Bias associated with calculating the gross nitrification rates in forested catchments using 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 to: Weitian Ding (ding.weitian.v2@s.mail.nagoya-u.ac.jp)

Abstract

A novel method 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 calculations include the approximation that the $\Delta^{17}O$ value of nitrate consumed through assimilation or denitrification in the forested soil 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 the GNR in our simulated calculation 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 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. 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) (net nitrification rate + consumption rate of NO_3^- (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. 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 studies have successfully estimated the GNR in water environments, such as lakes, using the $\Delta^{17}O$ values of NO_3^-, as a conservative tracer of 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 $NO_3^-_{bio}$ 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:

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

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 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 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 streams, the GNR in a forested catchment was estimated similarly to the estimation for water environments (Fang et al., 2015). Contrary to water environments, where 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 measured easily, it is often difficult to determine the $\Delta^{17}O$ values of NO_3^- consumed in soil layers. Consequently, past studies have approximated the values to be equal to the $\Delta^{17}O$ value of stream NO_3^- leached from

65 forested catchments without actual observation (Fang et al., 2015, Hattori et al., 2019,
66 Huang et al., 2020). However, such an approximation should be conducted with
67 extreme caution, as the $\Delta^{17}O$ values of soil NO_3^- are not always equal to those of
68 streams (Hattori et al., 2019, Rose, 2014, Nakagawa et al., 2018). To clarify the
69 details of the approximation and its impact on the final estimated GNR, we present an
70 accurate relationship between the $\Delta^{17}O$ of soil NO_3^- and the GNR, using basic isotope
71 mass balance equations. Thereafter, we present the estimated GNR for a forested
72 catchment whose $\Delta^{17}O$ values of soil NO_3^- were measured. Finally, we compared the
73 GNR estimated in this study with the GNR estimated from the $\Delta^{17}O$ values of stream
74 NO_3^-.
75

76 **2 Calculation**

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

79 $$NO_3^-_{deposition} + GNR = NO_3^-_{leaching} + NO_3^-_{uptake} + GDR \quad (2)$$

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

84 The isotope mass balance for each $\Delta^{17}O$ value of NO_3^- in the catchment can be
85 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^-, 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 show that the oxygen isotopic ratios of soil 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) reported that over 60 % of the soil exhibited $\Delta^{17}O$ values significantly higher than those of stream NO_3^- determined simultaneously ($\Delta^{17}O(NO_3^-)_{stream}$ = +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 $\delta^{18}O$ in 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 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 surface soil layers, implies that the estimated GNR in forested catchments obtained from Eq. 6 were inaccurate.

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).

147 To estimate the GNR, we divided the forest soils into 10 layers (i.e., 10 steps) in the
148 vertical direction. The $\Delta^{17}O$ values of NO_3^- gradually decreased with an increase in
149 depth, varying from +28.0 to +2.2 ‰ with a rate of −2.58 ‰ for each step (Fig. 1b).
150 Similarly, we assumed a gradual decrease with an increase in depth in the leaching
151 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
152 step) (Fig. 1c). This simulated the gradual net consumption of NO_3^- in accordance
153 with water flow in forested soils. The homogeneous soil was also divided into 10
154 layers in the vertical direction. The change in the leaching flux of NO_3^- with depth
155 was the same as that in the heterogeneous soil (Fig. 2c), whereas the $\Delta^{17}O$ values of
156 NO_3^- were constant at +2.2 ‰ in the layers (Fig. 2b).
157 Applying the total mass balance and isotope mass balance of NO_3^- shown in Eqs. 2
158 and 3 to each layer, we estimated the GNR (Figs. 1e and 2e) and total consumption
159 rate of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer. We assumed the
160 following. (1) The $\Delta^{17}O$ values of NO_3^- were constant in each layer. (2) The vertical
161 flow of NO_3^- in the soil layers proceeded downward from the surface to the final layer
162 (No. 10) with a uniform residence time in each layer. Finally, (3) the GNR and
163 consumption rate of NO_3^- (GDR + uptake) was 0 in the water layer. Thereafter, by
164 integrating the GNR determined for each layer, we estimated the total GNR in the
165 forested catchment.
166 The GNR estimated for the catchment with the homogeneous $\Delta^{17}O$ values in soil
167 NO_3^- 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. However, the GNR estimated for the catchment with the heterogeneous $\Delta^{17}O$ values in soil NO_3^- 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 $\Delta^{17}O(NO_3^-)_{stream}$. 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.

