

Dear Referee #2

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

**The authors assume in Equation (4),**

$$\Delta^{17}\text{O}(\text{NO}_3)\text{uptake} = \Delta^{17}\text{O}(\text{NO}_3)\text{denitrification} = \Delta^{17}\text{O}(\text{NO}_3)\text{stream}.$$

**However, this assumption is not necessarily correct. It requires the assumption that nitrates deposited from the atmosphere are first diluted by nitrification (increasing nitrate amount with decreasing D<sup>17</sup>O) and then (i.e., “afterward”), reduced in nitrate amount without changing D<sup>17</sup>O by uptake and/or denitrification.**

Who assumed Eq. (4) were the authors of the papers in which Eq. (6) had been used to estimate GNR, such as Fang et al. (2015), Hattori et al. (2019), and Huang et al. (2020). While none of the authors clarified that they had assumed Eq. (4) in their papers, Eq. (4) should be needed to derive Eq. (6) from Eqs. (2) and (3), as we presented in lines from 74 to 106. In addition, we also presented that this assumption (Eq. (4)) is not necessarily correct (lines from 63 to 65 and from 129 to 132). In short, you have the same opinion with us at least on this point. In response to your comment, we would like to revise our manuscript to emphasize that who assumed Eq. (4) were the authors of the papers in which Eq. (6) had been used to estimate GNR.

**Another reverse possibility could be that atmospheric nitrates are reduced in quantity through uptake and/or denitrification without changing D<sup>17</sup>O, and then nitrates are added through nitrification (by decreasing D<sup>17</sup>O). In this assumption, one could hypothesize:**

$$\text{D}^{17}\text{O\_uptake} = \text{D}^{17}\text{O\_denitrification} = \text{D}^{17}\text{O\_atm} \quad (\text{A1}),$$

and calculate GNR as follows:

$$\text{GNR} = \text{NO}_3\text{\_st} \times (\text{D}^{17}\text{O\_atm} - \text{D}^{17}\text{O\_st}) / \text{D}^{17}\text{O\_st} \quad (\text{A2}).$$

To compare using Equation (4) versus Equations A1 and A2, let's assume a system where 100 nitrates (assuming D<sup>17</sup>O is 24‰) are initially deposited. In this case, when suppose the stream water nitrate is also 100 but with D<sup>17</sup>O decreased to 3‰. Using the same assumption as the authors (using Eq. 4 and 6), GNR is calculated as 700 using Equation (6) in the manuscript (GNR = 100 x (24-3)/3 = 700). However, assuming A1 and A2, GNR can be calculated as 87.5 (GNR = 100 x (24-3)/24 = 87.5), which is an extremely lower result compared to another case. Yet, in both outcomes, the final stream water remains the same at 100 in nitrate amount and 3‰ in D<sup>17</sup>O of nitrate from the same starting point (100 of nitrate with D<sup>17</sup>O = 24‰). It is necessary to find a converging point by differentiation, and it can be understood that this is the “heterogeneous” method assumed by the authors in the manuscript with 10 soil layers. In the above-mentioned case, a

**GNR of ~208 will be the case when considering production and consumption occur simultaneously, as far as I calculated briefly (dividing layers > 1000).**

The equation A2 you wrote may be a typo. Under the assumption of A1, A2 should be:

$$\text{GNR} = \text{NO}_3\text{-st} \times (\text{D17O}_\text{atm} - \text{D17O}_\text{st}) / \text{D17O}_\text{atm} \quad (\text{RA2})$$

The equation A1 can be possible for forested catchments in which possible variations in both the leaching flux and  $\Delta^{17}\text{O}$  values of soil  $\text{NO}_3^-$  were not determined for each soil layer. When we apply the equation RA2 to the forested catchment we used for the simulation (i.e. the forested catchment studied by Hattori et al., 2019), we obtain much smaller GNR of  $2.4 \text{ kg of N ha}^{-1} \text{ y}^{-1}$  ( $\text{GNR} = 2.6 \times (28-2.2)/28 = 2.4$ ) compared to the GNR calculated by using Eq. (6) ( $83.6 \text{ kg of N ha}^{-1} \text{ y}^{-1}$ ;  $\text{GNR} = 7.0 \times (28-2.2)/2.2 = 83.6$ ) that had been used in the literatures (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020). Thus, you reached the same conclusion with us that the GNR estimated from Eq. (6) using the  $\Delta^{17}\text{O}$  values of stream nitrate was, to some extent, an overestimate of the actual GNR (L11-15 and L185-190).

