Bias ~~associated with~~in calculating ~~the~~ gross nitrification rates in forested catchments using the triple oxygen isotopic composition ($\Delta^{17}O$) of stream nitrate

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Abstract

A novel method has been proposed and applied in recent studies to quantify~~for quantifying the~~ gross nitrification rate (GNR) in forested catchments using the triple oxygen isotopic composition ($\Delta^{17}O$) of stream nitrate~~ leached from the catchments has been proposed and applied in recent studies~~. However, the equations used in ~~the~~ these calculations ~~include the approximation~~assume that the $\Delta^{17}O$ value of nitrate consumed through assimilation or denitrification in ~~the forested soil~~forest soils is equal to the $\Delta^{17}O$ value of stream nitrate. The GNR estimated from the $\Delta^{17}O$ value of stream nitrate was ~~more than six times~~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. ~~The $\Delta^{17}O$ values of the soil nitrate decreased with an increase in depth to that of the stream nitrate at the bottom. Most~~ Because most of the reported soil nitrate in forested catchments showed $\Delta^{17}O$ values higher than those of the stream nitrate~~ leached from the catchments. Thus~~, we concluded that the GNR estimated from the $\Delta^{17}O$ value of stream nitrate~~ in the forested catchments~~ was, to an extent, an overestimate of the actual GNR.

1 Introduction

Nitrate (NO_3^-) is an important nitrogen nutrient for primary production in ~~forested ecosystems~~soils. Nitrification is the microbial process that produces NO_3^- in forested ecosystems. Thus, quantifying the nitrification rate can assist in the evaluation of the present and future states of forested ecosystems. The net nitrification rate can be estimated from an increase in NO_3^- concentration during a certain period. However, the gross nitrification rate (GNR), which includes the ~~(~~net nitrification rate plus the~~+~~ consumption rate of NO_3^- (e.g., ~~that assimilated by plants or decomposed~~ through plant assimilation or denitrification)~~)~~, reflects the internal N cycling better than the net nitrification rate (Bengtsson et al., 2003), especially in forested ecosystems. Although the net nitrification rate is often negligible (Stark and Hart, 1997), the consumption rate is significant in forested ecosystems, such that the GNR often exceeds the net nitrification rate by several orders of magnitude (Verchot et al., 2001).

~~Recently, several~~Recent studies have successfully estimated the GNR in ~~water~~ aquatic environments, such as lakes, using the $\Delta^{17}O$ values of NO_3^-~~,~~ as a conservative tracer ~~of~~ to determine the mixing ratio between atmospheric nitrate ($NO_3^-{}_{atm}$) and biologically produced nitrate ($NO_3^-{}_{bio}$) (Tsunogai et al., 2011, 2018). The $NO_3^-{}_{atm}$ is deposited in the water environment, ~~and the~~while $NO_3^-{}_{bio}$ is produced through nitrification. The $NO_3^-{}_{bio}$ always shows the $\Delta^{17}O$ value close to 0 ‰ because its oxygen atoms are derived from either terrestrial O_2 or H_2O through nitrification. Contrarily, the $NO_3^-{}_{atm}$ always displays an anomalous enrichment in ^{17}O with $\Delta^{17}O$ value being approximately +26 ± 3 ‰ in Japan (Tsunogai et al., 2010, 2016; Ding et al., 2022, 2023) because of oxygen transfers from atmospheric ozone (Michalski et al., 2003; Nelson et al., 2018). Additionally, $\Delta^{17}O$ is almost stable during "mass-dependent" isotope fractionation processes (Michalski et al., 2004; Tsunogai et al., 2016). This is because possible variations in the $\delta^{17}O$ and $\delta^{18}O$ values during the processes of biogeochemical isotope fractionation follow the relation of $\delta^{17}O \approx 0.5\ \delta^{18}O$, which cancels out the variations in the $\Delta^{17}O$ value. Thus, regardless of the partial consumption through denitrification or assimilation after deposition in a water column, the $\Delta^{17}O$ can be used as a conservative tracer of $NO_3^-{}_{atm}$ to calculate the mixing ratio of $NO_3^-{}_{atm}$ to total NO_3^- ($NO_3^-{}_{atm}/NO_3^-{}_{total}$) in a water column using the following equation:

