BiasErrors associated with 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 for quantifying the gross nitrification rate (GNR) in ~~each~~ forested catchments using the triple oxygen isotopic composition ($\Delta^{17}O$) of stream nitrate ~~eluted~~leached from the catchments has been proposed and applied in ~~several~~ recent studies. However, the equations used in the calculations include the approximation that the $\Delta^{17}O$ value of nitrate ~~metabolized~~ consumed through ~~either~~ assimilation or denitrification ~~with~~in the forested soil is equal to the $\Delta^{17}O$ value of stream nitrate ~~in the stream~~. The GNR estimated from the $\Delta^{17}O$ value of stream nitrate was more than six times the ~~actual~~ GNR in our simulated calculation for a forested catchment where the soil nitrate ~~in the soil~~ had ~~exhibited~~ $\Delta^{17}O$ values ~~larger~~ higher than those ~~in~~ the stream nitrate. The $\Delta^{17}O$ values of the soil nitrate decreased~~while showing a decreasing trend~~ with an increase in depth to ~~increasing depths until~~ that of the stream nitrate at the bottom. ~~As most~~Most of the reported soil nitrate in forested catchments from past studies showed $\Delta^{17}O$ values higher than those of the stream nitrate ~~eluted~~leached from the ~~each~~ catchments.~~,~~ Thus, we concluded that the GNR estimated from the $\Delta^{17}O$ value of stream nitrate in the forested catchments was, to ~~some~~ an extent, an overestimate of the actual GNR.

1 Introduction

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

Recently, several studies have successfully estimated the GNR in water environments, such as lakes, using the $\Delta^{17}O$ values of NO_3^-, as a ~~conserved~~ conservative tracer of the mixing ratio between ~~the~~ atmospheric nitrate ($NO_3^-{}_{atm}$) ~~deposited into the water environment~~ and ~~the remineralized~~ biologically produced nitrate ($NO_3^-{}_{bio}$~~re~~) ~~produced through nitrification therein~~ (Tsunogai et al., 2011, 2018). The $NO_3^-{}_{atm}$ is deposited in the water environment, and the $NO_3^-{}_{bio}$ is produced through nitrification. ~~Although~~ The NO_3^- ~~re~~bio always shows the $\Delta^{17}O$ value~~s~~ close to 0 ‰ because its oxygen atoms ~~derive~~ are derived from either terrestrial O_2 or H_2O through nitrification.~~,~~ ~~In contrast~~Contrarily, the $NO_3^-{}_{atm}$ always displays an anomalous enrichment in ^{17}O with $\Delta^{17}O$ value~~s~~ 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. ~~Therefore~~Thus, regardless of the partial ~~metabolism~~ consumption through denitrification or assimilation after deposition in a water column, the $\Delta^{17}O$ can be used as a conservative~~ed~~ 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:

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

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 ~~each~~ the water environment, respectively. Using ~~both~~ 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 ~~has been~~was successfully estimated. This is because 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 application in water environments, the $\Delta^{17}O$ method has ~~also~~ been applied to forested catchments ~~for~~ to determine their GNR ~~determination~~ (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020). ~~By u~~Using the deposition flux of $NO_3^-{}_{atm}$ into the catchment ~~as well as~~and the ~~elution~~leaching flux of ~~both~~ unprocessed

$NO_3^-{}_{atm}$ and $NO_3^-{}_{bio}$~~re~~ from~~ the~~ streams, ~~which can be determined from the $\Delta^{17}O$ values of NO_3^- in stream water eluted from the catchment, ~~the GNR in~~ each~~ athe forested catchment ~~has been~~was estimated ~~in a manner similar~~similarly to the estimation for~~ the~~ water environments (Fang et al., 2015). Contrary to water environments, where the $\Delta^{17}O$ values of NO_3^- ~~with~~in the water layers are homogeneous in the water column due to the active vertical mixing of water ~~–~~and can be measured easily, it is often difficult to determine the $\Delta^{17}O$ values of~~ the~~ NO_3^- ~~metabolized~~ consumed in soil layers. Consequently, past studies have approximated the values to be equal to the $\Delta^{17}O$ value of stream NO_3^- ~~eluted~~leached from~~ each~~ forested catchments without actual observation (Fang et al., 2015, Hattori et al., 2019, Huang et al., 2020). However, such an approximation should be conducted with extreme caution, as the $\Delta^{17}O$ values of soil NO_3^- are not always equal to those of~~ the~~ streams ~~–~~(Hattori et al., 2019, Rose, 2014,~~ –~~ Nakagawa et al., 2018~~Osaka et al., 2010~~). To clarify the details of the approximation ~~along with~~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, ~~starting from the~~using basic isotope mass balance equations. Then, we present the ~~GNR~~ estimated GNR for a forested catchment ~~in which the~~whose $\Delta^{17}O$ values of soil NO_3^- ~~in soil are~~were measured. Finally, we compared the GNR estimated in this study with the GNR estimated from the $\Delta^{17}O$ values of stream NO_3^-.