**In reality, production and consumption occur simultaneously. Therefore, both cases may overestimate or underestimate GNR to an extreme.**

Both nitrification and consumption (uptake + denitrification) of  $\text{NO}_3^-$  usually occur simultaneously in forested soil, as you pointed out. This is the reason we done a simulated calculation for the case shown in Figure 1 in the manuscript, in which both nitrification and consumption of  $\text{NO}_3^-$  occur simultaneously in the soil.

**Thus, authors should consider this case considering equations A1 and A2, in addition to the case considered in this study.**

Thank you for your advice. The aim of this paper is to clarify that the GNR estimated by using Eq. (6) was not the only GNR that can be expected in each forested catchment. Rather, the GNR estimated by using Eq. (6) often overestimate actual GNR to some extent. We trust that the case shown in Figure 1 is sufficient to accomplish our aim shown above.

**Additionally, the authors have limited their verification of GNR calculation overestimation in their manuscript (underestimation in the case of A1 and A2 in this review report) to the soil profile. However, if pointing out such overestimation in GNR calculation methods, it would be better to also consider similar considerations for N cycling rates (e.g., GNR) calculated for lake systems, as advanced by the authors' group. Hasn't there been an overestimation for similar reasons in studies using nitrogen cycling rates in Lake systems, as shown**

**in Tsunogai et al. (2011 and 2018) and other previous research? In lake and/or river studies, might they have calculated rates assuming that nitrates are added by nitrification (increasing the amount and decreasing D<sup>17</sup>O), and then the amount reduces by uptake and denitrification without changing D<sup>17</sup>O “only once” within each observation period unit (monthly or quarterly)? Wouldn’t both assumptions based on Equation 4 and those similar to A1 and A2 be equally valid? Assuming simultaneous production and consumption as in lake mass balance calculations, converging to a single value might provide a more reliable N cycle rate. It should also be pointed out that the authors' group's previous N cycling research may have been overestimated.**

Your claim on our studies applying the  $\Delta^{17}\text{O}$  tracer to water environments is wrong. In case of the water environments, differ from forested catchments, the  $\Delta^{17}\text{O}$  values of  $\text{NO}_3^-$  were mostly homogeneous in the water column due to the active vertical mixing in the water column during cold seasons and storm events, as explained in lines 58-59. Additionally, the homogeneity of the  $\Delta^{17}\text{O}$  values had been verified through actual observation prior to calculating GNR (Tsunogai et al., 2011, 2018). Furthermore, the extent of heterogeneities of the  $\Delta^{17}\text{O}$  values in the water column had been evaluated in calculating GNR etc., so that the calculated values of GNR were reported with the ranges of errors (Tsunogai et al., 2011, 2018). These are the essential differences between the past studies on the water environments and those on the forested catchments using Eq. (6) to estimate GNR.

**Especially, since Tsunogai et al. (2018) concluded that the nitrogen cycle rate was faster compared to  $^{15}\text{N}$  tracer experiments, which makes their study significant, it is important to consider the possibility of overestimation. Overall, this manuscript should consider and comment also on the case of their application for other systems like lake/river.**

Your understanding on Tsunogai et al. (2018) is wrong. Please note that the difference in the fluxes between the  $\Delta^{17}\text{O}$  method and the  $^{15}\text{N}$  tracer method estimated in a water environment by Tsunogai et al. (2018) was only 20 % on the annual base, while the difference in the forested catchment between the calculation methods was more than 500 %. In addition, Tsunogai et al. (2018) had discussed the reason for the difference (20 %) between the  $\Delta^{17}\text{O}$  method and the  $^{15}\text{N}$  tracer method in detail in the paper and concluded that the differences in the period of observation (instantaneous for the  $^{15}\text{N}$  tracer method vs. long-range average for the  $\Delta^{17}\text{O}$  method) were primarily responsible for the discrepancy so that the reason was essentially different from the discrepancy in the forested catchment. We don't see any merit in discussing the water environments again in this manuscript.

**Based on the above two major comments, here are some suggestions for the cases considered in this study:**

**1. Consider that the case of Equation (4) may not always be correct.**

As we already explained, those who assumed Eq. (4) were the authors of the papers in which Eq. (6) had been used to estimate GNR, such as Fang et al. (2015), Hattori et al. (2019), and Huang et al. (2020). We presented that this assumption (Eq. (4)) is not necessarily correct, in lines from 63 to 65 and from 129 to 132, so that you have the same opinion with us on this point. In response to your comment, we would like to revise our manuscript to emphasize that who assumed Eq. (4) were the authors of the papers in which Eq. (6) had been used to estimate GNR.

