is model-based and lacks direct observational support. However, the model analysis presented in the paper is well thought out, so I wonder about potential assumptions that could lead to such a finding in the model (but potentially not in nature). One such issue might be that the abundance of active cells is not modeled (assumption iii, which is common). This may lead to large variations in cell specific rates and potentially fractionation effects. Are these likely candidates for the “structural limitations” mentioned on Line 398? If so, consider expanding on this in the discussion of your result. For example, rather low fractionation has been reported by Perez-Rodriguez et al. 2017 (<https://doi.org/10.1016/j.gca.2017.05.014>) and by Kritee et al. 2012 (<https://doi.org/10.1016/j.gca.2012.05.020>), and Kritee’s figure 1 suggests lower epsilons at low cell specific rates. Nevertheless, the values reported here are much lower, so a broader discussion of existing supporting or contradictory experimental evidence would be welcome (including past work by some of the authors).

We are aware of the unexpectedly low isotope effects for NO₃⁻ reduction via denitrification, and have investigated this phenomenon extensively to ensure its robustness. We can assure the reviewer of the validity of our modeling

framework by presenting the case of a deep-sea station in the Bering Sea, originally reported in Lehmann et al. (2007; doi:10.1016/j.gca.2007.07.025), where we estimated $^{15}\epsilon_{\text{Den1}}$ to be approximately 21‰ (i.e., the model is capable of capturing high porewater-level NO_3^- reduction isotope effects when they occur). We speculate that the release of NO_2^- into porewater during the first step of denitrification plays a key role in lowering the estimated $^{15}\epsilon_{\text{Den1}}$ in Lake Lucerne sediments, as shown by the comparison of the one-step versus three-step denitrification approaches presented in section 4.4. A more extensive assessment of these isotope dynamics across a variety of benthic environments will be presented in a follow-up paper.

The main objective of the present paper is to introduce and validate the model framework, including technical details. Nonetheless, we value the suggested input on assumption (iii), and the consequent effects on cell-specific rates and non-constant isotope effects over depth, and will explicitly add that we did not consider variability in cell-specific rates under this assumption. While these considerations are valid, our measured $\delta^{15}\text{N-NO}_3^-$ profile lacks a sharp gradient below the oxycline, suggesting a suppressed isotope effect for denitrification also in natural settings and not due to model artifacts. This is further confirmed by the comparison of model outputs for the one-step and three-step denitrification approaches (section 4.4). During this assessment, the model assumptions regarding cell densities and rates were kept unaltered, indicating that the primary reason for the low estimated $^{15}\epsilon_{\text{Den1}}$ in the three-step denitrification approach likely lies in the release of NO_2^- during denitrification.

Lastly, the “structural limitations” mentioned on Line 398 (in the original MS) refer to all model assumptions listed in section 2.3, including processes not explicitly considered within the model, and the parameterization of modelled processes. These structural limitations increase model uncertainty relative to observation error, which combines sampling and analysis uncertainties.

Below are a few more comments:

Model formulation

The mineralization of organic matter is modeled depending on intrinsic rate constants for aerobic and anaerobic mineralization reactions, as well as for sulfate reduction and denitrification. Different pathways are modeled depending on the availability of dissolved electron acceptors, as well as inhibition of anaerobic mineralization by O_2 and NO_3 . Notably, the mineralization rate is modeled to not depend on the availability of reactive organic matter.

While this manuscript clearly expands beyond previous work, I suggest to revise the wording on line 77-79 and 765/766. For example, the work by Rooze and Meile also incorporated (some) stepwise process descriptions that emphasize the role of nitrite

as intermediate, and uses data (though not porewater profiles) for validation. Also, some existing models deal more comprehensively with the coupling between different elemental cycles (see below), and the applicability of this particular model to other environments really depends on how substantial this coupling is. For example, I did not see how reduced products from anaerobic mineralization reactions are accounted for (other than ammonium). As a consequence, the model will underestimate the use of nitrate and O₂ through their reoxidation reaction. It seems that in the particular application for Lake Lucerne, this may not affect the results greatly (compare rates of anaerobic mineralization to those of aerobic mineralization in Figure 3, indicating that there is little anaerobic mineralization). However, these are modeled rates, and not accounting for the use of O₂ to oxidize hydrogen sulfide or reduced metals limits the applicability of the model to a broader range of environments.

