https://doi.org/10.5194/bg-2022-236
Preprint. Discussion started: 12 January 2023

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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

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https://doi.org/10.5194/bg-2022-236
Preprint. Discussion started: 12 January 2023

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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),

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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 $\Delta^{17}O$ 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 $\Delta^{17}O$ value close to 0 ‰ because its oxygen atoms come from either terrestrial O_2 or H_2O through nitrification, $NO_3^-_{atm}$ displays an anomalous enrichment in ^{17}O with a $\Delta^{17}O$ 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, $\Delta^{17}O$ 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, $\Delta^{17}O$ 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:

$$[NO_3^-_{atm}]/[NO_3^-_{total}] = [NO_3^-_{atm}]/([NO_3^-_{re}] + [NO_3^-_{atm}]) = \Delta^{17}O_{water}/\Delta^{17}O_{atm} \quad (1)$$

where $\Delta^{17}O_{atm}$ and $\Delta^{17}O_{water}$ denote the $\Delta^{17}O$ 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 $\Delta^{17}O$ value of NO_3^- in a lake water column and the deposition rate of

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$NO_3^-{}_{atm}$ 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 $NO_3^-{}_{atm}$ into the catchment as well as the elution flux of both unprocessed $NO_3^-{}_{atm}$ and $NO_3^-{}_{re}$ 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:

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

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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 $\Delta^{17}O$ value of NO_3^- in the catchment can also be calculated using the same method:

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

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 in each catchment, that of $NO_3^-_{re}$ 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 $\Delta^{17}O$ 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:

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

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

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

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

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

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 $\Delta^{17}O$ values of NO_3^- metabolized in each catchment should be homogeneous and therefore correspond with those of NO_3^- 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 $\Delta^{17}O$ values of NO_3^- 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 $\Delta^{17}O$ values of soil NO_3^- 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 $\Delta^{17}O$ values of NO_3^- in forested soils, we estimated GNR for two simulated forested soils: that with a vertically heterogeneous $\Delta^{17}O$ of NO_3^- (Fig. 1a) and that with a vertically homogeneous $\Delta^{17}O$ of NO_3^- (Fig. 2a). Because Hattori et al. (2019) reported the

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104 $NO_3^-{}_{deposition}$ 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},
105 $\Delta^{17}O(NO_3^-)_{atm}$ to be +28.0 ‰, and $\Delta^{17}O(NO_3^-)_{stream}$ to be +2.2 ‰ in the forested
106 catchment, we adopted the same parameter in the simulated calculation in this study.
107 We divided the soils in the heterogeneous forest soils into 10 layers in the vertical
108 direction simulating the soils observed by Hattori et al. (2019), where the $\Delta^{17}O$ values
109 of NO_3^- gradually decreased with increasing depths, showing the $\Delta^{17}O$ values from
110 +28.0 to +2.2 ‰ with a decrease rate of +2.58 ‰ for each step (Fig. 1b). Similarly,
111 we assumed gradual decrease with increasing depths in the leaching flux of NO_3^-, i.e.,
112 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
113 step (Fig. 1c). In the homogeneous forest soils, we also divided the forested soils into
114 10 layers in the vertical direction. The vertical changes in the leaching flux of NO_3^-
115 were the same as those in the heterogeneous soils (Fig. 2c), whereas the $\Delta^{17}O$ values
116 of NO_3^- were constant to be +2.2 ‰ in the soil layers (Fig. 2b).
117 Applying the total mass balance and isotope mass balance of NO_3^- shown in Eqs. 2
118 and 3 to each layer, we estimated both GNR (Figs. 1e and 2e) and total metabolic rate
119 of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer assuming that: (1) the $\Delta^{17}O$
120 values of NO_3^- were constant in each layer, (2) the vertical flow of NO_3^- in the soil
121 layers was only downward from surface to the water layer with a uniform residence
122 time in each layer, and (3) the GNR and metabolic rate of NO_3^- (GDR + uptake) was
123 zero in the water layer (the layers beyond the no. 10 soil layer). Then, by integrating
124 the GNR determined for each layer, we can estimate the total GNR in each forested

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catchment. Although the GNR simulated for the catchment with the homogeneous $\Delta^{17}O$ 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 $\Delta^{17}O$ values of NO_3^- in the forested soils (Fig. 1e). As a result, we conclude that the distribution of the $\Delta^{17}O$ values of NO_3^- in the forested soils can significantly affect the overall GNR in forested catchments as calculated from the $\Delta^{17}O$ of stream NO_3^-.