**Consider also the case assuming Equations A1 and A2 provided in this review report.**

As we already explained, the aim of this paper is to clarify that the GNR estimated by using Eq. (6) was not the only GNR that can be expected in each forested catchment. Rather, the GNR estimated by using Eq. (6) often overestimate actual GNR to some extent. We trust that showing the case Figure 1 is sufficient to accomplish our aim.

**2. Instead of comments using other group's case as an example, verify the calculation process and resulting GNR in the more general system.**

As presented in lines from 191 to 201, our conclusion is that it is impossible to estimate reliable GNR in each ecosystem (e.g., forested catchments, lakes, glaciers) in general using  $\Delta^{17}\text{O}$  as a tracer without measurement on the  $\Delta^{17}\text{O}$  values of  $\text{NO}_3^-$  actually consumed in each ecosystem. It is impossible to present the calculation process in the more general system without actual observation.

**3. Not only soil profile cases, but also consider possible changes for the other systems led by their research group (e.g., Tsunogai et al. 2011 Biogeos; Tsunogai et al. 2018 L&O).**

In case of the water environments, differ from forested catchments, the  $\Delta^{17}\text{O}$  values of  $\text{NO}_3^-$  were mostly homogeneous in the water column due to the active vertical mixing in the water column during cold seasons and storm events, as we explained in lines 58-59. Additionally, the homogeneity of the  $\Delta^{17}\text{O}$  values had been verified through actual observation prior to calculating GNR (Tsunogai et al., 2011, 2018). Furthermore, the extent of heterogeneities of the  $\Delta^{17}\text{O}$  values in the water column had been evaluated in calculating GNR etc., so that the calculated values of GNR were reported with the ranges of errors (Tsunogai et al., 2011, 2018). These are the

essential differences between the past studies on the water environments and those on the forested catchments using Eq. (6) to estimate GNR. We don't see any merit in discussing the water environments again in this manuscript.

**I also note that the current manuscript seems to criticize other groups' research, which may be due to language issues, so I want to avoid pointing out each by each in this review report. However, it might be worthwhile for the authors to reflect similar self-criticism on their group's previous nitrogen cycle research. To be honest, the current manuscript feels like an incomplete consideration that criticizes others' research one-sidedly. I also note that a similar modification of the calculation way for GNR based on D17O, considering both the production and consumption of nitrate simultaneously, has been already considered/published in another paper (Hattori et al. 2023).**

Because we found a problem in applying the  $\Delta^{17}\text{O}$  method to forested catchments by using Eq. (6) to estimate GNR as Fang et al. (2015) did, we just ignored and did not estimate GNR in our subsequent manuscripts studying forested catchments using  $\Delta^{17}\text{O}$  of  $\text{NO}_3^-$  as a tracer, such as Nakagawa et al. (2018) and Ding et al. (2022, 2023). Because no one pointed out the problem to use Eq. (6) in calculating GNR in forested catchments, however, apparently overestimated GNR by using Eq. (6) became "normal" in the papers subsequent to Fang et al. (2015) (Hattori et al., 2019, Huang et al., 2020), which seems to have reduced reliability of the  $\Delta^{17}\text{O}$  method. We trust this paper is worthy of publication in Biogeosciences to clarify the problem inherited in this method.

Concerning to Hattori et al. (2023), please note that the first preprint of our paper was published on 12 Jan 2023 (<https://bg.copernicus.org/preprints/bg-2022-236/>). Because this was 5 months earlier than the submission of Hattori et al. (2023), who should "consider" must be the authors of Hattori et al. (2023).

#### **Title: It is better to replace "error" with "bias"?**

Thank you for your advice. We would like to revise the "error" to "bias" in the revised manuscript.

#### **L149: Why 10 layers? If you consider fewer or more layers, do you expect any changes?**

Thank you for your questions. Dividing the forested soils into more layers can enhance the precision of the simulated GNR. By dividing the forested soils into 10, 20, 30, 50, 100, and 1000 layers, the simulated GNR was 13.0, 11.4, 11.0, 10.5, 10.3,

and 10.1 kg of N ha<sup>-1</sup> y<sup>-1</sup>, respectively. We would like to add this information to the revised manuscript.

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

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