$$[NO_3^-{}_{atm}]/[NO_3^-{}_{total}] = [NO_3^-{}_{atm}]/([NO_3^-{}_{bio}] + [NO_3^-{}_{atm}]) = \Delta^{17}O/\Delta^{17}O_{atm} \qquad (1)$$

where the $\Delta^{17}O_{atm}$ and $\Delta^{17}O$ denote the $\Delta^{17}O$ values of $NO_3^-{}_{atm}$ and NO_3^- dissolved in the water environment, respectively. Using the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratio estimated from the $\Delta^{17}O$ value of NO_3^- in a lake water column and the deposition rate of $NO_3^-{}_{atm}$ into the lake, the GNR (i.e., production rate of $NO_3^-{}_{bio}$) ~~was~~ can be successfully estimated. This ~~is~~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 partial consumption of NO_3^- has little influence on the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratio in~~ the lake water column (Tsunogai et al., 2011, 2018).

In addition to applications in water environments, the $\Delta^{17}O$ method has been applied to forested catchments to determine ~~their~~ GNR (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020). Using the deposition flux of $NO_3^-{}_{atm}$ into the catchment and the leaching flux of unprocessed $NO_3^-{}_{atm}$ and $NO_3^-{}_{bio}$ ~~from~~ via streams, the GNR in a forested catchment was estimated similarly to the estimation for water environments (Fang et al., 2015). ~~Contrary to~~However, unlike in water environments, where the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratio of nitrate consumed in the water column ~~the $\Delta^{17}O$ values of NO_3^- in the water layers are homogeneous in the water column due to the active vertical mixing of water and~~ can be easily measured ~~easily~~, it is often difficult to determine the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratio~~$\Delta^{17}O$ values~~ of NO_3^- consumed in soil layers. Consequently, past studies have approximated ~~the~~ these values ~~to be~~as equal to ~~the $\Delta^{17}O$ value~~those of stream NO_3^- leached from forested catchments without actual observation (Fang et al., 2015, Hattori et al., 2019, Huang et al., 2020). ~~However, such~~Such an approximation should be ~~conducted~~ used with extreme caution, as the $NO_3^-{}_{atm}/NO_3^-{}_{total}$ ratio (~~-~~$\Delta^{17}O$ values) of soil NO_3^- are not always equal to those of stream~~s~~ NO_3^- (Hattori et al., 2019, Rose, 2014, Nakagawa et al., 2018). To clarify the details of the approximation and its impact on the final estimated GNR, we present an accurate relationship between the $\Delta^{17}O$ of soil NO_3^- and the GNR, using basic isotope mass balance equations. Thereafter, we present possible range of variation in the ~~estimated~~ GNRs estimated for a forested catchment~~whose~~, using parameters such as $\Delta^{17}O$ values of ~~soil~~ stream NO_3^- ~~were~~reported in a past study ~~measured~~. Finally, we compared the GNRs estimated in this study with ~~the GNR estimated~~those obtained from the $\Delta^{17}O$ values of stream NO_3^-.

2 Calculation

The total mass balance equation of NO_3^- including the GNR in catchments can be expressed as follows:

$$NO_3^-{}_{deposition} + GNR = NO_3^-{}_{leaching} + NO_3^-{}_{uptake} + GDR \quad (2)$$

where $NO_3^-{}_{deposition}$, GNR, $NO_3^-{}_{leaching}$, $NO_3^-{}_{uptake}$, and GDR denote the deposition flux of NO_3^- into the catchment, GNR in the catchment, leaching flux of NO_3^- from the catchment, uptake rate of NO_3^- in the catchment, and gross denitrification rate in the catchment, respectively.

