

Dear Referee #2

Thank you very much for your valuable comments and questions on our manuscript. We would like to respond to each of your comments one by one.

I suggest that Ding et al. elaborate on the assumptions of their simulation. For example, is it realistic to assume that $\Delta^{17}\text{O}$ decreases linearly with depth? In this scenario, the nitrate (NO_3) flux down the soil profile would consist of mostly unprocessed atmospheric NO_3 (at least until halfway down the depth profile). This does not appear to align with the empirical data used in the simulation from Hattori et al.

Thank you for your question. First, note that the linear variation model used in the simulated calculation is one of the possible variations in the $\Delta^{17}\text{O}$ values of soil NO_3^- in forested catchments. It is impossible to decide whether the linear variation model was realistic until the downward water flux, together with the concentration and $\Delta^{17}\text{O}$ value of NO_3^- , is determined for each soil layer.

However, the simultaneous observations of the oxygen isotopes of soil NO_3^- and stream NO_3^- (Hattori et al., 2019; Osaka et al., 2010; Figs. 1 and 2) implied that the homogeneous model assumed in past studies, in which the $\Delta^{17}\text{O}$ values of soil NO_3^- were homogeneous and always equal to the mean $\Delta^{17}\text{O}$ value of stream NO_3^- as we explained in the submitted manuscript, was unrealistic. This is why we proposed the linear variation model as a possible alternative model for the simulated calculation.

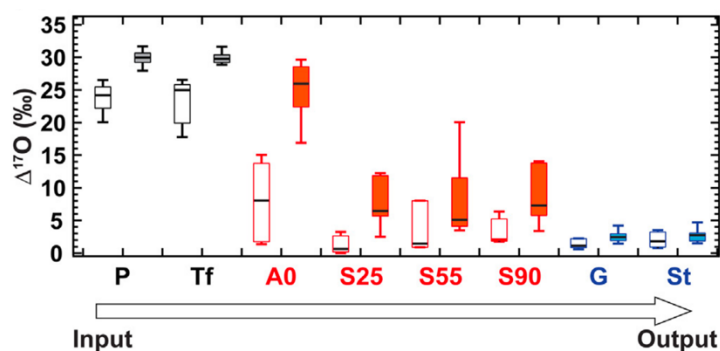


Figure 1. Vertical distribution of $\Delta^{17}\text{O}$ values of NO_3^- in precipitation, each soil layer (0 cm, 25 cm, 55 cm, and 90 cm), ground water, and stream water (Cited from Hattori et al., 2019).

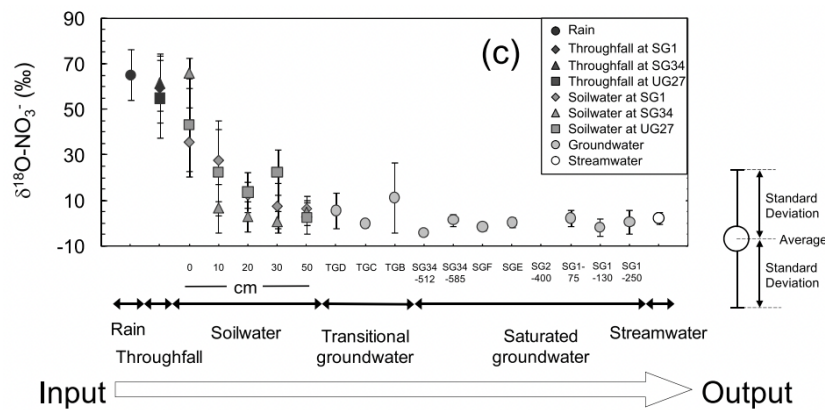


Figure 2. Vertical distribution of $\delta^{18}\text{O}$ values of NO_3^- in the rain, each soil layer (0 cm, 10 cm, 20 cm, 30 cm, and 50 cm), ground water, and stream water (Cited from Osaka et al., 2010).

How would the simulation results change if D17O dramatically decreased from deposition to the first soil depth used in the simulation? Existing data show that D17O of NO_3^- can be low in the uppermost soil horizons (<5 per mille in the upper 7 cm, Yu and Elliott 2018; 2 per mille +/- 1.1 per mille from 0-30 cm, Costa et al., 2011), which suggests that the assumption of a linear decrease of D17O from deposition to “water” (i.e., the lowest soil depth in the simulation; Figure 1) is not necessarily valid.

