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Interactive comment on "Evidence for microbial mediated nitrate cycling within floodplain sediments during groundwater fluctuations" *by* Nicholas J. Bouskill et al.

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See for example the extensive work by Paul Brooks, Michelle Baker, Mark Williams

The manuscript under review examines how nitrate is produced and transformed at the capillary fringe during annual fluctuations in the water table. We are familiar with the large body of Paul Brooks' work, however, this work primarily concerns itself with the measurement and modeling of the subnivial nitrogen cycle, as well as examining surface hydrological processes. Much of this work is not necessarily pertinent to the current manuscript. Mark Williams similarly takes a broad approach to examining hydrologically-induced changes in the nitrogen cycle, however, as far as we are

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aware does not work around the capillary fringe either. Michelle Baker's work primarily focuses on carbon and nitrogen cycling within riverine biogeochemical hotspots (i.e., riparian areas of rivers corridors, and in-stream hyporheic zones) rather than around the capillary fringe. While these authors' outstanding work has informed our broader thinking on the terrestrial nitrogen cycle, their body of work is not immediately applicable to the current manuscript, which is why these papers have not been cited. Indeed, there are few studies in the literature that we can find, taking a mechanistic approach to understanding the nitrogen cycle at capillary fringes that serve as relevant citations for the current work. This also goes against the reviewer's supposition our work is not novel. While there are a number of manuscripts examining nitrogen dynamics around the hyporheic zone of streams (e.g., Bohlke et al., Biogeochem. 2009; Zarnetske et al., JGR, 2011) and rivers (e.g., Clilverd et al., Biogeochem, 2008; Hinkle et al., J. Hydro. 2001), and oxygen transformations around the capillary fringe itself (Haberer et al., J. Contamin. Hydrol), we can, in fact, find few manuscripts that examines the importance of hydrological fluctuations around the capillary fringe with respect to nitrogen cycling. The manuscripts that examine nitrogen cycling around the capillary fringe (e.g., Abit et al., Geoderma, 2008), all of which are referenced in the current manuscript (Pg. 2, Ln. 5 - 6) do not take a similar mechanistic approach as described here.

Where are the microbial data needed to test the main predictions of the model (e.g. Fig 4)? There are skilled microbial ecologists on this team and working on this site. I didn't find the model results compelling in the absence of microbial data, especially given the poor performance of the model in predicting nitrate at the three (!) depths where it was apparently compared (Fig. 4).

There has been a significant amount of microbial work performed at this site (e.g., Anantharaman et al., Nat. Comm, 2016; Hug et al., ISME, 2015; Jewell et al., ISME, 2016; Wrighton et al., ISME, 2014), all of which is extremely useful for initializing the reaction network for this, and other, models (as pointed out on Pg. 3, Ln 20, and dis-

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cussed further on Pg. 14). For example, one of the questions we set out to address in the current manuscript concerns the interactions between different heterotrophic and autotrophic metabolisms promoting N-transformations and loss (denitrification vs. anaerobic ammonium oxidation, see Pg. 3, Ln 5). The notion that anammox is important in this environment comes directly from molecular evidence profiling the community within the naturally reduced zones of the floodplain (e.g., Jewell et al., ISME, 2016). These areas, located just below the capillary fringe, have high abundance of chemolithoautotrophic metabolisms, however, little information exists comparing the importance of different metabolisms to nitrogen loss. Several manuscripts have tackled these questions within marine environments (e.g., Babbin et al., Science, 2014; Koeve & Kahler, Biogeosciences, 2010) using measurements and models, but as far as we are aware, this has not been extended to terrestrial systems. Furthermore, because feedbacks between biotic and abiotic systems are inherently non-linear, and therefore cannot be addressed directly by molecular studies, we believe a mathematical model of interacting microbial guilds informed by these prior studies is a plausible approach to address these interactions.

However, we have yet to find microbial data that is applicable for benchmarking microbial models. Microbial models represent the active portion of the microbial community, and are simplified representations of microbial guilds using several traits, and imposed trade-offs. Therefore, commonly collected microbial metrics are not comparable to modeled metrics. For example, measurements of biomass (via chloroformfumigation) account for microbial and fungal biomass and additional labile compounds from non-living sources (e.g., plant residue), and frequently overestimate biomass. Modeled biomass, on the other hand, represents the products of growth of the metabolisms considered (never the full community).

Molecular markers of microbial activity (e.g., mRNA measurements) show some promise as benchmarks of specific modeled microbial processes, but at this stage require more work to determine the factors that control the regulation of mRNA. Previous

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work has shown a lack of correlation between the production of mRNA and the activity of the pathway encoded by that mRNA. Post transcriptional modification pathways play and important role in determining the balance between transcription and translation. More specific incubation experiments around the capillary fringe (for example, the use of random isotope pairing techniques to differentiate anammox from denitrification), would be very useful for parsing out metabolisms of importance, however, were beyond the scope of the current study.

Nonetheless, we believe benchmarks for microbial models are an important area to highlight in this manuscript, and have included a section in the discussion that explicitly deals with the benchmarking needs for models of this type.