We thank the reviewer for the insightful input on the coupling of different element cycles and their role in the sedimentary redox zone. While we aimed to make the model as comprehensive as possible, the large number of parameters and limited field measurement data currently define the feasibility limits of the parameter estimation with adequate accuracy. As the model code is publicly available on GitLab, other users are encouraged to revise and extend the model for their specific needs, including the addition of new state variables and reactions, provided that they can be constrained by field data.

Taking these considerations into account, we will update the manuscript accordingly (line numbers still refer to the original manuscript):

- L77-79: "To date, only limited efforts have been made to develop comprehensive benthic isotope models that integrate multiple N-transformation processes in a stepwise manner, and assess the expression of their isotope effects in the porewater of aquatic sediments, validated with observational data (Denk et al., 2017; Rooze and Meile, 2016)."

- L765-767: "Overall, this study presents one of the first comprehensive diagenetic N isotope models that explicitly incorporate multiple transformation pathways in a stepwise manner and are validated against field measurements."

- Add a point to the list of model assumptions in section 2.3 stating that we do not consider re-oxygenation of reduced compounds other than NH₄⁺.

All state variables considered I believe are solutes, i.e. the code does not track any solids. This shortens the simulation time and makes the MCMC feasible. However, it also implies that bioturbation does not account for solid phase mixing. Thus, it is

not surprising that changing D_b has largely no effect on the model results (section 4.2, scenario D). As a consequence, I suspect this is largely a result caused by the model structure and unlikely to be true in reality. Furthermore, it is not clear how the value of D_{bio} was determined. Its current value drops from $1.16 \times 10^{-9} \text{ m}^2/\text{s}$ at $z=0$ to $4.26 \times 10^{-10} \text{ m}^2/\text{s}$ at 1 cm depth. Thus, it essentially only exceeds molecular diffusion in the top cm. However, non-diffusive mixing typically exceeds solute transport by molecular diffusion more substantially, and bioturbation tends to often dominate the movement of solid phases near the sediment-water interface. Thus, this value should be justified for solutes and if this is hard to do, then consider toning down conclusions related to bioturbation. And because there are no solid phases accounted for, the model also ignores the effect of the precipitation of FeS (maybe not a huge pool, but reported present (e.g. Horw Bay; Table 2 in Urban et al. 1999 [https://doi.org/10.1016/S0016-7037\(98\)00306-8](https://doi.org/10.1016/S0016-7037(98)00306-8)), affecting the extent of use of electron acceptors such as nitrate and dissolved oxygen.

We agree with the reviewer that the complexity of modeling bioirrigation and its effects on solute diffusion extends beyond the simple diffusion enhancement represented in our model. Among other simplifications, our model does not take into account the solid phase, and we will emphasize this limitation in the model assumptions of the revised manuscript.

Our assessment of bioirrigation focused primarily on identifying the model sensitivity to bioturbation, as the magnitude of its effect on solute diffusion remains highly uncertain (as stated in section 4.2). However, we respectfully disagree with the reviewer's statement that "changing D_b has largely no effect on the model results (section 4.2, scenario D)". While the effects on isotopic composition and fit quality was indeed small (Lines 532-533 of the original manuscript), altering the D_b value (i.e., the magnitude of solute-diffusion enhancement due to bioirrigation) has a pronounced effect on the modelled rate parameters (Lines 530-531).

The manuscript says that rates of N cycling were measured, but I didn't see those results. The rate of (total) organic matter mineralization is on the order of 450 uM N/d near the surface, which translate into approximately 3 uM/L/d or $120 \text{ nmol/cm}^3/\text{hr}$. (Figure 3: I assume these rates are in uM nitrogen/d because different N processes are being compared. Please clarify if these are in mol C). These rate estimates are about 100 times larger than the $1 \text{ nmolC/cm}^3/\text{hr}$ reported in Fiscal et al. 2019 (<https://doi.org/10.5194/bg-16-3725-2019>) for Lake Lucerne surface sediment. Please clarify the evidence supporting your numbers (measured rates; or fitting profiles using low estimates of transport as discussed above?)