2 Calculation

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

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

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

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

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

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

If the $\Delta^{17}O$ values of the NO_3^- in the forested soil layers, where the NO_3^- was ~~metabolized~~ consumed through ~~either~~ assimilation ~~(by plants and other organisms)~~ or denitrification, are equal to the $\Delta^{17}O$ values of NO_3^- in the stream, we could obtain Eq. 4 ~~can be expressed as follows~~:

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

Consequently, by combining Eqs. 3 and 4, we could obtain the following relationship:

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

We could estimate the GNR using Eq. 6 obtained from Eqs. 2 and 5 because we can approximate the $\Delta^{17}O$ values of $NO_3^-{}_{bio}$~~re~~ produced through nitrification ($\Delta^{17}O(NO_3^-)_{nitrification}$) to ~~be~~ 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 ~~for~~ to quantify~~ing~~ the GNR in the ~~each~~ 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 the stream~~, as presented in Eq. 4 to obtain Eq. 6. While the number of simultaneous observations of the oxygen isotopes of NO_3^- in both 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 ~~limited~~ observations show that the oxygen isotopic ratios of soil NO_3^- are mostly higher than 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) reported that ~~more than~~over 60 % of the soil exhibited $\Delta^{17}O$ values significantly higher than those of stream NO_3^- determined simultaneously ($\Delta^{17}O(NO_3^-)_{stream}$ ~~$\Delta^{17}O$~~ = +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 ~~decreasing~~ trend in the vertical distribution ~~had been found~~was observed in $\delta^{18}O$ in another forested catchment, from ~~greater than~~above +35 ‰ at the surface soil to less than +10 ‰ at depths of 30–50 cm from the soil surface (Osaka et al., 2010). ~~Besides~~In addition, most of the soil NO_3^- also exhibited $\delta^{18}O$ values higher than those ~~in~~ of the stream NO_3^- (Osaka et al., 2010). ~~Furthermore,~~ 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~~, reporting $\Delta^{17}O$ values~~. They reported significantly higher $\Delta^{17}O$ values in soil NO_3^- (+9.1 ± 5.8 ‰ on average) than ~~in the~~those of stream NO_3^- (+0.5 ‰ on average) ~~eluted~~leached from the forested catchment. As most ~~of the~~ fine roots and root biomass ~~is~~are concentrated in the top 10 cm of the soil in forested catchments (Jackson et al., 1996; Li et al., 2020), most uptake reactions 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 ~~the~~ surface soil layers, implies~~y~~ that the estimated GNRs in ~~the~~ forested catchments obtained from Eq. 6 were inaccurate.

To demonstrate the impact of the differences in the $\Delta^{17}O$ values of~~between~~ soil NO_3^- and stream NO_3^- on the GNR and present~~, along with presenting~~ the problems associated with the approximation to obtain Eq. 6, we estimated the GNR of~~for~~ two simulated forested soils. The $\Delta^{17}O$ of the first soil ~~— one~~ with NO_3^- decreased ~~showing a decreasing trend in $\Delta^{17}O$ down~~ to the $\Delta^{17}O$ of the stream NO_3^- (heterogeneous soil) (Figs. 1a and 1b). The second soil ~~and one~~ with NO_3^- showing the same $\Delta^{17}O$ values as those of the stream NO_3^- (homogeneous soil) (Figs. 2a and 2b). ~~With Because~~ Hattori et al. (2019) report~~ing~~ed 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~~ir~~ forested catchment they studie~~y~~d.~~,~~ W~~w~~e adopted the same values in ~~our~~ the present calculation to simulate the same forested soil. All the symbols (e.g., GNR) used ~~in this study~~here were ~~in accordance~~consistent with those of Hattori et al. (2019)~~ as well~~.

