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Dr. Frank Hagedorn
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Title: Bias in calculating gross nitrification rates in forested catchments using the triple oxygen isotopic composition ($\Delta17O$) of stream nitrate
Authors: Weitian Ding et al.
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Dear Dr. Frank Hagedorn:

Thank you very much for handling our manuscript. We would like to thank the referees as well for the constructive comments on our manuscript. We have carefully studied the comments and revised the manuscript accordingly. We include below point-by-point responses to the comments, and detailed descriptions of the modifications we made to the manuscript. Besides, we also uploaded the revised manuscript in MS Word, in which all the revisions from BGD version were recorded. We hope that with these changes you will find our revised manuscript appropriate for publication in your journal.

Sincerely yours,
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Response to the referee #1:

My recommandations made for the previous version of the manuscript were largely applied. The suggestion of trying a different profile of nitrate with soil depth was also realised, but only reported in the authors' answers and not in the article itself. The idea goes into the conclusion, but the abstract still only reports on the one simulation (verb "was" in its last sentence). The abstract therefore leaves the question open how generalisable the conclusion is. And essentially it would be.

Thank you for your comment. We added the simulated calculation in the manuscript as follows (P2, L6-9; P11, L192-204 in revised manuscript).

The GNR estimated from the $\Delta^{17}O$ value of stream nitrate was significantly higher than the GNRs in our simulated calculations for a forested catchment where the soil nitrate had $\Delta^{17}O$ values higher than those the stream nitrate.

Furthermore, even if we assumed non-linear variation for the leaching flux of soil NO_3^-, in which the leaching flux of soil NO_3^- increased with soil depth from layers 1 to 5 with an increasing rate of 0.44 kg of N ha^{-1} y^{-1} $layer^{-1}$, while the leaching flux decreased with soil depth from layers 6 to 10 with a decreasing rate of 1.32 kg of N ha^{-1} y^{-1} $layer^{-1}$ (Table S3), the newly estimated total GNR (19.1 kg of N ha^{-1} y^{-1}) was still comparable with that estimated for the forested catchment with the heterogeneous soil shown by Figure 1 (13.0 kg of N ha^{-1} y^{-1}). As a result, we concluded that the differences in the $\Delta^{17}O$ values of the soil NO_3^- consumed in a forested catchment from that of stream NO_3^- resulted in a significant deviation in the GNR estimated using Eq. 6 from the actual GNR. In addition, the most important parameter to determine GNR was the $\Delta^{17}O$ values of NO_3^- consumed in soil layers. That is, the other parameters such as the number of layers and the vertical changes in the leaching flux of soil NO_3^- had little impact on total GNR.

L. 10: could be simplified to "with depth" (dropping "an increase in").

Thank you for your comment. To improve the flow of abstract, we removed the sentence “The $\Delta^{17}O$ values of the soil nitrate decreased with an increase in depth to that of the stream nitrate at the bottom” from the abstract in the revised manuscript.

L. 19-20: "forest ecosystems" could be replaced by "soils" (first because this is not only valid for forests, second because it avoids to repeat too many times "forest ecosystems").

Thank you for your comment. We revised this in the revised manuscript as follows (P2, L15 in the revised manuscript).

Nitrate (NO_3^-) is an important nitrogen nutrient for primary production in soils.

L. 24: a parenthesis inside another parenthesis could be avoided simply by putting a comma between both parts.

Thank you for your comment. We revised this in the revised manuscript as follows (P2, L19-23 in the revised manuscript).

However, the gross nitrification rate (GNR), which includes the net nitrification rate plus the consumption rate of NO_3^- (e.g., through plant assimilation or denitrification), reflects the internal N cycling better than the net nitrification rate (Bengtsson et al., 2003), especially in forested ecosystems.

L. 31: the comma before "as a" does not seem justified.

Thank you for your comment. We removed the comma in the revised manuscript as follows (P3, L28 in the revised manuscript).

Recent studies have successfully estimated the GNR in aquatic environments, such as lakes, using the $\Delta^{17}O$ values of NO_3^- as a conservative tracer to determine the mixing ratio between atmospheric nitrate ($NO_3^-{}_{atm}$) and biologically produced nitrate ($NO_3^-{}_{bio}$) (Tsunogai et al., 2011, 2018).

L. 52: "also" should be introduced between "this is" and "because" (as it is just on more reason and not the single reason why the method is applicable).

Thank you for your comment. We revised the sentence in the revised manuscript as follows (P4, L49-52 in the revised manuscript).

This approach works because the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratios are homogeneous in the water column due to the active vertical mixing; thus, we can constrain the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratios of NO_3^- consumed in the lake water column (Tsunogai et al., 2011, 2018).

L. 55: "applications" could be plural.

Thank you for your comment. We revised the sentence in the revised manuscript as follows (P4, L53-55 in the revised manuscript).