If we estimated the downward water flux at each soil layer, together with the concentration and $\Delta^{17}O$ 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 $\Delta^{17}O$ 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 $\Delta^{17}O$ value of NO_3^-, it is difficult to assume that the $\Delta^{17}O$ 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 $\Delta^{17}O$ values of soil NO_3^- have been considered.

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4 Conclusion

Past studies proposed the $\Delta^{17}O$ method to determine GNR in each forested catchment. The equations used in the calculation presupposed that the $\Delta^{17}O$ 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 $\Delta^{17}O$ values of NO_3^- in forested soils before applying the $\Delta^{17}O$ 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.

Acknowledgments

The authors are grateful to the members of the Biogeochemistry Group, Nagoya University, for their valuable support throughout this study. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan under grant numbers 22H00561, 17H00780, 22K19846, the Yanmar Environmental Sustainability Support

https://doi.org/10.5194/bg-2022-236
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Association, and the river fund of the river foundation, Japan. Weitian Ding would like to take this opportunity to thank the "Nagoya University Interdisciplinary Frontier Fellowship" supported by Nagoya University and JST, the establishment of university fellowships towards the creation of science technology innovation, Grant Number JPMJFS2120.

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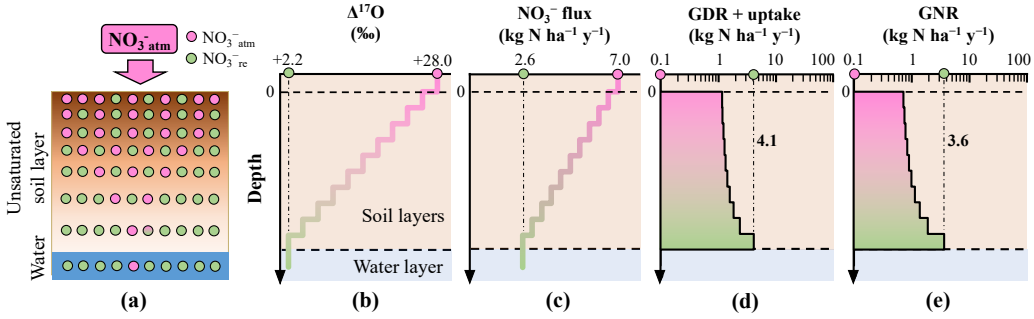

240 **Figure. 1**. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil where the distribution
241 of the $\Delta^{17}O$ values of NO_3^- is heterogeneous (a). Vertical distribution of the following
242 parameters in the forested soil: the simulated $\Delta^{17}O$ values of NO_3^- (b), simulated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

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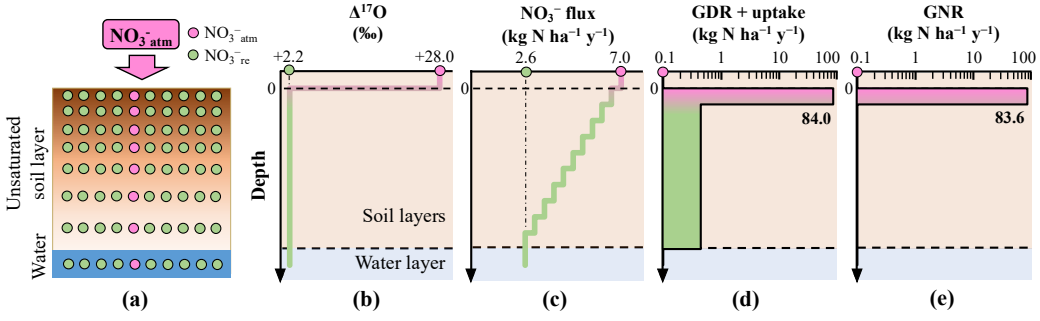

246 **Figure. 2**. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil where the distribution
247 of the $\Delta^{17}O$ values of NO_3^- is homogeneous (a). Vertical distribution of the following
248 parameters in the forested soil: the simulated $\Delta^{17}O$ values of NO_3^- (b), simulated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