To estimate the GNR, w~~W~~e divided ~~the soils in~~ the ~~heterogeneous~~ forest soils into 10 layers (i.e., 10 steps) in the vertical direction.~~, simulating the soils observed by Hattori et al. (2019), in which t~~The $\Delta^{17}O$ values of NO_3^- gradually decreased with ~~increasing~~ an increase in depth, varying from +28.0 to +2.2 ‰ with a rate of −~~decrease of +~~2.58 ‰ for each step (Fig. 1b). Similarly, we assumed a gradual decrease with an increasing in depth in the leaching flux of NO_3^-, ~~i.e.,~~ (from 7 to 2.6 kg of N ha^{-1} y^{-1} at~~with~~ a rate of ~~decrease of~~ −0.44 kg of N ha^{-1} y^{-1} ~~for each~~per step) (Fig. 1c).~~, which~~This simulated the gradual net consumption of NO_3^- in accordance with water flow in forested soils. ~~In t~~The homogeneous ~~forest~~ soil~~s,~~ was~~e~~ also divided ~~the forested soils~~ into 10 layers in the vertical direction. The change ~~with depth~~ in the leaching flux of NO_3^- with depth was the same as that in the heterogeneous soil~~s~~ (Fig. 2c), whereas the $\Delta^{17}O$ values of NO_3^- were constant at +2.2 ‰ in the ~~soil~~ 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 ~~both~~ the GNR (Figs. 1e and 2e) and total consumption~~metabolic~~ rate of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer. We assumed~~ing~~ the following.~~s:~~ (1) The $\Delta^{17}O$ values of NO_3^- were~~are~~ constant in each layer.~~,~~ (2) The vertical flow of NO_3^- in the soil layers proceeded downward from the surface to the ~~water~~ final layer (No. 10) with a uniform residence time in each layer.~~, and~~ Finally, (3) the GNR and consumption~~metabolic~~ rate of NO_3^- (GDR + uptake) was~~is~~ zero in the ~~soil/~~water layer ~~(layers beyond the no. 10 soilfinal layer) (No. 10)~~. Thereafter~~n~~, by integrating the GNR determined for each layer, we estimated the total GNR in ~~each~~ the forested catchment.

Although the 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), exactly equal to that estimated by Hattori et al. (2019) using Eq. 6 ~~(Fig. 2e)~~, the ~~total~~ GNR estimated for the catchment with the heterogeneous $\Delta^{17}O$ values in soil NO_3^- ~~(heterogeneous soil)~~ was considerably lower ~~a much smaller~~ (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 $\Delta^{17}O(NO_3^-)_{stream}$.~~simulated for the catchment with the heterogeneous $\Delta^{17}O$ values in soil NO_3^- (Fig. 1e).~~ Even if ~~we increased~~ the number of ~~the~~ layers in the forested soils was increased ~~in~~to 20, 30, 50, 100, and 1000 to enhance the precision of the GNR simulated for the catchment with the heterogeneous ~~$\Delta^{17}O$ values in~~ soil ~~NO_3^-~~, 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.~~s:~~ (1) P~~p~~ast studies estimated~~ing~~ the GNR using Eq. 6 ~~did~~ approximated the $\Delta^{17}O$ values of soil NO_3^- consumed in the forested catchments were~~was~~ homogeneous and always equal to that of stream NO_3^- ~~mathematically and~~. (2) T~~t~~he differences between the $\Delta^{17}O$ values of the soil NO_3^- ~~metabolized~~ 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.

By combining the mass balance and isotope mass balance shown in Eqs. 2 and 3, Eq. 7 ~~can be~~was obtained ~~as the equation~~ to estimate the GNR accurately:

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

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

Note that tThe 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 the forested catchments. It is impossible to decide determine whether the linear variation was realistic or not until the downward water flux, along with the concentration and $\Delta^{17}O$ values of NO_3^-, wasis 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) impliedy that the approximation of the $\Delta^{17}O$ values of the soil NO_3^- metabolized through assimilation or denitrification to be always equal to the $\Delta^{17}O$ valuethat of the stream NO_3^-, shown in (Fig. 2b), wasis unrealistic.