By combining the total mass balance and isotope mass balance shown in Eqs. 2 and 3, Eq. 7 was 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 "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 GNR

189 estimated from stream NO_3^- using Eq. 6 exceeded the GNR estimated from soil NO_3^-
190 using Eq. 7, to an extent. Therefore, the GNR estimated from Eq. 6 was overestimated
191 to an extent.
192 The linear variation in the leaching flux and $\Delta^{17}O$ values of soil NO_3^- used in the
193 simulated calculations (Fig. 1) is just one of many possible variations in forested
194 catchments. It is impossible to determine whether the linear variation was realistic or
195 not until the downward water flux, along with the concentration and $\Delta^{17}O$ value of
196 NO_3^-, was determined for each soil layer. However, the simultaneous observations of
197 the oxygen isotopes of soil NO_3^- and stream NO_3^- (Hattori et al., 2019; Osaka et al.,
198 2010; Nakagawa et al., 2018; Rose, 2014) implied that the approximation of the $\Delta^{17}O$
199 values of soil NO_3^- to that of the stream NO_3^- (Fig. 2b) was unrealistic.
200 If we estimate the downward water flux at each soil layer, with the NO_3^-
201 concentration and $\Delta^{17}O$ value of NO_3^- in each soil layer using, e.g., a tension-free
202 lysimeter (Inoue et al., 2021), we could estimate the vertical change in the leaching
203 flux of NO_3^- for each soil layer along with the $\Delta^{17}O$ of soil NO_3^-. Thereafter,
204 applying Eq. (7) to each layer, we can more accurately estimate the GNR for the
205 forested catchment, by integrating the GNR estimated for each soil layer with a more
206 accurate NO_3^- consumption rate of the forested catchment. Without such an
207 observation of the distribution and leaching flux of NO_3^-, with the $\Delta^{17}O$ values in
208 forest soil, the GNR estimated using Eq. (6), assuming that the $\Delta^{17}O$ values of soil

209 NO_3^- are always equal to those of stream NO_3^-, should be reported with significant
210 errors in which the possible variations in the $\Delta^{17}O$ values of soil NO_3^- are considered.
211
212 **4 Conclusion**
213 Past studies have proposed the $\Delta^{17}O$ method for determining the GNR in forested
214 catchments. The equations used in the calculation presuppose that the $\Delta^{17}O$ values of
215 NO_3^- consumed in forested soils are homogeneous and equal to those of the stream
216 NO_3^-. However, in reality, the values are often heterogeneous and do not always
217 equal those of the stream. It is essential to clarify/verify the $\Delta^{17}O$ values of NO_3^- in
218 forested soils and streams before applying the $\Delta^{17}O$ values of stream NO_3^- to estimate
219 the GNR.
220
221 *Data availability.* All data are presented in the Supplement.
222
223 *Author contributions.* WD, UT, and FN designed the study. WD and UT performed
224 data analysis and wrote the paper.
225
226 *Competing interests.* The authors declare that they have no conflict of interest.
227
228 *Acknowledgments*