We thank the reviewer for the careful attention to this comparison. We confirm that all rates are expressed in units of N and not C; therefore, the rates in Figure 3 are indeed uM N d^{-1} . The conversion provided by the reviewer

appears to be off by a factor of 10^3 (i.e., $3 \mu\text{mol/L/d} = 0.125 \text{ nmol/cm}^3/\text{h}$), which brings our estimates much closer (within less than an order of magnitude) to those reported by Fiskal et al. (2019). Nonetheless, a key difference between our study and the study by Fiskal et al (2019) lies in the depth resolution of the profiles. As the oxycline and nitracline in the sediments of Lake Lucerne are located within the top 1-1.5 cm, our finer resolution allows us to better resolve these steep gradients and thereby better constrain N-cycling reactions occurring within the upper few mm of the sediment.

The measured rates were obtained using ^{15}N tracer incubations, which provide rate estimates under above-natural substrate concentrations (potential rates). Thus, we used these data only to quantify the relative importance of denitrification, anammox and DNRA. The ratios among these processes were used as priors for the respective f factors listed in Table C1.

The model description is clear, but I have some questions about the process descriptions

- denitrification and nitrification: these processes are implemented as multi-step processes, with the rates of the steps following the initial step modeled as a fraction of the rate of that first step. This is done “to prevent unrealistic rates” (e.g. L180). But it also predetermines the “sharing” of NO_2^- as a key intermediate between different processes, and, for example, with the Monod dependency on nitrite, the second step of denitrification could not occur at a rate higher than what is fueled by nitrate. This may not be a huge issue for N dynamics and I consider this to be a reasonable model simplification, but can you discuss the implications in particular for the isotopic signatures?

This comment is based on a misunderstanding of our model parameterization, which we clarify below for denitrification.

Parameterizing the denitrification model with rate parameters k_{Den1} , k_{Den2} and k_{Den3} is mathematically equivalent to our parameterization with k_{Den1} , f_{Den2Den1} and f_{Den3Den1} , as these parameters are linked through the invertible transformations $k_{\text{Den2}} = f_{\text{Den2Den1}} k_{\text{Den1}}$ and $k_{\text{Den3}} = f_{\text{Den3Den1}} k_{\text{Den1}}$ (line 783). Therefore, identical results can be produced with both parameterizations for any values of k_{Den1} , k_{Den2} and k_{Den3} . The reviewer seems to interpret the factors f as “fractions” smaller than 1, which is not the case; the priors (see Appendix C) do not constrain these values to be <1 .

The issue of “unrealistic rates” arises because, during inference, the rate parameters of the second and third steps can grow indefinitely without producing unreasonable concentration profiles, as their rates are limited by the first reaction step. Increasing the rate parameters of the succeeding steps

thus only reduces the concentrations of intermediate reaction compounds without strongly modifying overall rates.

In Bayesian inference, such identifiability problems are addressed through prior information, since the data alone may not sufficiently constrain all rate parameters (unless the intermediate compound is measured and inconsistent with very small concentrations). The k_{Den} rate coefficients can vary very strongly from one system to the other, as they are proportional to the product of the bacterial abundance and their growth rate, both of which were not explicitly modeled, are system specific and can vary widely. To account for this, we used a uniform prior for k_{Den1} . This approach, however, is not suitable for k_{Den2} and k_{Den3} for the reason mentioned above, so informative priors are needed. Because higher bacterial densities for the first step are typically correlated with higher densities in the following steps, the ratios $k_{\text{Den2}}/k_{\text{Den1}}$ ($= f_{\text{Den2Den1}}$) and $k_{\text{Den3}}/k_{\text{Den1}}$ ($= f_{\text{Den3Den1}}$) are less variable between systems than the absolute rate coefficients (k_{Den2} and k_{Den3}) themselves. Thus, it is easier to formulate “universal” priors for these ratios (the factors f) than for the absolute rates, preventing the inference process to “move” towards unrealistically large values without requiring assumptions about bacterial densities.

We will emphasize this reasoning in the revised manuscript by adding the following text: “The re-parameterization of the second and third steps using the f_{Den2Den1} and f_{Den3Den1} factors corresponds to exactly the same model without any approximation or simplification. It serves solely to facilitate the specification of priors, as more knowledge is typically available about ratios of maximum rates (i.e., $f_{\text{Den2Den1}} = k_{\text{Den2}}/k_{\text{Den1}}$) than about the absolute maximum rates themselves.”

