Ideas and perspectives: Errors associated with the gross nitrification rates in forested catchments calculated from the triple oxygen isotopic composition ($\Delta^{17}$O) of stream nitrate

Weitian Ding\(^1\), Urumu Tsunogai\(^1\), Fumiko Nakagawa\(^1\)

\(^1\)Graduate School of Environmental Studies, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

Corresponding author: Weitian Ding (ding.weitian.v2@s.mail.nagoya-u.ac.jp)
Abstract

A novel method to quantify the gross nitrification rate (GNR) in each forested catchment using the triple oxygen isotopic composition ($\Delta^{17}O$) of stream nitrate eluted from the catchment has been proposed and used in recent studies. However, the equations used in the calculations assumed homogeneous $\Delta^{17}O$ values of nitrate being metabolized through either assimilation or denitrification within the forested soil layers without particular discussions. The GNR estimated from the $\Delta^{17}O$ of stream nitrate using the equations was more than six times the actual GNR in our simulated calculation for a forested catchment where the $\Delta^{17}O$ values of nitrate being metabolized in the soil were heterogeneous and showed a decreasing trend with increasing depths. Therefore, we should verify that the $\Delta^{17}O$ values of nitrate being metabolized are homogeneous in forested soils or estimate the possible range of errors using $\Delta^{17}O$ of stream nitrate to estimate the GNR.

1 Introduction

Nitrate ($NO_3^-$) is a crucial nutrient in forest ecosystems that often limits primary production. Nitrification is the microbial process that generates $NO_3^-$ from the ammonium in a forested ecosystem; therefore, the nitrification rate is an important parameter to be quantified when evaluating each forest ecosystem’s present and future states. The total rate of $NO_3^-$ production, gross nitrification rate (GNR), reflects internal N cycling better than the net nitrification rate (Bengtsson et al., 2003),
especially in forest ecosystems where the GNR often exceeds the net nitrification rate by order of magnitude (Stark and Hart, 1997; Verchot et al., 2001).

Recently, several studies successfully determined GNR in each water environment using the Δ^{17}O values of NO$_3^-$ as a conserved tracer for the mixing ratio between the atmospheric nitrate (NO$_3^-$$_{atm}$) deposited into each water environment and remineralized nitrate (NO$_3^-$$_{re}$) produced through nitrification therein (Tsunogai et al., 2011, 2018). Although NO$_3^-$$_{re}$ always has a Δ^{17}O value close to 0‰ because its oxygen atoms come from either terrestrial O$_2$ or H$_2$O through nitrification, NO$_3^-$$_{atm}$ displays an anomalous enrichment in $^{17}$O with a Δ^{17}O value being approximately $+26$‰ (Tsunogai et al., 2010, 2016) because of oxygen transfers from atmospheric ozone (Michalski et al., 2003; Nelson et al., 2018). Additionally, Δ^{17}O is almost stable during “mass-dependent” isotope fractionation processes (Michalski et al., 2004; Tsunogai et al., 2016); therefore, regardless of partial metabolism through denitrification or assimilation after deposition, Δ^{17}O can be used as a conserved tracer of NO$_3^-$$_{atm}$ to calculate the mixing ratio of NO$_3^-$$_{atm}$ within total NO$_3^-$ (NO$_3^-$$_{atm}$/NO$_3^-$$_{total}$) using the following equation:

$$\frac{[NO_3^-_{atm}]/[NO_3^-_{total}]}{\Delta^{17}O_{atm}/\Delta^{17}O_{water}} = \frac{\Delta^{17}O_{water}}{\Delta^{17}O_{atm}}$$

where Δ^{17}O$_{atm}$ and Δ^{17}O$_{water}$ denote the Δ^{17}O values of NO$_3^-$$_{atm}$ and NO$_3^-$ dissolved in each water environment, respectively. Using both the NO$_3^-$$_{atm}$/NO$_3^-$$_{total}$ ratio estimated from the Δ^{17}O value of NO$_3^-$ in a lake water column and the deposition rate of
NO$_3^-$ into the lake, past studies successfully estimated GNR therein (Tsunogai et al., 2011, 2018).

In addition to water environments, the $\Delta^{17}$O method has been further applied to forested catchments to determine GNR (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020; Riha et al., 2014). By using the deposition flux of NO$_3^-$ into the catchment as well as the elution flux of both unprocessed NO$_3^-$ and NO$_3^-$ via stream, which can be determined from the $\Delta^{17}$O values of NO$_3^-$ in stream water eluted from the catchment, GNR in each forested catchment has been determined in a manner similar to the water environments (Fang et al., 2015). Applying the $\Delta^{17}$O method to forested soils, where the $\Delta^{17}$O values of NO$_3^-$ are often heterogeneous (Costa et al., 2011; Hattori et al., 2019), should be done with extreme caution, in contrast to water environments where the $\Delta^{17}$O values of nitrate were largely homogeneous. We present an accurate relationship between the $\Delta^{17}$O and GNR starting from the basic isotope mass balance equations to explain the problem of using the $\Delta^{17}$O method in such heterogeneous environments.