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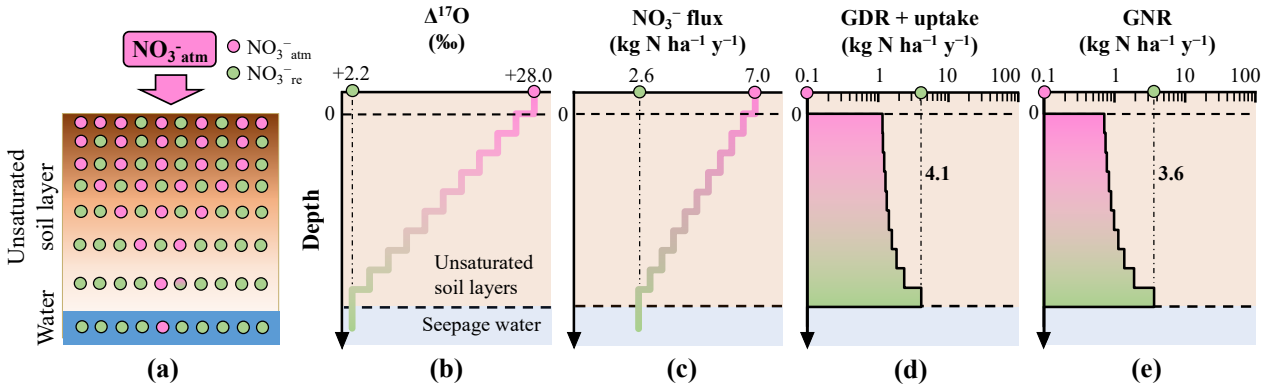


326 **Figure. 1**. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil with heterogeneous

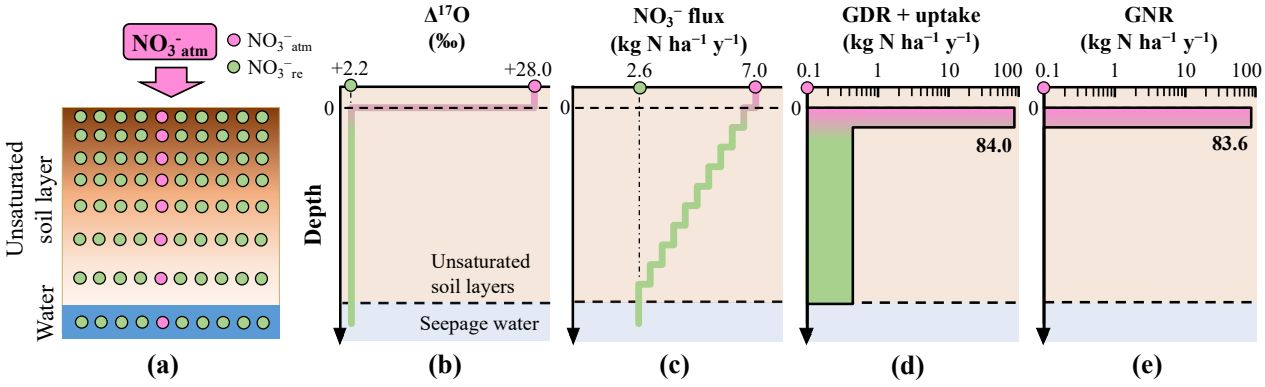
327 distribution of $\Delta^{17}O$ values of NO_3^- (a). Vertical distribution of the following

328 parameters in the forested soil: simulated $\Delta^{17}O$ values of NO_3^- (b), simulated leaching

329 flux of NO_3^- (c), estimated NO_3^- consumption rate (GDR + uptake) (d), and estimated

330 GNR (e).

331



332 **Figure. 2**. Distribution of $NO_3^-{}_{atm}$ in the simulated forested soil with homogeneous

333 distribution of $\Delta^{17}O$ values of NO_3^- (a). Vertical distribution of the following

334 parameters in the forested soil: simulated $\Delta^{17}O$ values of NO_3^- (b), simulated leaching

335 flux of NO_3^- (c), estimated NO_3^- consumption rate (GDR + uptake) (d), and estimated

336 GNR (e).