By combining the mass balance and isotope mass balance shown in Eqs. 2 and 3, Eq. 7 can be obtained to accurately estimate the 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 $\Delta^{17}O$ values of NO_3^- in forested soil, from which the NO_3^- was metabolized through either assimilation or denitrification. As most of the soil NO_3^- measured to date exhibit $\Delta^{17}O$ values higher than those of the stream NO_3^- eluted from each catchment (Hattori et al., 2019, Rose, 2014), the GNR estimated from stream NO_3^- using Eq. 6 is higher than the GNR estimated from soil NO_3^- using Eq. 7, to some extent. In other words, the GNR estimated from Eq. 6 overestimated the GNR in each forested catchment to some extent.

If we estimated the downward water flux at each soil layer, together with the NO_3^- concentration and $\Delta^{17}O$ value of NO_3^- in each soil layer through the methods such as those using, e.g., a tension-free lysimeter (Inoue et al., 2021), we could estimate the vertical change in the leaching flux of NO_3^- for each soil layer along with the $\Delta^{17}O$ value of soil NO_3^- in each layer. Thereaftern, applying Eq. (7) toin each layer, we can more accurately more accurately estimate the GNR for the forested catchment more accurately, by integrating the GNR estimated for each soil layer together with a more accurate NO_3^- consumptionmetabolic rate of NO_3^- (GDR + uptake) of the forested catchment. However, wWithout such an observation of the distribution and the leaching flux of the $\Delta^{17}O$ value of NO_3^- ,together with the $\Delta^{17}O$ values in forest soil, it is difficult to assume that the $\Delta^{17}O$ values of soil NO_3^- are always equal to those of stream NO_3^-; thus, the GNR estimated by 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 ~~to determine~~for determining the GNR in ~~each~~ forested catchments. The equations used in the calculation presuppose that the $\Delta^{17}O$ values of NO_3^- consumed in forested soils are homogeneous and equal to those of ~~NO_3^- in~~ the stream NO_3^-.~~;~~ H~~h~~owever, in reality, the values are often heterogeneous and do not always equal ~~to~~ those ~~corresponding to~~of the stream. It is essential to clarify/verify the $\Delta^{17}O$ values of NO_3^- in~~the~~ forested soils and streams before applying the ~~stream NO_3^--~~$\Delta^{17}O$ values of stream NO_3^- to estimate the 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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References

Bengtsson, G., Bengtson, P. and Månsson, K. F.: Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity, Soil Biol. Biochem., 35(1), 143–154, doi:10.1016/S0038-0717(02)00248-1, 2003.

Ding, W., Tsunogai, U., Nakagawa, F., Sambuichi, T., Sase, H., Morohashi, M., and Yotsuyanagi, H.: Tracing the source of nitrate in a forested stream showing elevated concentrations during storm events, Biogeosciences, 19, 3247–3261, https://doi.org/10.5194/bg-19-3247-2022, 2022.

Ding, W., Tsunogai, U., Nakagawa, F., Sambuichi, T., Chiwa, M., Kasahara, T., and Shinozuka, K.: Stable isotopic evidence for the excess leaching of unprocessed atmospheric nitrate from forested catchments under high nitrogen saturation, Biogeosciences, 20, 753–766, https://doi.org/10.5194/bg-20-753-2023, 2023.

Fang, Y., Koba, K., Makabe, A., Takahashi, C., Zhu, W., Hayashi, T., Hokari, A. A., Urakawa, R., Bai, E., Houlton, B. Z., Xi, D., Zhang, S., Matsushita, K., Tu, Y., Liu, D., Zhu, F., Wang, Z., Zhou, G., Chen, D., Makita, T., Toda, H., Liu, X., Chen, Q., Zhang, D., Li, Y. and Yoh, M.: Microbial denitrification dominates nitrate losses from forest ecosystems, Proc. Natl. Acad. Sci. U. S. A., 112(5), 1470–1474, doi:10.1073/pnas.1416776112, 2015.

Hattori, S., Nuñez Palma, Y., Itoh, Y., Kawasaki, M., Fujihara, Y., Takase, K. and Yoshida, N.: Isotopic evidence for seasonality of microbial internal nitrogen cycles in a temperate forested catchment with heavy snowfall, Sci. Total Environ., 690, 290–299, doi:10.1016/j.scitotenv.2019.06.507, 2019.