2 Calculation

The total mass balance equation of NO$_3^-$ including GNR in each catchment can be expressed as follows:

$$\text{NO}_3^-\text{deposition} + \text{GNR} = \text{NO}_3^-\text{leaching} + \text{NO}_3^-\text{uptake} + \text{GDR}$$

(2)
where NO$_3^-$ deposition, GNR, NO$_3^-$ leaching, NO$_3^-$ uptake, and GDR denote the deposition flux of NO$_3^-$ into each catchment, the gross nitrification rate in each catchment, the leaching flux of NO$_3^-$ from each catchment, the uptake rate of NO$_3^-$ in each catchment, and the gross denitrification rate in each catchment, respectively.

The isotope mass balance for each $\Delta^{17}\text{O}$ value of NO$_3^-$ in the catchment can also be calculated using the same method:

$$\text{NO}_3^-\text{deposition} \times \Delta^{17}\text{O(NO}_3^-)_{\text{atm}} + \text{GNR} \times \Delta^{17}\text{O(NO}_3^-)_{\text{nitrification}} = \text{NO}_3^-\text{leaching} \times \Delta^{17}\text{O(NO}_3^-) + \text{NO}_3^-\text{uptake} \times \Delta^{17}\text{O(NO}_3^-)_{\text{uptake}} + \text{GDR} \times \Delta^{17}\text{O(NO}_3^-)_{\text{denitrification}}$$ (3)

where $\Delta^{17}\text{O(NO}_3^-)_{\text{atm}}$, $\Delta^{17}\text{O(NO}_3^-)_{\text{nitrification}}$, $\Delta^{17}\text{O(NO}_3^-)_{\text{stream}}$, $\Delta^{17}\text{O(NO}_3^-)_{\text{uptake}}$, and $\Delta^{17}\text{O(NO}_3^-)_{\text{denitrification}}$ denote the $\Delta^{17}\text{O}$ value of NO$_3^-$ atm deposited in each catchment, that of NO$_3^-$ produced through nitrification, that of NO$_3^-$ eluted from each catchment, that of NO$_3^-$ assimilated by plants and other organisms in each catchment, and that of NO$_3^-$ decomposed through denitrification in each catchment, respectively.

If the $\Delta^{17}\text{O}$ values of NO$_3^-$ were homogeneous in forested soils where NO$_3^-$ was metabolized through either assimilation (by plants and other organisms) or denitrification, Eq. 4 can be expressed as follows:

$$\Delta^{17}\text{O(NO}_3^-)_{\text{stream}} = \Delta^{17}\text{O(NO}_3^-)_{\text{uptake}} = \Delta^{17}\text{O(NO}_3^-)_{\text{denitrification}}$$ (4)

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

$$\text{NO}_3^-\text{deposition} \times \Delta^{17}\text{O(NO}_3^-)_{\text{atm}} + \text{GNR} \times \Delta^{17}\text{O(NO}_3^-)_{\text{nitrification}} = (\text{NO}_3^-\text{leaching} + \text{NO}_3^-\text{uptake} + \text{GDR}) \times \Delta^{17}\text{O(NO}_3^-)_{\text{stream}}$$ (5)
We could estimate GNR using Eq. 6 obtained from Eqs. 2 and 5 because we can approximate the Δ¹⁷O values of NO₃⁻ re produced through nitrification

\[(\Delta ^{17}O(NO_3^-)_{\text{nitrification}}) \text{ to be 0 (Michalski et al., 2003; Tsunogai et al., 2010):}\]

\[
GNR = NO_3^- \text{deposition} \times (\Delta ^{17}O(NO_3^-)_{\text{ann}} - \Delta ^{17}O(NO_3^-)_{\text{stream}})/\Delta ^{17}O(NO_3^-)_{\text{stream}} \tag{6}
\]

The Eq. 6 corresponds to that used in previous studies for quantifying GNR in each forested catchment (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020; Riha et al., 2014).

3 Results and Discussion

The Δ¹⁷O values of NO₃⁻ metabolized in each catchment should be homogeneous and therefore correspond with those of NO₃⁻ in the stream, as presented in Eq. 4 to obtain Eq. 6. However, many of the forested catchments do not satisfy this condition needed to obtain Eqs. 4–6. In the studied forested soils, Hattori et al. (2019) found a decreasing trend in the Δ¹⁷O values of NO₃⁻ together with the depth, from more than +20‰ at the surface soil to less than +3‰ at depths of 25 to 90 cm from the soil surface. Furthermore, most of the Δ¹⁷O values of soil NO₃⁻ differed from those in the stream eluted from the catchment (+2.2‰ on average) in Hattori et al. (2019).