In addition to applications in water environments, the $\Delta^{17}O$ method has been applied to forested catchments to determine GNR (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020).

L. 115: using the verb "differ" does not seem correct here. Please revise the sentence, reconsidering also the word "difficult" (something like "very limited" may be better).

Thank you for your comment. We revised the sentence in the revised manuscript as follows (P7, L113-115 in the revised manuscript).

Different from water environments, vertical mixing of water/soil is limited in forested soil, so the $\Delta^{17}O$ values of soil NO_3^- are often heterogeneous.

L. 175 ff: this sentence is not well formed: as I understand it, one subject ("studies") would have two verbs ("estimated" and "were"). Please revise.

Thank you for your comment. We revised the sentence in the revised manuscript as follows (P10, L181-184 in the revised manuscript).

This result allows us to further verify that past studies estimating GNR using Eq. 6 implicitly approximated that $\Delta^{17}O$ values of soil NO_3^- consumed in forested catchments were homogeneous and always equal to those of stream NO_3^-.

L. 206: the last sentence of the section repeats facts already described. My suggestion is to delete it and go directly to the conclusion.

Thank you for your comment. We deleted the sentences in the revised manuscript.

L. 229: there should be no comma after "referees".

Thank you for your comment. We revised this in the revised manuscript as follows (P13, L241-242 in the revised manuscript).

We thank Dr. Joel Bostic, Dr. Lucy Rose and other two anonymous referees for their valuable remarks on an earlier version of this paper.

Response to the referee #3:

The paper provides a set of model calculations estimating the GNR of a catchment, for the community to consider the vertical profiles of D17O in soil layers. The results presented, however, are way too vague and handwaving, particularly the vertical profiles of D17O and nitrate flux taken. I suggest the authors reconsider the validity of the model and assumptions and provide the needed justification.

1. GNR is largely affected by the vertical gradient of D17O in the soil. The main data the authors quoted are from Hattori et al. (2019), who provided limited information on the soil nitrate at depths. The authors should analyze that data in detail and compare to the gradient taken in the current work. Same for nitrate concentration.

Thank you for the comment. Our simulation was done for the forested catchment reported by Hattori et al. (2019), as presented in the manuscript. While the $\Delta^{17}O$ value of $NO_3^-{}_{atm}$ was +28.0 ‰, the $\Delta^{17}O$ value of $NO_3^-{}_{stream}$ (nitrate leaching from the catchment) was +2.2 ‰ on the average in the catchment. In addition, they found a decreasing trend in the $\Delta^{17}O$ values of soil NO_3^- with depth throughout their observation. Specifically, the measured mean $\Delta^{17}O$ values (average values of summer and winter season) of the soil NO_3^- was +17, +4, +3, and +5 ‰ at depths of 0, 25, 55, and 90 cm from the soil surface, respectively. Similar decreasing trend had been found in the other forested catchment as well (Rose, 2014). These are the reasons we used the $\Delta^{17}O$ values of soil NO_3^- showing decreasing trend with soil depth in our original simulations (linear variation), shown by Figures 1b and 2b in the manuscript.

While Hattori et al. (2019) reported the concentration of soil NO_3^- for each layer showing little vertical variation in the forested catchment, they didn't measure the water flux in the catchment. Thus, it is difficult to constrain the vertical changes in the leaching flux of soil NO_3^- from each layer in the forested catchment. Still, the deposition flux of NO_3^- was 7.0 kg of N ha^{-1} y^{-1} and the final leaching flux of NO_3^- via stream was estimated to be 2.6 kg of N ha^{-1} y^{-1} in the forested catchment (Hattori et al., 2019). In addition, the water flux always showed gradual decreasing trend with depth in various forested catchments (e.g., Christiansen et al., 2006). Thus, we used the linear decreasing variation in the leaching flux of soil NO_3^- in our simulations, shown by Figures 1c and 2c in the manuscript. Similar decreasing trend in the leaching flux of soil NO_3^- had been found in the other forested catchments as well (Callesen et al., 1999; Inoue et al., 2021). None of the vertical profiles of $\Delta^{17}O$ and leaching flux of soil NO_3^- adopted in our model were "handwaving".

In response to your comment, we made a new simulated calculation in which the forested soil layers were divided vertically into 5 layers to increase the vertical gradient in the $\Delta^{17}O$ values between the layers (Table R1). While GNR was increased to 29.6 kg of N ha^{-1} y^{-1} from the original (13.0 kg of N ha^{-1} y^{-1}), it was still significantly smaller than the GNR calculated by using Eq.6 (83.6 kg of N ha^{-1} y^{-1}). This additional simulated calculation also supports our conclusion that the GNR estimated from the $\Delta^{17}O$ value of stream nitrate in forested catchments can be an overestimate of the actual GNR.