The isotope mass balance for each $\Delta^{17}O$ value of NO_3^- in the catchment can be expressed using a similar equation:

$$NO_3^-{}_{deposition} \times \Delta^{17}O(NO_3^-)_{atm} + GNR \times \Delta^{17}O(NO_3^-)_{nitrification} = NO_3^-{}_{leaching} \times \Delta^{17}O(NO_3^-)_{stream} + NO_3^-{}_{uptake} \times \Delta^{17}O(NO_3^-)_{uptake} + GDR \times \Delta^{17}O(NO_3^-)_{denitrification} \quad (3)$$

where $\Delta^{17}O(NO_3^-)_{atm}$, $\Delta^{17}O(NO_3^-)_{nitrification}$, $\Delta^{17}O(NO_3^-)_{stream}$, $\Delta^{17}O(NO_3^-)_{uptake}$, and $\Delta^{17}O(NO_3^-)_{denitrification}$ denote the $\Delta^{17}O$ value of $NO_3^-{}_{atm}$ deposited into the catchment, that of the $NO_3^-{}_{bio}$ produced through nitrification, that of the NO_3^- leached from the catchment, that of the NO_3^- assimilated by plants and other organisms in the catchment, and that of the NO_3^- decomposed through denitrification in the catchment, respectively.

If the $\Delta^{17}O$ values of the NO_3^- in the forested soil layers, where the NO_3^- was consumed through assimilation or denitrification, are equal to the $\Delta^{17}O$ values of NO_3^- in the stream, we could obtain Eq. 4:

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

Consequently, by combining Eqs. 3 and 4, we could obtain Eq. 5:

$$NO_3^-{}_{deposition} \times \Delta^{17}O(NO_3^-)_{atm} + GNR \times \Delta^{17}O(NO_3^-)_{nitrification} = (NO_3^-{}_{leaching} + NO_3^-{}_{uptake} + GDR) \times \Delta^{17}O(NO_3^-)_{stream} \quad (5)$$

We could estimate the GNR using Eq. 6 obtained from Eqs. 2 and 5 because we can approximate the $\Delta^{17}O$ values of $NO_3^-{}_{bio}$ produced through nitrification ($\Delta^{17}O(NO_3^-)_{nitrification}$) to 0 (Michalski et al., 2003; Tsunogai et al., 2010):

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

Eq. 6 corresponds to the equations used in previous studies to quantify the GNR in the forested catchments (Eq. 4 in Fang et al., 2015; Eq. 8 in Hattori et al., 2019; Eq. 4 in Huang et al., 2020).

3 Results and Discussion

The $\Delta^{17}O$ values of NO_3^- in forested soil layers should be equal to those of stream NO_3^- in Eq. 6, as presented in Eq. 4 to obtain Eq. 6. While the number of simultaneous observations of the oxygen isotopes of NO_3^- in the soil and stream in a given forested catchment is limited (Hattori et al., 2019, Osaka et al., 2010, Rose, 2014, Nakagawa et al., 2018), the observations showed that the oxygen isotopic ratios of soil NO_3^- are often heterogeneous. In addition, the oxygen isotopic ratios of soil NO_3^- mostly exceeded those of stream NO_3^-. mostly exceed those of stream NO_3^-. Differ from water environments, vertical mixing of water/soil is difficult in forested soil, so the $\Delta^{17}O$ values of soil NO_3^- are often heterogeneous. For example, Hattori et al. (2019) found a decreasing $\Delta^{17}O$ trend in soil NO_3^- with depth, ranging from over +20 ‰ at the surface to less than +3 ‰ at depths of 25–90 cm.~~Hattori et al. (2019) reported that over~~ Additionally, more than 60 % of the soil samples exhibited $\Delta^{17}O$ values significantly higher than those of stream NO_3^- determined simultaneously ($\Delta^{17}O(NO_3^-)_{stream}$ = +1 to +3 ‰). ~~In addition, they found a decreasing $\Delta^{17}O$ trend in soil NO_3^- with depth, declining from greater than +20 ‰ at the surface to less than +3 ‰ at depths of 25–90 cm from the surface.~~ A similar trend in the vertical distribution was observed in the $\delta^{18}O$ values of NO_3^- in ~~another~~another forested catchment, from above +35 ‰ at the surface soil to less than +10 ‰ at depths of 30–50 cm from the soil surface (Osaka et al., 2010). In addition, most of the soil NO_3^- also exhibited $\delta^{18}O$ values higher than those of the stream NO_3^- (Osaka et al., 2010). Rose (2014) monitored the horizontal distribution of the $\Delta^{17}O$ of soil NO_3^- by randomly setting 15 tension-free lysimeters at depths of 0–10 cm in a 39-ha forested catchment. They reported significantly higher $\Delta^{17}O$ values in soil NO_3^- (+9.1 ± 5.8 ‰ on average) than those of stream NO_3^- (+0.5 ‰ on average) leached from the forested catchment. As most fine roots and root biomass are concentrated in the top 10 cm of the soil in forested catchments (Jackson et al., 1996; Li et al., 2020), most assimilation (uptake reactions) of NO_3^- should occur in that top 10 cm of soil. Consequently, the significant difference in the $\Delta^{17}O$ values between soil NO_3^- and stream NO_3^-, particularly in surface soil layers, implies that the estimated GNRs in forested catchments obtained from Eq. 6 were inaccurate.