We disagree, however, with the reviewer's assertion that the model performs poorly in failing to capture the nitrate dynamics. The model does not capture the totality of the nitrate dynamics in the current configuration. This is because the model is being run to examine the extent of biological nitrogen loss from the different depths. We make this point in the materials & methods (Pg. 7 Ln 7 - 8), the results (e.g., Pg. 10, Ln 8 - 11) and discussion. From this perspective, comparison with the Rayleigh calculations from the isotopic data, the model actually performs reasonably in capturing the nitrate dynamics as catalyzed by different microbiological guilds and as a function of the oxygen dynamics, and organic matter/ nitrate concentrations. It is quite possible to configure the model to account for all of the nitrate loss from biological dynamics (as shown in the supplemental figure 4), or under variable electron donor ratios (supplemental figure 5), however, the broad conclusions from the isotopic data suggests that this would be incorrect, and again, highlights the utility of using isotope data to benchmark this model. It is possible that this point is not made clear enough in the current text. Therefore we have added additional text to the discussion to emphasize this point. For comparison, we have also run the model to simulate both abiotic (dilution) and biotic pathways. These simulations are given in the supplemental figures and discussed further in the text. Finally, in order to compare how well the model captures the data, we have run

statistical tests represented in a Taylor Diagram also included in the supplemental, and further discussed in the text.

The microbial simulations come off as entirely speculative given that there are no data presented, as does the speculation as to the importance of annamox vs. canonical denitrification vs. chemolithotrophic processes. Contrary to the conclusion (P17 25), I don't think the authors can make any concrete claims as to the mechanisms driving the patterns observed, especially given that the nitrate isotope fractions are not well constrained for these pathways, and that there is enormous variation in nitrate isotope fractionation during denitrification.

Our conclusions are drawn predominantly from the simulations, and the conditions under which these simulations are performed. With regards to understanding the importance of nitrogen loss via heterotrophic denitrification Vs. chemolithoautotrophic anaerobic ammonia oxidation, this question is driven primarily by recent molecular microbiology work at this site showing a relatively high abundance of chemolithoautotrophic metabolisms in the groundwater (Jewell et al., ISME, 2016; Frontiers in Microbiology, 2016), and high abundance of ammonia-oxidizing archaea (Hug et al., Envion. Micro, 2015) and heterotrophic denitrifiers (Anantharamam et al., Nat. Comm. 2016) at shallower depths. We do point out in the text that the spin-up conditions (i.e., a low water table fostering aerobic conditions) prior to the water table perturbation simulations can select against obligate anaerobes (such as the anammox bacteria), and for faculative aerobe such as the heterotrophic bacteria.

From this perspective, we do not believe that our interpretation of the model simulations is speculative. The development of the model is informed by prior studies at the same site (Pg. 2 Ln 25), the model parameters are taken from literature values of representative organisms (aerobic and anaerobic ammonia oxidizers & faculative heterotrophs, see supplemental tables), the broad conclusions of the model simulations (i.e., the % of

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biotic N-loss Vs. abiotic dilution) are supported by isotopic benchmarks (from Rayleigh fractionation calculations, Pg. 10 Ln 10, and simple mixing calculations, see supplemental material) , and the final question, as to the general importance of anammox Vs. denitrification to N-loss, is supported by prior ecophysiological data and mechanistic modeling. The discussion also goes into more details as to the broader conclusions (i.e., from Pg. 15. Ln 20 onwards) we make from the study. We have, however, added additional text to make it clear that these conclusions are based primarily on model simulations.

The isotopic data has not been used to attempt to parse between the two different pathways. As with previous studies examining the contributions of anammox vs denitrification to nitrogen loss (e.g., Babbin et al., Science, 2014; Koeve & Kahler, Biogeosciences, 2010), we've employed a mechanistic model. As with previous models, it is a simplification of real-world conditions, yet captures some of the more important traits related to fitness under fluctuating environments. Hence, the output is therefore theoretical, rather than speculative, yet corresponds to findings of previous studies attempting to parse out the factors determining nitrogen loss from discrete end members.

We believe that this study therefore supplies suitable impetus for follow up experimental work based on the model output. Furthermore, modifications to the baseline model presented here (for example, incorporating dynamic energy budgets based on the thermodynamic approach explained in the text) could be used to examine why there is such variability in isotopic fractionation from an ecological and metabolic perspective.

The spatial replication of the field data seems inadequate given the heterogeneity of the system under study. Why are no isotope measurements from the vadose zone and shallow soils reported? This seems critical to get at the question of biogeochemical processing of N vs. dilution or mixing that comes up throughout the paper, and the enormous spatial heterogeneity of nitrate isotope compositions that is increasingly

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documented in the literature. What is the composition of the water that is posited to be diluting the sediment zone of interest? There was almost no discussion of the hydrology of the site and potential source waters, which are critical for getting at this point. To interpret the isotopes, you would need to consider mixing rather than pure dilution unless you could demonstrate that you were mixing nitrate-rich vs. nitrate-free water. This is especially critical in the context of the heterogeneity in buried organic lenses that has been demonstrated at this site.