- the rate expression of reaction 1b (Table 1) follows a Monod dependency on both of the ammonia involved forming the N_2O . This contrasts with pretty much all the other reactions (including the O_2 dependency in the same reaction), in which the reaction stoichiometry is not accounted for in the rate laws. Is there any experimental evidence that supports this formulation? And maybe more importantly what are the implications of this choice?

We include limitations in all consumed compounds to prevent a process from continuing when one of the reactants reaches zero concentrations (as this would lead to negative concentrations calculated by the model). For reaction 1b, the quadratic dependence provides a meaningful limitation for *both* ammonia molecules at low concentrations.

While this is a convenient feature for the bulk model (which does not distinguish isotopes), it becomes absolutely essential if we distinguish

isotopes. Table 1 summarizes only the partial model for the ^{14}N species. The complete model formulation in Table A1 expands to the combinations $^{14}\text{NH}_4^+$, $^{14}\text{NH}_4^+$, $^{14}\text{NH}_4^+$ $^{15}\text{NH}_4^+$, $^{15}\text{NH}_4^+$ $^{14}\text{NH}_4^+$ and $^{15}\text{NH}_4^+$ $^{15}\text{NH}_4^+$. The two mixed terms ($^{14}\text{NH}_4^+$ $^{15}\text{NH}_4^+$ and $^{15}\text{NH}_4^+$ $^{14}\text{NH}_4^+$) are combined as $2x(^{14}\text{NH}_4^+$ $^{15}\text{NH}_4^+)$. We would like to point out that the partial rates r' below Table A1 have "1" in the numerator of the nitrogen limitation term, which is multiplied by the combinations listed in the rightmost column of the table; thus, the model formulation guarantees explicit limitation in both $^{14}\text{NH}_4^+$ and $^{15}\text{NH}_4^+$, which is absolutely essential to avoid the occurrence of negative concentrations as outlined above and for consistent mass balances (this latter point also motivated the inclusion of $^{15}\text{NH}_4^+$ $^{15}\text{NH}_4^+$ which, despite their very low concentration, is needed for a consistent model).

The same argument applies to the second step of denitrification and is implemented analogously (see Table A1). We also note a misprint in Table 1, equation [2] for denitrification: $[^{14}\text{NO}_2^-]$ should read $[^{14}\text{NO}_2^-]^2$. We will correct this error.

As explained above, separate limitations are already needed for conceptual reasons (to formulate a consistent model); there is not much experimental evidence for the exact functional form of these limitation terms. This is also the case for all other limitation terms in the model. We also implemented exponential instead of Monod-type limitations in the model, but the results did not substantially change.

- anammox: this process includes not only the $\text{NO}_2^- + \text{NH}_4^+ \rightarrow \text{N}_2$ reaction, but also the production of nitrate from nitrite. If I understand correctly, the parameter f_{side} (Table A1) is therefore representing the 0.3 $\text{NO}_3^-/\text{NH}_4^+$ (Line 205). Please clarify; also define $[s]$ and $[m]$ in table A1.

We confirm that the production of NO_3^- from NO_2^- is what we define with the parameter " f_{side} ". We will revise the caption of Table A1 to clarify the two anammox reactions: "Anammox encompasses both the comproportionation of NH_4^+ and NO_2^- to N_2 , defined as the main (" m ") reaction, and the production of NO_3^- from NO_2^- , defined as the side (" s ") reaction".

To ensure clarity also in the text, we will include this information in section 2.2, where we describe the modelled transformation processes (Line 204): "Anammox is modelled to include both the comproportionation of NH_4^+ and NO_2^- to N_2 (main reaction, " m "), and the NO_3^- production via NO_2^- oxidation (side reaction, " s ") (where 0.3 mol NO_3^- are produced per 1 mol NH_4^+ and 1.3 mol NO_2^-)."

- Table A1: define gamma_Den1, _Den2, _Den3. Please clarify so it is clear why R is $^{15}\text{N}/^{14}\text{N}$, instead of e.g. $^{15}\text{N}/(^{14}\text{N}+^{15}\text{N})$.

We thank the reviewer for pointing out this oversight. We will:

- clarify this by adding “ γ ” on line 238 so that assumption (vii) will read: “OM composition is approximated by the Redfield ratio (C:N:P = 106:16:1), used to estimate the fraction of NH_4^+ released during OM mineralization, γ ”

- revise the caption of Table A1 to include the meaning of γ : “The γ parameter defines the fraction of NH_4^+ released during OM mineralization for each reaction.”