To demonstrate the possible change in GNR per the variation in the Δ¹⁷O values of NO₃⁻ in forested soils, we estimated GNR for two simulated forested soils: that with a vertically heterogeneous Δ¹⁷O of NO₃⁻ (Fig. 1a) and that with a vertically homogeneous Δ¹⁷O of NO₃⁻ (Fig. 2a). Because Hattori et al. (2019) reported the
NO$_3^-$ deposition to be 7.0 kg of N ha$^{-1}$ y$^{-1}$, NO$_3^-$ leaching to be 2.6 kg of N ha$^{-1}$ y$^{-1}$, Δ$^{17}$O(NO$_3^-$) atm to be +28.0 ‰, and Δ$^{17}$O(NO$_3^-$) stream to be +2.2 ‰ in the forested catchment, we adopted the same parameter in the simulated calculation in this study. We divided the soils in the heterogeneous forest soils into 10 layers in the vertical direction simulating the soils observed by Hattori et al. (2019), where the Δ$^{17}$O values of NO$_3^-$ gradually decreased with increasing depths, showing the Δ$^{17}$O values from +28.0 to +2.2 ‰ with a decrease rate of +2.58 ‰ for each step (Fig. 1b). Similarly, we assumed gradual decrease with increasing depths in the leaching flux of NO$_3^-$, i.e., from 7 to 2.6 kg of N ha$^{-1}$ y$^{-1}$ with a decrease rate of 0.44 kg of N ha$^{-1}$ y$^{-1}$ for each step (Fig. 1c). In the homogeneous forest soils, we also divided the forested soils into 10 layers in the vertical direction. The vertical changes in the leaching flux of NO$_3^-$ were the same as those in the heterogeneous soils (Fig. 2c), whereas the Δ$^{17}$O values of NO$_3^-$ were constant to be +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 GNR (Figs. 1e and 2e) and total metabolic rate of NO$_3^-$ (GDR + uptake) (Figs. 1d and 2d) in each layer assuming that: (1) the Δ$^{17}$O values of NO$_3^-$ were constant in each layer, (2) the vertical flow of NO$_3^-$ in the soil layers was only downward from surface to the water layer with a uniform residence time in each layer, and (3) the GNR and metabolic rate of NO$_3^-$ (GDR + uptake) was zero in the water layer (the layers beyond the no. 10 soil layer). Then, by integrating the GNR determined for each layer, we can estimate the total GNR in each forested...
catchment. Although the GNR simulated for the catchment with the homogeneous
Δ^{17}O values of NO$_3^-$ in the forested soils showed a value of 83.6 kg of N ha$^{-1}$ y$^{-1}$
equal to that estimated by Hattori et al. (2019) (Fig. 2e), the total GNR became a
much smaller value of 13.0 kg of N ha$^{-1}$ y$^{-1}$ simulated for the catchment with the
heterogeneous Δ^{17}O values of NO$_3^-$ in the forested soils (Fig. 1e). As a result, we
conclude that the distribution of the Δ^{17}O values of NO$_3^-$ in the forested soils can
significantly affect the overall GNR in forested catchments as calculated from the
Δ^{17}O of stream NO$_3^-$. If we estimated the downward water flux at each soil layer, together with the
concentration and Δ^{17}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 Δ^{17}O value of each NO$_3^-$. Then, applying
the mass balance and isotope mass balance shown in Eqs. 2 and 3 in each layer, we
can estimate a more accurate GNR of the forested catchment by integrating the GNR
estimated for each soil layer together with the more accurate metabolic rate of NO$_3^-$(GDR + uptake) of the forested catchment. However, without such observation on the
distribution of the Δ^{17}O value of NO$_3^-$, it is difficult to assume that the Δ^{17}O values of
NO$_3^-$ were homogeneous in forested soils where NO$_3^-$ was metabolized, so that the
GNR should be reported with errors in which possible variations in the Δ^{17}O values of
soil NO$_3^-$ have been considered.
4 Conclusion

Past studies proposed the Δ^{17}O method to determine GNR in each forested catchment. The equations used in the calculation presupposed that the Δ^{17}O values of NO_3^- in forested soils were homogeneous, however, they are often heterogeneous and showing a decreasing trend with increasing depths. It must be essential to clarify/verify the distribution of the Δ^{17}O values of NO_3^- in forested soils before applying the Δ^{17}O values of stream NO_3^- to estimate 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


**Figure. 1.** Distribution of NO$_3^-$ atm in the simulated forested soil where the distribution of the Δ$^{17}$O values of NO$_3^-$ is heterogeneous (a). Vertical distribution of the following parameters in the forested soil: the simulated Δ$^{17}$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 where the distribution of the Δ$^{17}$O values of NO$_3^-$ is homogeneous (a). Vertical distribution of the following parameters in the forested soil: the simulated Δ$^{17}$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).