Thank you for your comment. In response to your request, we made a new simulated calculation in which the $\Delta^{17}\text{O}$ values of NO_3^- were decreased from +28.0 ‰ to +5.0 ‰ at the first layer and then gradually decreased with the subsequent nine layers, from +5.0 ‰ to +2.2 ‰ with a decrease rate of +0.31 ‰ for each step (Table 1). As a result, the simulated GNR (36.1 kg of N $\text{ha}^{-1} \text{y}^{-1}$) was significantly smaller than the GNR (83.6 kg of N $\text{ha}^{-1} \text{y}^{-1}$) calculated using Eq. 6, in which the $\Delta^{17}\text{O}$ values of NO_3^- in the forested soils were homogeneous.

Table 1. $\Delta^{17}\text{O}$ values of NO_3^- , leaching flux of NO_3^- , total metabolic rate of NO_3^- (GDR + uptake), and GNR in the simulated forested soil where the distribution of $\Delta^{17}\text{O}$ values of NO_3^- is heterogeneous with the values decreased from +28.0 to +5.0 ‰ at the first layer, and gradually decreased with the later 9 layers, from +5.0 ‰ to +2.2 ‰.

Depth layer	$\Delta^{17}\text{O}$ ‰	NO_3^- flux	GDR +uptake	GNR
kg of N ha ⁻¹ y ⁻¹				
0	28.0	7.0	0.0	0.0
1	5.0	6.6	32.6	32.2
2	4.7	6.1	0.9	0.4
3	4.4	5.7	0.9	0.4
4	4.1	5.2	0.9	0.4
5	3.8	4.8	0.9	0.4
6	3.4	4.4	0.9	0.4
7	3.1	3.9	0.9	0.4
8	2.8	3.5	0.9	0.4
9	2.5	3.0	0.9	0.4
10	2.2	2.6	0.9	0.4
11	2.2	2.6	0.0	0.0
Total				36.1

I also request the authors elaborate on the assumption that there is no biological processing in the water layer. Is this water layer the stream? Or is it intended to be the saturated zone of the soil? If it is the latter, is it reasonable to assume there is no processing along flowpaths between the saturated zone and the stream? How would the simulation results change if this assumption was violated?

The name of the final layer does not influence the estimated GNR. What we called the "water layer" in the submitted manuscript was the layer where the biological processing of NO_3^- was negligible. If the biological processing was significant in the water layer, it can be integrated into the other layers where biological processing was active.

While Ding et al. present a simulation that suggests inaccurate GNR estimates produced by the triple oxygen isotope approach at the watershed scale, I suggest the authors expand the scope of their premise to make it more broadly relevant. GNR, as calculated by soil scientists in soil cores (which has been done with D17O, Yu and Elliott 2018, along with many others using d15N), are not equal to gross nitrification rates as calculated at the watershed scale using streamwater NO3. I think this difference needs to be made clear and this manuscript represents a potential outlet to do so.

Thank you for your comments. Yu and Elliott (2018) sampled various soil cores for incubating with fertilizer NO_3^- enriched in ^{17}O to estimate GNR using the isotopic mass balance. Because the $\Delta^{17}\text{O}$ values of soil NO_3^- in the incubation bottles were homogeneous, the estimated GNR should be relevant. However, $\Delta^{17}\text{O}$ values of soil NO_3^- on the catchment scale were heterogeneous and different from the mean $\Delta^{17}\text{O}$ value of stream NO_3^- , as explained in our manuscript. Thus, the GNRs estimated on the catchment scale by the past studies were most likely inaccurate; hence, we should consider that significant errors were included in the estimated GNRs. We would like to add this to the revised manuscript. Thank you.

For example, if D17O is a conservative tracer, a decrease in D17O of NO_3 between deposition and streamwater requires addition of or dilution by terrestrial NO_3 , which has $\text{D17O} = 0$ (or approximately equal to 0). By this logic, Hattori's D17O data indicate that the streamwater NO_3 measured must have had terrestrial NO_3 added (i.e., nitrification) or have been diluted by terrestrial NO_3 along its flowpath from deposition to stream. I think this example illustrates the difference between GNR as measured in a soil core and GNR as measured at the watershed scale. Perhaps it would be more appropriate to give a different name to the watershed scale metric that can be calculated using eq. 6.

We could obtain an accurate estimated GNR of the forested catchment scale if we estimated the downward water flux and the concentration and $\Delta^{17}\text{O}$ value of NO_3^- in each soil layer, as presented in the submitted manuscript. If we can obtain such an accurate catchment scale GNR, the GNR will be comparable to that estimated from the core scale and then integrated into the catchment scale.

