Dear Referee #1

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

First, it is not necessary to assume that the distribution of 17O of nitrate along the soil profile is homogeneous or heterogeneous when apply the nitrate oxygen isotope method to forest soils. In fact, the assumption of the method is that the plants or microbes access the same nitrate source in forest soil with denitrifiers (Fang et al., 2015, PNAS). This assumpition is not identical to the assumption that the spatial distribution of 17O of nitrate along the soil profile is homogeneous, as demonstrated by the authors (Fig. 2).

Your understanding of the nitrate isotope method is wrong. The study by Fang et al. (2015) (and other subsequent studies listed in our submitted manuscript) estimated the GNR of forested catchments using Eq. 6:

$$GNR = {NO_3^-}_{deposition} \times (\Delta^{17}O(NO_3^-)_{atm} - \Delta^{17}O(NO_3^-)_{stream})/\Delta^{17}O(NO_3^-)_{stream} \quad (6)$$

where $\Delta^{17}O(NO_3^-)_{atm}$, $\Delta^{17}O(NO_3^-)_{stream}$, and ${NO_3^-}_{deposition}$ denote the $\Delta^{17}O$ value of ${NO_3^-}_{atm}$ deposited onto each catchment, the $\Delta^{17}O$ value of NO_3^- eluted from each catchment (stream NO_3^-), and the deposition flux of NO_3^- into each catchment, respectively. To estimate GNR for each forested catchment using Eq. 6, $\Delta^{17}O(NO_3^-)_{stream}$ should be equal to $\Delta^{17}O(NO_3^-)_{uptake}$ (the $\Delta^{17}O$ value of NO_3^- assimilated by plants) and $\Delta^{17}O(NO_3^-)_{denitrification}$ (the $\Delta^{17}O$ value of NO_3^- decomposed through denitrification), as explained in our submitted manuscript. That is, the studies that used Eq. 6 to estimate the GNR assumed Eq. 4 in the investigated forested catchments.

$$\Delta^{17}O(NO_3^-)_{stream} = \Delta^{17}O(NO_3^-)_{uptake} = \Delta^{17}O(NO_3^-)_{denitrification} \quad (4)$$

Because most metabolic reactions (GDR + uptake) of NO_3^- occurred in soil layers within forested catchments, the studies that estimated GNR using Eq. 6 assumed that soil NO_3^- was homogeneous at the $\Delta^{17}O$ values and equal to $\Delta^{17}O(NO_3^-)_{stream}$. None of them disclosed this assumption in their papers. Therefore, to clarify this assumption together with its significant impact on the final GNR estimated, we presented GNRs estimated for a forested catchment in which the $\Delta^{17}O$ values of NO_3^- in soil layers had been clarified.

Second, it is not correct to assume that the distribution of 17O of nitrate along the soil profile is homogeneous (Fig. 2). 17O has been rarely measured along the soil profile.

We agree that the $\Delta^{17}O$ values of soil NO_3^- are not always homogeneous in forested catchments. Nevertheless, the study by Fang et al. (2015) and the subsequent studies used Eq. 6, in which the $\Delta^{17}O$ values of soil NO_3^- were assumed to be homogeneous

and always equal to $\Delta^{17}O(NO_3^-)_{stream}$, to estimate GNR in each forested catchment, as explained above. As a result, we submitted this manuscript to disclose this critical issue in their estimated GNRs.

The one and only study shows a sharp decrease in 17O of nitrate in the top soil and remains relatively constant in the soil from 25 to 95 cm (Hattori et al., 2019, Sci. Total Environment). Thus, the assumption made by the author was not supported the field observation.

First, the accurate vertical distribution of downward NO_3^- flux had not been clarified in the forested catchment because Hattori et al. (2019) did not monitor the downward water flux in the forested catchment. Thus, in the submitted manuscript, we did not propose any model that represents an accurate vertical distribution of both downward NO_3^- flux and $\Delta^{17}O$ values of soil NO_3^- in the forested catchment studied by Hattori et al. (2019). The linear variation model for both downward flux and the $\Delta^{17}O$ values of soil NO_3^- adopted in the simulated calculation is one of the possible variations in soil NO_3^- in the forested catchment studied by Hattori et al. (2019). The homogenous model could be the case as well. Still, the $\Delta^{17}O$ data of soil NO_3^- reported by Hattori et al. (2019), in which >80% of soil nitrate showed $\Delta^{17}O$ values higher than those in the stream eluted from the catchment (+2.2‰ on average; Fig. 1), implying that the estimated GNR using Eq. 6, in which the $\Delta^{17}O$ values of NO_3^- metabolized in soil layers were assumed to be always equal to $\Delta^{17}O(NO_3^-)_{stream}$, was most likely inaccurate in the forested catchment.

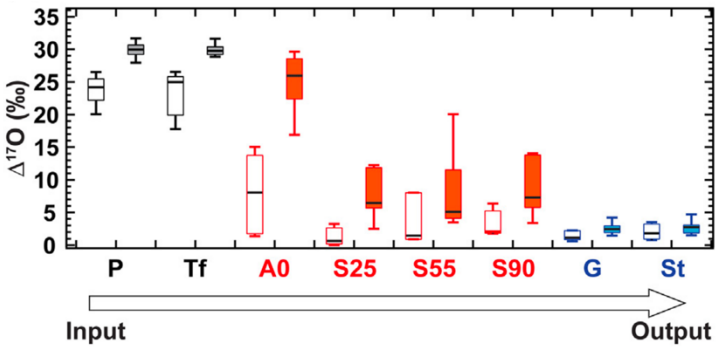

Figure 1. Vertical distribution of $\Delta^{17}O$ values of NO_3^- in precipitation, each soil layer (0 cm, 25 cm, 55 cm, and 90 cm), ground water, and stream water. Open symbols denote values for summer (June–September), and solid figures denote values for winter (January–April). Box-plot black lines indicate the mean values. Box-plot Box-plot lower and upper boundaries indicate the lower (25%) and upper (75%) quartiles of data in each component, respectively. Whiskers denote the minimum and maximum values reported in each component. The white arrow represents the flux of NO_3^- inputs and outputs in the ecosystem (Cited from Hattori et al., 2019).