To demonstrate the impact of this approximation on GNR estimation, we simulated GNR for two different forest soils within the same catchment. In the first scenario, soil NO_3^- exhibited a $\Delta^{17}O$ value close to that of $\Delta^{17}O(NO_3^-)_{atm}$ at the surface, which decreased to the $\Delta^{17}O$ of stream NO_3^- at depth (heterogeneous soil) (Figs. 1a and 1b). In the second scenario, soil NO_3^- had $\Delta^{17}O$ values equal to those of stream NO_3^- throughout the soil profile (homogeneous soil) (Figs. 2a and 2b).

To simulate the forested catchment studied by Hattori et al. (2019), we used the same parameters values for the current calculation, including 7.0 kg N ha^{-1} y^{-1} for $NO_3^-{}_{deposition}$, 2.6 kg N ha^{-1} y^{-1} for $NO_3^-{}_{leaching}$, +28.0 ‰ for $\Delta^{17}O(NO_3^-)_{atm}$, and +2.2 ‰ for $\Delta^{17}O(NO_3^-)_{stream}$. All symbols (e.g., GNR) are consistent with those used by Hattori et al. (2019).

To estimate GNR in each forest soil type, we divided the soils into 10 vertical layers (i.e., 10 steps). In the heterogeneous soil, the Δ17O values of NO_3^- gradually decreased with depth, from +28.0‰ to +2.2‰, at a rate of −2.58‰ per step (Fig. 1b). In the homogeneous soil, $\Delta^{17}O$ values of NO_3^- were constant at +2.2‰ across all layers (Fig. 2b). 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).

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).

Applying the total mass balance and isotope mass balance equations (Eqs. 2 and 3) to each layer, we estimated GNR (Figs. 1e and 2e) and the total consumption rate of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer. In this calculation, we assumed the following: (1) $\Delta^{17}O$ values of NO_3^- were constant in each layer; (2) vertical flow of NO_3^- in soil layers proceeds downward from the surface to the final layer (No. 10);

and (3) GNR and the NO_3^- consumption rate (GDR + uptake) are 0 in layers beyond the final layer. By summing the GNR determined for each layer, we estimated the total GNR in the forested catchment.