Table R1. $\Delta^{17}O$ values of NO_3^-, leaching flux of NO_3^-, total consumption rate of NO_3^- (GDR + uptake), and GNR in the simulated forested soil where the distribution of $\Delta^{17}O$ values of NO_3^- is heterogeneous with the values in accordance with the measured $\Delta^{17}O$ mean values of soil NO_3^- at different depth as reported by Hattori et al. (2019).

Depth	$\Delta^{17}O$	NO_3^- flux	GDR +uptake	GNR
layer	‰	kg of N ha^{-1} y^{-1} $layer^{-1}$		
0	28	7.0	0.0	0.0
1	17	6.1	5.4	4.5
2	4	5.2	20.8	19.9
3	3	4.4	2.6	1.7
4	5	3.5	-0.9	-1.7
5	2	2.6	6.1	5.2
6	2	2.6	0	0
Total			34.0	29.6

We added the information about the assumed NO_3^- leaching flux to the revised manuscript as follows (P9-10, L160-170; P11, L192-204 in the revised manuscript).

To estimate GNR in each layer, both the $\Delta^{17}O$ value and the NO_3^- leaching flux in soil are required. While Hattori et al. (2019) reported soil NO_3^- concentrations for each layer, indicating little vertical variation within the forested catchment, they did not measure the catchment water flux. Consequently, it is difficult to constrain the NO_3^- leaching flux for each layer of forest soil. Nevertheless, $NO_3^-{}_{deposition}$ was 7.0 kg N ha^{-1} y^{-1} and $NO_3^-{}_{leaching}$ was 2.6 kg N ha^{-1} y^{-1} in the catchment (Hattori et al., 2019). Additionally, because water fluxes decrease gradually with depth in various forest settings (e.g., Christiansen et al., 2006), we assumed a gradual decrease in NO_3^-, leaching flux from 7.0 to 2.6 kg N ha^{-1} y^{-1} at a rate of −0.44 kg N ha^{-1} y^{-1} per layer (Figs. 1c and 2c). Similar trends in the NO_3^- leaching flux of soil have been observed in other forested catchments (Callesen et al., 1999; Inoue et al., 2021).

Furthermore, even if we assumed non-linear variation for the leaching flux of soil NO_3^-, in which the leaching flux of soil NO_3^- increased with soil depth from layers 1 to 5 with an increasing rate of 0.44 kg of N ha^{-1} y^{-1} $layer^{-1}$, while the leaching flux decreased with soil depth from layers 6 to 10 with a decreasing rate of 1.32 kg of N ha^{-1} y^{-1} $layer^{-1}$ (Table S3), the newly estimated total GNR (19.1 kg of N ha^{-1} y^{-1}) was still comparable with that estimated for the forested catchment with the heterogeneous soil shown by Figure 1 (13.0 kg of N ha^{-1} y^{-1}). As a result, we concluded that the differences in the $\Delta^{17}O$ values of the soil NO_3^- consumed in a forested catchment from that of stream NO_3^- resulted in a significant deviation in the GNR estimated using Eq. 6 from the actual GNR. In addition, the most important parameter to determine GNR was the $\Delta^{17}O$ values of NO_3^- consumed in soil layers. That is, the other parameters such as the number of layers and the vertical changes in the leaching flux of soil NO_3^- had little impact on total GNR.

2. If I understand the model correctly, the authors implicitly assumed steady-state that the soil nitrate profile is nonvarying, inconsistent with variable profiles seen by Hattori et al.

Thank you for your comment. Please note that the y-axes in our simulated models were layers, not depths. While the $\Delta^{17}O$ values of NO_3^- always showed decreasing trends with depths irrespective to the seasons, $\Delta^{17}O$ values of soil NO_3^- showed significant temporal variation at each depth (Hattori et al., 2019). This was the reason why the layers were adopted for the y-axes in our models, instead of depths.
As a result, the specific depth of each layer varies over time. In addition, the relation between depth and layer is not always linear. The temporal variation found in the vertical distributions of $\Delta^{17}O$ values can be explained by this model as well without contradiction because the $\Delta^{17}O$ values of soil NO_3^-, while showing large temporal variation at each depth, always showed decreasing trend with depth throughout their observation (Hattori et al., 2019).
On the other hand, those who used Eq.6, such as Fang et al. (2015), Hattori et al. (2019), and Huang et al. (2020), implicitly assumed the $\Delta^{17}O$ values of NO_3^- in the soil, where GDR and uptake occurred, to be "steady state" at the $\Delta^{17}O$ value of stream NO_3^- (+2.2 ‰), while actual $\Delta^{17}O$ values of soil NO_3^- were variable temporally and generally higher than +2.2 ‰, as you point out. This was the reason we concluded that GNR estimated by using Eq.6 was highly inaccurate and submitted this manuscript.