Third, I agree that it is the distribution of 17O of nitrate along the soil profile is highly hetrogeneous, as nitrification is dominant in surface soils, and deposited nitrate may enter soil from the forest floor. However, it is not correct to assume that 17O of nitrate decreased linearly with soil depth (Fig. 1).

The linear variation in the $\Delta^{17}O$ values and the downward flux of soil NO_3^- in the simulated calculation are possible variations in soil NO_3^- in the forested catchment. It is impossible to decide whether the linear variation model was correct until the downward water flux, together with the concentration and $\Delta^{17}O$ values of soil NO_3^-, is determined for each soil layer, as previously explained.

The field observation by Hattori et al did not support this assumption. This may be main reason for unrealstically low gross nitrification rate (13 kg N/ha.yr) as calculated by the authors, in the study forest with modate to high N deposition (16 kg N.ha.yr). Nitrification must be strongly active in this forest, which was supported by high soil nitrate concentrations and a large seasonal varation in 15N and 17O of nitrate (Fig. 3 of Hattori et al.).

Again, the linear variation in the $\Delta^{17}O$ values and the downward flux of soil NO_3^- in the simulated calculation are one of the possible variations in soil NO_3^- in forested catchments. The estimated GNR (13.0 kg of N ha^{-1} y^{-1}) was also one of the possible values. It is impossible to decide whether the linear variation model was realistic until the downward water flux, together with the concentration and $\Delta^{17}O$ values of soil NO_3^-, is determined for each soil layer, as previously explained.

Concerning the concentrations of soil nitrate in the forested catchment reported by Hattori et al. (2019), note that the concentration was not so high, at least in Japanese forested catchments. While the mean concentrations of soil nitrate ranged from 0.8 to 2.3 mg/L (from 13 to 37 uM; Fig. 2) in the study by Hattori et al. (2019), they were 398 uM in the KJ catchment (Nakagawa et al., 2018) and 51 uM in the Matsuzawa catchment (Osaka et al., 2010). Values of >100 uM have also been reported in OYS-O, OYS-M, and TM catchments in Japan (Fang et al., 2015).

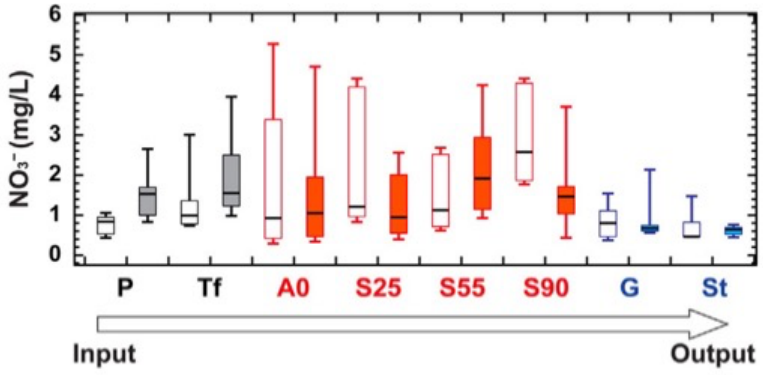

Figure 2. Vertical distribution of NO_3^- concentration in precipitation, each soil layer (0 cm, 25 cm, 55 cm, and 90 cm), ground water, and stream water. Box-plot black lines indicate the mean values (Cited from Hattori et al., 2019).

In fact, the nitrate oxgen isotope method admit high heterogeneity of soil nitrfication in both spatially and seasonally. And it is difficult and almost impossible to capture these heterogeneities. **However, these heterogeneities can be integrated to streamwater. The nitrate oxygen isotope method takes this advantage of it.**

Your understanding of the nitrate isotope method is wrong. As presented in the submitted manuscript, the deviations in the $\Delta^{17}O$ values of NO_3^- consumed actually through metabolic reactions (especially uptake reactions) from the mean $\Delta^{17}O$ values of stream NO_3^- significantly impacted the estimated GNR. Considering that most of the root biomass is concentrated in the top 10 cm of soils in forested catchments (Jackson et al., 1996), most uptake reactions should occur at the top 10 cm of soil layers and not in the stream. Therefore, possible heterogeneity in the $\Delta^{17}O$ values of soil NO_3^- in forested catchments should be considered in estimating GNR. Furthermore, although oxygen isotopes of soil NO_3^- in forested catchments have rarely been measured along the soil profile, all studies (in which oxygen isotopes were determined for both soil NO_3^- and stream NO_3^- simultaneously) showed that the oxygen isotopes of soil NO_3^- were significantly deviated from those of the stream NO_3^- (Hattori et al., 2019; Osaka et al., 2010; Nakagawa et al., 2018), implying that the GNRs of forested catchments estimated using Eq. 6 were inaccurate.

Using Eq. 6 to estimate GNR in forested catchments, it is necessary to verify that the $\Delta^{17}O$ values of soil NO_3^- were always equal to those of stream NO_3^- or to estimate the possible range of errors.

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

Sincerely,
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