To demonstrate the impact of the differences in the $\Delta^{17}O$ values of soil NO_3^- and stream NO_3^- on the GNR and present the problems associated with the approximation to obtain Eq. 6, we estimated the GNR of two simulated forested soils. The $\Delta^{17}O$ of the first soil with NO_3^- decreased to the $\Delta^{17}O$ of the stream NO_3^- (heterogeneous soil) (Figs. 1a and 1b). The second soil with NO_3^- showing the same $\Delta^{17}O$ values as those of the stream NO_3^- (homogeneous soil) (Figs. 2a and 2b). Hattori et al. (2019) reported the $NO_3^-{}_{deposition}$ as 7.0 kg of N ha^{-1} y^{-1}, $NO_3^-{}_{leaching}$ as 2.6 kg of N ha^{-1} y^{-1}, $\Delta^{17}O(NO_3^-)_{atm}$ as +28.0 ‰, and $\Delta^{17}O(NO_3^-)_{stream}$ as +2.2 ‰ in the forested catchment they studied. We adopted the same values in the present calculation to simulate the same forested soil. All the symbols (e.g., GNR) used here were consistent with those of Hattori et al. (2019).

To estimate the GNR, we divided the forest soils into 10 layers (i.e., 10 steps) in the vertical direction. The $\Delta^{17}O$ values of NO_3^- gradually decreased with an increase in depth, varying from +28.0 to +2.2 ‰ with a rate of −2.58 ‰ for each step (Fig. 1b). Similarly, we assumed a gradual decrease with an increase in depth in the leaching flux of NO_3^-, (from 7 to 2.6 kg of N ha^{-1} y^{-1} at a rate of −0.44 kg of N ha^{-1} y^{-1} per step) (Fig. 1c). This simulated the gradual net consumption of NO_3^- in accordance with water flow in forested soils. The homogeneous soil was also divided into 10 layers in the vertical direction. The change in the leaching flux of NO_3^- with depth was the same as that in the heterogeneous soil (Fig. 2c), whereas the $\Delta^{17}O$ values of NO_3^- were constant at +2.2 ‰ in the layers (Fig. 2b).

Applying the total mass balance and isotope mass balance of NO_3^- shown in Eqs. 2 and 3 to each layer, we estimated the GNR (Figs. 1e and 2e) and total consumption rate of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer. We assumed the following. (1) The $\Delta^{17}O$ values of NO_3^- were constant in each layer. (2) The vertical flow of NO_3^- in the soil layers proceeded downward from the surface to the final layer (No. 10) with a uniform residence time in each layer. Finally, (3) the GNR and consumption rate of NO_3^- (GDR + uptake) was 0 in the water layer. Thereafter, by integrating the GNR determined for each layer, we estimated the total GNR in the forested catchment.

The total GNR estimated for the catchment with the homogeneous $\Delta^{17}O$ values in soil NO_3^- (homogeneous soil) was 83.6 kg of N ha^{-1} y^{-1} (Fig. 2e), which was exactly equal to that estimated by Hattori et al. (2019) using Eq. 6. 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^-. However, the total GNR estimated for the catchment with the heterogeneous $\Delta^{17}O$ values in soil NO_3^- (heterogeneous soil) was considerably lower (13.0 kg of N ha^{-1} y^{-1}; Fig. 1e), while the same parameters with the homogeneous $\Delta^{17}O$ values in soil NO_3^- were used for $NO_3^-{}_{deposition}$, $NO_3^-{}_{leaching}$, $\Delta^{17}O(NO_3^-)_{atm}$, and $\Delta^{17}O(NO_3^-)_{stream}$.

As we increased the number of layers in the forest soils to 20, 30, 50, 100, and 1000, the estimated GNR for the heterogeneous soil decreased to 11.4, 11.0, 10.5, 10.3, and 10.1 kg N ha^{-1} y^{-1}, respectively. Moreover, when we changed the calculation method from stepwise summation to integration, the estimated GNR was 11.2 kg N ha^{-1} y^{-1}. Even if the number of layers in the forested soils was increased to 20, 30, 50, 100, and 1000 to enhance the precision of the GNR simulated for the catchment with the heterogeneous soil, the GNR was 11.4, 11.0, 10.5, 10.3, and 10.1 kg of N ha^{-1} y^{-1}, respectively. Consequently, we concluded the following. (1) Past studies estimated the GNR using Eq. 6 approximated the $\Delta^{17}O$ values of soil NO_3^- consumed in the forested catchments were homogeneous and always equal to that of stream NO_3^-. (2) The differences between the $\Delta^{17}O$ values of the soil NO_3^- consumed in a forested catchment and that of stream NO_3^- resulted in a significant deviation in the GNR estimated using Eq. 6 from the actual GNR. 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.