Huang, S., Wang, F., Elliott, E. M., Zhu, F., Zhu, W., Koba, K., Yu, Z., Hobbie, E. A., Michalski, G., Kang, R., Wang, A., Zhu, J., Fu, S. and Fang, Y.: Multiyear Measurements on $\Delta^{17}O$ of Stream Nitrate Indicate High Nitrate Production in a Temperate Forest, Environ. Sci. Technol., 54(7), 4231–4239, doi:10.1021/acs.est.9b07839, 2020.

Inoue, T., Nakagawa, F., Shibata, H. and Tsunogai, U.: Vertical Changes in the Flux of Atmospheric Nitrate From a Forest Canopy to the Surface Soil Based on $\Delta^{17}O$ Values, J. Geophys. Res. Biogeosciences, 126(4), 1–18, doi:10.1029/2020JG005876, 2021.

Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E. and Schulze, E. D.: A global analysis of root distributions for terrestrial biomes, Oecologia, 108(3), 389–411, doi:10.1007/BF00333714, 1996.

Li, F. L., McCormack, M. L., Liu, X., Hu, H., Feng, D. F., and Bao, W. K.: Vertical fine-root distributions in five subalpine forest types shifts with soil properties across environmental gradients, Plant Soil, 456, 129–143, https://doi.org/10.1007/s11104-020-04706-x, 2020.

Michalski, G., Scott, Z., Kabiling, M. and Thiemens, M. H.: First measurements and modeling of $\Delta^{17}O$ in atmospheric nitrate, Geophys. Res. Lett., 30(16), 3–6, doi:10.1029/2003GL017015, 2003.

Michalski, G., Meixner, T., Fenn, M., Hernandez, L., Sirulnik, A., Allen, E. and Thiemens, M.: Tracing Atmospheric Nitrate Deposition in a Complex Semiarid Ecosystem Using $\Delta^{17}O$, Environ. Sci. Technol., 38(7), 2175–2181, doi:10.1021/es034980+, 2004.

Nakagawa, F., Tsunogai, U., Obata, Y., Ando, K., Yamashita, N., Saito, T., Uchiyama, S., Morohashi, M. and Sase, H.: Export flux of unprocessed atmospheric nitrate from temperate forested catchments: A possible new index for nitrogen saturation, Biogeosciences, 15(22), 7025–7042, doi:10.5194/bg-15-7025-2018, 2018.

Nelson, D. M., Tsunogai, U., Ding, D., Ohyama, T., Komatsu, D. D., Nakagawa, F., Noguchi, I. and Yamaguchi, T.: Triple oxygen isotopes indicate urbanization affects sources of nitrate in wet and dry atmospheric deposition, Atmos. Chem. Phys., 18(9), 6381–6392, doi:10.5194/acp-18-6381-2018, 2018.

Osaka, K., Ohte, N., Koba, K., Yoshimizu, C., Katsuyama, M., Tani, M., Tayasu, I. and Nagata, T.: Hydrological influences on spatiotemporal variations of $\delta^{15}N$ and $\delta^{18}O$ of nitrate in a forested headwater catchment in central Japan: Denitrification plays a critical role in groundwater , J. Geophys. Res. Biogeosciences, 115(G2), n/a-n/a, doi:10.1029/2009jg000977, 2010.

Rose, L. A.: Assessing the nitrogen saturation status of appalachian forests using stable isotopes of nitrate [PhD thesis, University of Pittsburgh]. Retrieved from http://d-scholarship.pitt.edu/22783/1/LRose_ETD_081914_revised1.pdf.

Stark, J. M. and Hart, S. C.: High rates of nitrification and nitrate turnover in undisturbed coniferous forests, Nature, 385(6611), 61–64, doi:10.1038/385061a0, 1997.

Tsunogai, U., Komatsu, D. D., Daita, S., Kazemi, G. A., Nakagawa, F., Noguchi, I. and Zhang, J.: Tracing the fate of atmospheric nitrate deposited onto a forest ecosystem in Eastern Asia using $\Delta^{17}O$, Atmos. Chem. Phys., 10(4), 1809–1820, doi:10.5194/acp-10-1809-2010, 2010.

Tsunogai, U., Daita, S., Komatsu, D. D., Nakagawa, F. and Tanaka, A.: Quantifying nitrate dynamics in an oligotrophic lake using $\Delta^{17}O$, Biogeosciences, 8(3), 687–702, doi:10.5194/bg-8-687-2011, 2011.