Nitrate accumulates and dissipates only in the depths currently under investigation (i.e., 2 - 3 m below surface depth), with little evidence from this study or from previous studies that nitrate accumulates at shallower or deeper depths. Measurements of nitrate in the vadose zone were below detection (figure 2), it is also unlikely, given infiltration rates at this site ($\sim$ 3 cm yr-1, Pg. 14, Ln 2), that nitrate from shallower soils are transported to = 2 m and below. This is further supported by recent work at the site adding $\sim$ 2500 gallons of deuterium-enriched snow (δD $\sim$ 2200 per mil), for the purpose of examine water infiltration into the vadose zone around the well used in the current study. Snowmelt last 6 days and δD rapidly infiltrated to $\sim$ 1 – 1.5 m, with very little deuterium signal seen below 1.5 m. Therefore, the transportation of nitrate from the vadose zone to the capillary fringe was not considered to be of importance in the current study. Similarly, nitrate below the 3 m line has been shown to be very low. Fig. 2 shows nitrate data for 3.14 m below surface depth, the lower bound of the current data set, with nitrate concentration ranging from 60 to 700 micromoles. Below this, into the background aquifer, nitrate ranges from undetectable up to 80 micromoles, as reported in previous studies (Zachara et al, J. Cont. Hydrol. 2013; Yabusaki et al., ES&T, 2017). This is alluded to in the main text (Pg. 3, Ln 14), however, we have rewritten this statement to make it clearer. Finally, and further emphasizing the nitrate-deficient conditions in the groundwater, a recent NO3 injection experiment injected $\sim$ 2 mM of nitrate into the groundwater intending to stimulate chemolithoautotrophic metabolisms (Jewell et al., ISME, 2016; Frontiers in Microbiology, 2016). Prior to the injection, nitrate concentrations ranged from undetectable to $\sim$ 70 μM. Post-injection, the nitrate

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was entirely consumed within the first 1 m downgradient.

In summary, the reason that no isotope measurements were made in the vadose zone or background aquifer was that nitrate was often below detection limits of the technique. This would also minimize the likelihood of nitrate from outside the depths studied contributing significantly to the observations reported here.

I am very surprised that the authors report nitrate concentrations of 5 mM surely they must mean 5 micromolar or 5 mg NO3- L-1?

The mM units are correct. Nitrate is measured routinely at this site by ion chromatography according to approaches reported in the main text (Pg. 4, Ln 10 -11). Data from previous years also shows the large accumulation of nitrate (to mM concentrations) in the unsaturated zone pore water are a recurring phenomenon. An explanation for such high nitrate concentrations is the presence of a natural reduced zone around this well (as discussed on Pg. 14, Ln 11). Organic matter concentrations are very high in these zones, Janot et al., ES&T, 2016, recorded organic matter in these regions with OC concentrations as high as 1.7 %. We can therefore use a back-of-theenvelope calculation to estimate nitrogen availability from the OM in these regions. Considering a measured C:N ratio for the relevant depths of 7 (Conrad et al., unpublished) and a bulk density of $\sim$ 2 g cm-3, OM in these naturally reduced regions could yield 0.004 g-N cm-3, or 290 mM of nitrogen. Using a conservative mineralization rate of 2 % per year would therefore yield $\sim$ 6 mM of nitrogen.

This high nitrogen yield therefore makes this site an excellent candidate to study biological hotspots of activity.

The manuscript is riddled with errors. In the title alone there is a grammatical error and a misspelling of one of the author names. I urge the lead author to give the paper a

proper proof reading before sending out for review!

The manuscript has been proofread again. However, I (the lead-author) am unable to identify the spelling mistake. Looking at both the file for submission, and the file uploaded to the Biogeosciences-Discussions website, all authors names are spelt correctly.

For example, P8 line 18, 15N is given as -1.8. At line 23, this same value is referred to but the minus sign is missing. This oversight has been fixed.

There are many more examples... P4 30: “Samples of pore gas from 2 m bsd” do you mean below 2 m? The abbreviation bsd stands for below surface depth, and is defined on page 3, line 31.

How many depths were sampled? Seven depths were sampled (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.14), and this information can be found in the first paragraph of the materials and methods (Pg. 4, Ln 2).

P9 15: what do you mean by “highest (most reduced) value” This simply refers to the highest enrichment recorded, however, might be confusing, therefore has been reworded.

The message in Fig. 3 is not at all clear as presented. Try putting the same values (15N, e.g.) on a common plot so we can compare the trends among depths over time. We are unsure as to what is unclear here. The figure shows the corresponding enrichment in the 15N18O-nitrate accompanied by the trajectory in nitrate concentration over time. The left yaxis represents the isotopic composition of nitrate (15N/ 18O), and is the same axes (from - 10 to +20 per mil) across all three plots, while the right y-axis is the concentration of nitrate from 0 - 8 mM, and again, is the same axis across all three plots. We are therefore not sure as to the value of re-plotting these figures by 15N.

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P13 20: Need citation A citation has been added

P15 25: “the measured in $N2O$ peak” This has been reworded.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-212/bg-2017-212-AC1-supplement.pdf

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-212, 2017.