By combining the total mass balance and isotope mass balance shown in Eqs. 2 and 3, Eq. 7 was obtained to accurately estimate the total GNR:

$$GNR = NO_3^-{}_{leaching} - NO_3^-{}_{deposition} + (NO_3^-{}_{deposition} \times \Delta^{17}O(NO_3^-)_{atm} - NO_3^-{}_{leaching} \times \Delta^{17}O(NO_3^-)_{stream}) / \Delta^{17}O(NO_3^-)_{soil} \quad (7)$$

where $\Delta^{17}O(NO_3^-)_{soil}$ denotes the "average" $\Delta^{17}O$ of NO_3^- consumed through assimilation or denitrification in the forested catchment. Most of the soil NO_3^- measured to date exhibited $\Delta^{17}O$ values higher than those of stream NO_3^- leached from the catchments (Hattori et al., 2019, Rose, 2014). Consequently, the total GNR estimated from stream NO_3^- using Eq. 6 exceeded the total GNR estimated from soil NO_3^- using Eq. 7, to an extent. Therefore, the total GNR estimated from Eq. 6 was overestimated to an extent.

~~The linear variation in the leaching flux and $\Delta^{17}O$ values of soil NO_3^- used in the simulated calculations (Fig. 1) is just one of many possible variations in forested catchments. It is impossible to determine whether the linear variation was realistic or~~

not until the downward water flux, along with the concentration and $\Delta^{17}O$ value of NO_3^-, was 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; Nakagawa et al., 2018; Rose, 2014) implied that the approximation of the $\Delta^{17}O$ values of soil NO_3^- to that of the stream NO_3^- (Fig. 2b) was unrealistic.

If we can estimate the downward water flux at each soil layer, along with the NO_3^- concentration and $\Delta^{17}O$ value of NO_3^- in each soil layer using, e.g., a tension-free lysimeter (Inoue et al., 2021), we could estimate the vertical change in the NO_3^- leaching flux of NO_3^- for each soil layer, along with the $\Delta^{17}O$ values of soil NO_3^-. Thereafter, applying Eq. (7) to each layer, we can more accurately estimate the total GNR for the forested catchment accurately, by integrating the GNR estimated for each soil layer, together with a more accuratethe NO_3^- consumption rate inof the forested catchment. Without such an observation of the distribution and leaching flux of NO_3^-, with the $\Delta^{17}O$ values in forest soil, the GNR estimated using Eq. (6), assuming that the $\Delta^{17}O$ values of soil NO_3^- are always equal to those of stream NO_3^-, should be reported with significant errors in which the possible variations in the $\Delta^{17}O$ values of soil NO_3^- are considered.

4 Conclusion

Past studies have proposed the $\Delta^{17}O$ method for determining the GNR in forested catchments. The equations used in the calculation presuppose implicitly assumed that the $\Delta^{17}O$ values of NO_3^- consumed in forested soils are homogeneous and equal to those of the stream NO_3^-. However, ~~in reality,~~ the values are often heterogeneous and do not always equal those of the stream in forested soils. It is essential to clarify/verify the $\Delta^{17}O$ values of NO_3^- in forested soils and streams before applying the $\Delta^{17}O$ values of stream NO_3^- to estimate the total GNR.

Data availability. All data are presented in the Supplement.

Author contributions. WD, UT, and FN designed the study. WD and UT performed data analysis and wrote the paper.

Competing interests. The authors declare that they have no conflict of interest.