Tsunogai, U., Miyauchi, T., Ohyama, T., Komatsu, D. D., Nakagawa, F., Obata, Y., Sato, K. and Ohizumi, T.: Accurate and precise quantification of atmospheric nitrate in streams draining land of various uses by using triple oxygen isotopes as tracers, Biogeosciences, 13(11), 3441–3459, doi:10.5194/bg-13-3441-2016, 2016.

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.

Verchot, L. V., Holmes, Z., Mulon, L., Groffman, P. M. and Lovett, G. M.: Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types, Soil Biol. Biochem., 33(14), 1889–1901, doi:10.1016/S0038-0717(01)00095-5, 2001.

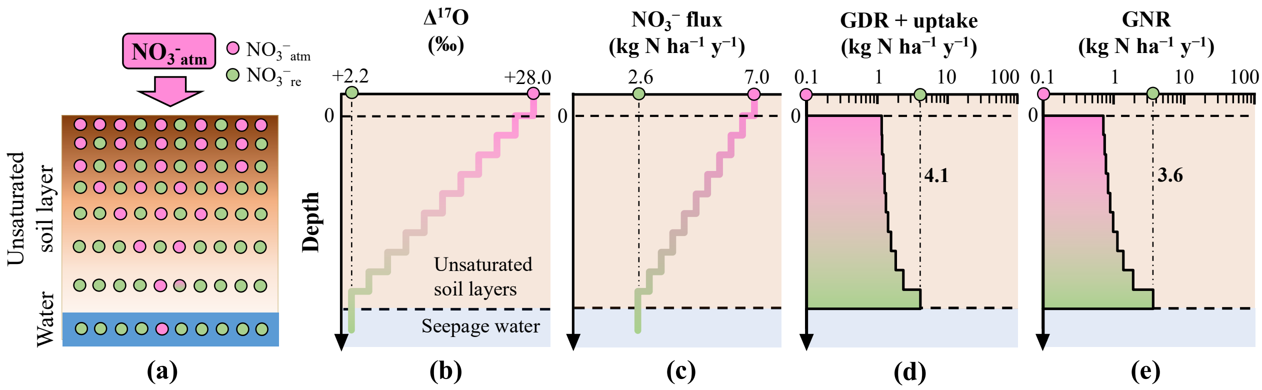

Figure. 1. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil ~~where the~~with heterogeneous distribution of~~ the~~ $\Delta^{17}O$ values of NO_3^- ~~is heterogeneous~~ (a). Vertical distribution of the following parameters in the forested soil: ~~the~~ simulated $\Delta^{17}O$ values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

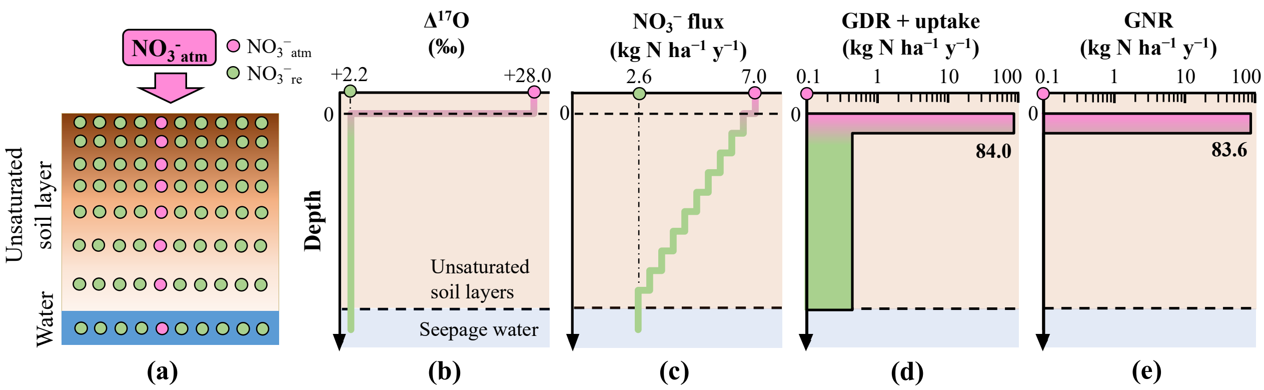

Figure. 2. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil with homogeneous ~~where the~~ distribution of~~ the~~ $\Delta^{17}O$ values of NO_3^- ~~is homogeneous~~ (a). Vertical distribution of the following parameters in the forested soil: the simulated $\Delta^{17}O$ values of NO_3^-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