We added this information to the revised manuscript as follows (P9, L149-159 in the revised manuscript).

Note that the y-axes in the models were layers, not depths (Tables S1, S2, and S3). While the $\Delta^{17}O$ values of soil NO_3^- always showed decreasing trends with depths irrespective to the seasons, $\Delta^{17}O$ values of soil NO_3^- showed significant temporal variation at each depth (Hattori et al., 2019). This was the reason why the layers were adopted for the y-axes in our models, instead of depths. As a result, the specific depth of each layer varies over time. In addition, the relation between depth and layer is not always linear. The temporal variation found in the vertical distributions of $\Delta^{17}O$ values in the forested catchment (Hattori et al., 2019) can be explained by our model as well without contradiction because the $\Delta^{17}O$ values of soil NO_3^-, while showing large temporal variation at each depth, always showed decreasing trend with depth throughout their observation (Hattori et al., 2019).

3. No discussion on the nitrate resident time in the soil column. To have the model working requires GDR/uptake/GNR time scale less than the transport/diffusion time in each layer.

Thank you for the comment. As already discussed in past studies (Tsunogai et al., 2011; 2018), the GNR can be calculated from the isotopic mass balance (Eq. (3); $NO_3^-{}_{deposition} \times \Delta^{17}O(NO_3^-)_{atm} + GNR \times \Delta^{17}O(NO_3^-)_{nitrification} = NO_3^-{}_{leaching} \times \Delta^{17}O(NO_3^-)_{stream} + NO_3^-{}_{uptake} \times \Delta^{17}O(NO_3^-)_{uptake} + GDR \times \Delta^{17}O(NO_3^-)_{denitrification}$), so that the parameter of residence time in the soil column is not necessary for calculating GNR. This is the merit to determine $\Delta^{17}O$ of NO_3^- for those we can constrain the values of $\Delta^{17}O(NO_3^-)_{uptake}$ and $\Delta^{17}O(NO_3^-)_{denitrification}$.

4. The obtained GNR increases with depths (Figure 1e). Nitrification is minimal at low oxygen conditions. How significant is the nitrification in deep soils?

Because Hattori et al. (2019) didn't report the water flux for each soil layer, the linear variation in the leaching flux and $\Delta^{17}O$ values of soil NO_3^- used in the simulated calculations (Figure 1) is just one of the many possible vertical variations in forested catchments. Thus, the calculated vertical distribution of GNR was also one of many possible distributions.

Nevertheless, it is not surprising that the nitrification rates in deeper layers are comparable to those in surface layers in the forested catchments with high precipitation, because soil water is generally enriched in O_2 throughout the soil layers in such high precipitation area. For example, using the ^{15}N-pool dilution technique in three different forest soil layers (organic layer (surface layers), 0–10 cm depth, 10–40 cm depth) at Hoglwald Forest (Bavaria, Germany), Matejek et al. (2010) found active gross nitrification rate, up to 1600 ± 700 μmol N m^{-2} d^{-1} $layer^{-1}$ at the depths of 10–40 cm, while 600 ± 200 at the depths of 0–10 cm and 2000 ± 300 μmol N m^{-2} d^{-1} $layer^{-1}$ at the organic layer. Such active nitrification in deep layers in the literatures also implied that the vertical distribution of GNR estimated by using Eq. 6 (Figure 2e) was unrealistic, in which GNR should be concentrated only at the surface soil layers.

1. I believe NO3- flux reported is the flux at the layer boundary and GDR+uptake/GNR are in the layer. And so, the GDR+uptake/GNR unit should be kgN/ha/y/layer. Please clarify.

Thank you for your advice. We revised the units in Figures 1, 2, Tables S1, S2 and S3.

2. Figures 1 and 2 captions: I believe (b) and (c) are assumed, not simulated.

Thank you for your comment. We revised these in the revised manuscript.

3. Line 115: Different from …

Thank you for your comment. We revised the sentence in the revised manuscript as follows (P7, L113-115 in the revised manuscript).

Different from water environments, vertical mixing of water/soil is limited in forested soil, so the $\Delta^{17}O$ values of soil NO_3^- are often heterogeneous.

4. Line 173-175: A best is to do integral, not summation and to play with different D17O and nitrate flux gradients on the GNR.

Thank you for your comment. Compared to the summation, integration can enhance the precision of the simulated GNR. By dividing the forested soils into 10 layers, the result of integration for the simulated GNR for the forested catchment with the profile shown by Figure 1 was 11.2 kg of N ha^{-1} y^{-1} ($\int_0^{10} \frac{31.52-2.27x}{28-2.58x} - 0.44dx$).

We added this information to the revised manuscript as follows (P11, L190-192 in the revised manuscript).

Moreover, when we changed the calculation method from stepwise summation to integration, the estimated GNR was 11.2 kg N ha^{-1} y^{-1}.

Reference

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