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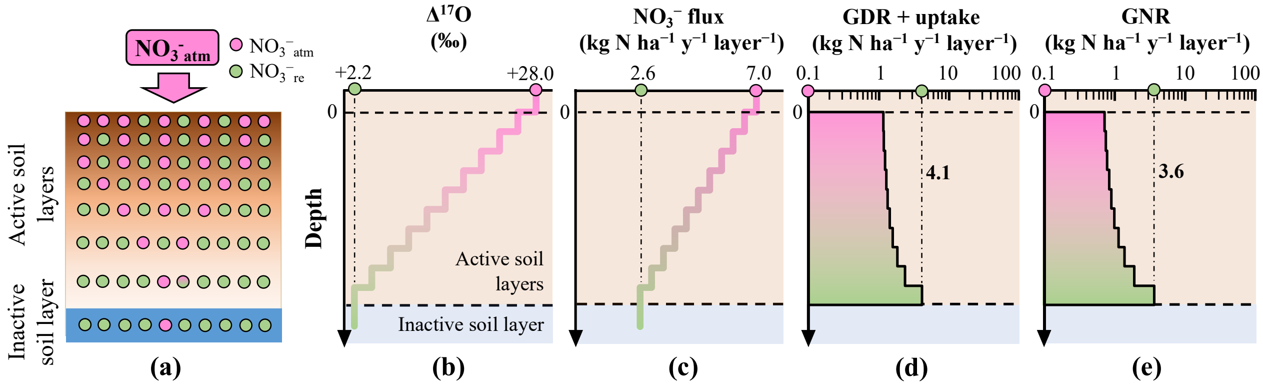

Figure. 1. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil with heterogeneous distribution of $\Delta^{17}O$ values of NO_3^- (a). Vertical distribution of the following parameters in the forested soil: ~~simulated~~ assumed $\Delta^{17}O$ values of NO_3^- (b), ~~simulated~~ assumed leaching flux of NO_3^- (c), estimated NO_3^- consumption rate (GDR + uptake) (d), and estimated GNR (e).

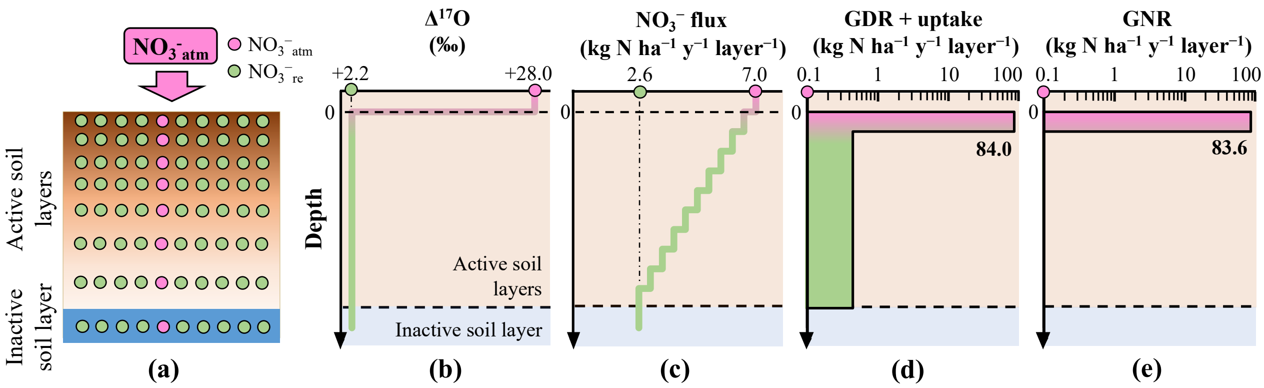

Figure. 2. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil with homogeneous distribution of $\Delta^{17}O$ values of NO_3^- (a). Vertical distribution of the following parameters in the forested soil: ~~simulated~~ assumed $\Delta^{17}O$ values of NO_3^- (b), ~~simulated~~ assumed leaching flux of NO_3^- (c), estimated NO_3^- consumption rate (GDR + uptake) (d), and estimated GNR (e).