- change the caption to reflect the real definition of the R term as $^{15}\text{N}/(^{14}\text{N}+^{15}\text{N})$.

Model/parameter analysis

The Bayesian inference analysis is well done and very helpful. For example, I was rather skeptical that a , b from the Ji et al. paper can be directly used in the sediment (p.8, 18). However, these parameters are apparently not impacting the results a great deal. This is a great example of the value of assessing the impact of the parameters on the model outcome.

We are grateful that the reviewer appreciates our efforts that went into validating and assessing the impact of the parameters on the model output. We emphasize that in other systems with different properties, the impact of each parameter may vary, and should be assessed prior to applying the model to isotope dynamics.

Visualization/presentation of results

Several figures are difficult to read without magnifying them on the screen. For example, in Figure 3 I had a difficult time identifying which line and process belong together. If another color scheme is not feasible, consider putting information identifying the relevant processes into the figure caption.

We acknowledge the difficulty in identifying and distinguishing the distinct profiles and will improve the graphics to the best of our ability. We will adopt a color-vision-deficiency-friendly palette, increase line widths and font size. We provide here an example of Figure 3 to show how we plan to change the figures to make it easier to distinguish the profiles.

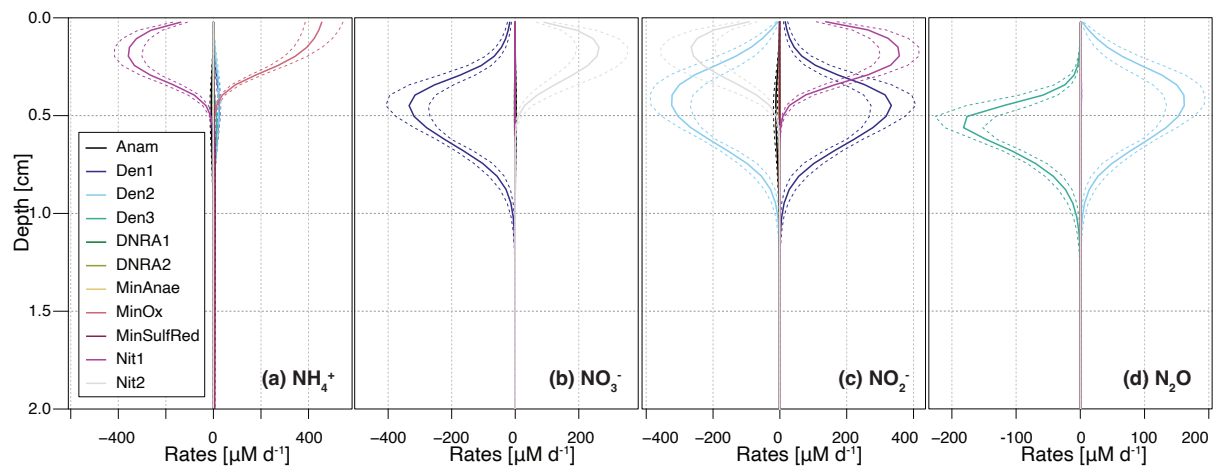


Figure S1: spell out what the solid (posterior) and dashed (prior) lines represent, and what ess= ... means in the titles of each panel

We thank the reviewer for pointing out the missing information. We will revise the caption to “Figure S1. Marginal prior distributions (dashed) and marginal posterior distributions (solid) for all parameters estimated in the Base scenario. The effective sample size (ess; the approximate number of independent posterior sample points) for each parameter is also reported.”

Line 242: in the reactions of the manuscript you refer to Mn^{2+} , but here is it Mn^{3+} .

We thank the reviewer for noting this inconsistency. We will replace “than oxidation by iron(III), Fe^{3+} , and manganese, Mn^{3+} , in some lacustrine systems” with “than oxidation by iron(III), Fe^{3+} , and manganese, Mn^{4+} , in some lacustrine systems” in lines 241-242. This is to ensure consistency with the equation provided in Table 1, where Mn^{4+} (in MnO_2) is reduced to Mn^{2+} . For clarity, our model does not include Mn(III) intermediates.