Specific comments:

-Lines 16-17: needs reference. Is NO_3 often limiting? Or NH_4 ? Or just N more generally?

Thank you for your comment. We would like to revise the sentence here as follows. Nitrate is one of the important nitrogen nutrients for primary production in forested ecosystems.

-Line 24: I suggest a different word than “determined”. Perhaps “quantified” or “estimated”

Thank you for your comment. We would like to use “estimated” here.

-Line 24: What is a “water environment”? A stream? A pond? A lake? Soil water?

Thank you for your comment. We would like to use “lake” here.

-Line 30-31: I suggest providing a range of D17O values of atmospheric NO₃. 26 per mille is a good average, but it can be much lower.

Thank you for your comment. We would like to add the range of $\Delta^{17}\text{O}$ values of atmospheric NO₃⁻ here.

-Line 32: What does “almost stable” mean?

Thank you for your question. It is not stable in the strict sense; however, it is stable on the per mil scale.

-Eq. 1: I suggest a different subscript for “water”. Readers might see “water” and think you are measuring the triple oxygen isotopes of O in H₂O.

Thank you for your question. We would like to use “ $\Delta^{17}\text{O}$ ” here.

-Line 46: Riha et al. 2014 was not a forested catchment. It was an urban watershed study.

Thank you for your comment. We would like to revise the reference.

-Line 54: reference for NO₃ being homogenous?

Thank you for your question. We would like to add the references (Tsunogai et al., 2011, 2018).

-Lines 95-99: Where were the soil data collected in relation to the stream for Hattori et al.?

Sampling points of soil water (shaded rectangle) and stream water (white inverted triangle) were shown in Fig. 3.

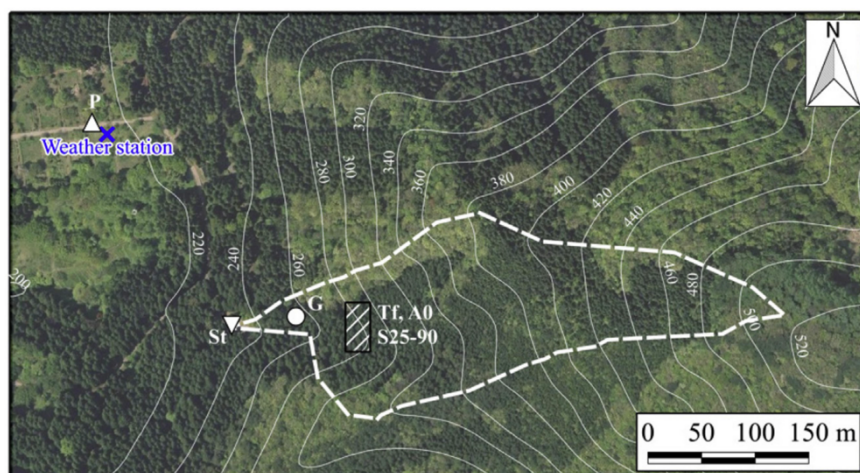


Figure 3. Map and sampling points (Cited from Hattori et al., 2019).

Is it reasonable to assume there was no processing along the flow paths from soil to stream?

Because the downward water flux has not been determined for each soil layer in the forested catchment, the accurate NO_3^- processing along the flow path from soil to stream is unknown. Again, the linear variation model is one of the possible variation models. We would like to emphasize this in the revised manuscript.

-Line 136: Are you missing a word at the end of this sentence? Perhaps add “sources” to the end.

Thank you for your question. We would like to revise the sentence as follows.

If we estimated the downward water flux at each soil layer, together with the concentration and $\Delta^{17}\text{O}$ value of NO_3^- in each soil layer using a tension-free lysimeter (Inoue et al., 2021), we could estimate the vertical changes in the leaching flux of NO_3^- for each soil layer together with the $\Delta^{17}\text{O}$ value of NO_3^- in each soil layer.

We would like to thank you for the helpful comments and questions. We hope that our responses to your comments are satisfactory.

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Reference

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Tsunogai, U., Miyauchi, T., Ohyama, T., Komatsu, D. D., Ito, M. and Nakagawa, F.: Quantifying nitrate dynamics in a mesotrophic lake using triple oxygen isotopes as tracers, *Limnol. Oceanogr.*, 63, S458–S476, doi:10.1002/lno.10775, 2018.

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