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Errors 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 catchment using the triple oxygen isotopic composition ($\Delta^{17}O$) of stream nitrate eluted from the catchment 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 through either assimilation or denitrification within the forested soil is equal to the $\Delta^{17}O$ value of 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 nitrate in the soil exhibited $\Delta^{17}O$ values larger than those in the stream while showing a decreasing trend with increasing depths until that of stream nitrate at the bottom. As 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 from each catchment, we concluded that the GNR estimated from the $\Delta^{17}O$ value of stream nitrate in the forested catchments was, to some extent, an overestimate of the actual GNR.

1 Introduction

Nitrate (NO_3^-) is one of the important nitrogen nutrients for primary production in forested ecosystems. Nitrification is the microbial process that produces NO_3^- in each forested ecosystem. Thus, quantifying the nitrification rate can assist in the evaluation of the present and future states of each forest ecosystem. While the net nitrification rate can be estimated from the increase in NO_3^- concentration during a certain period,

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

the gross nitrification rate (GNR), which includes the net nitrification rate and the metabolic rate of nitrate (e.g., 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 the net nitrification rate is negligible (Stark and Hart, 1997) while the metabolic rate is significant so that the GNR often exceeds the net nitrification rate by order of magnitude (Verchot et al., 2001).

Recently, several studies have successfully estimated GNR in water environments such as lakes, using the $\Delta^{17}O$ values of NO_3^- as a conserved tracer of the mixing ratio between the atmospheric nitrate ($NO_3^-{}_{atm}$) deposited into the water environment and the remineralized nitrate ($NO_3^-{}_{re}$) produced through nitrification therein (Tsunogai et al., 2011, 2018). Although $NO_3^-{}_{re}$ always shows the $\Delta^{17}O$ values close to 0 ‰ because its oxygen atoms derive from either terrestrial O_2 or H_2O through nitrification, $NO_3^-{}_{atm}$ always displays an anomalous enrichment in ^{17}O with $\Delta^{17}O$ values being approximately +26 ± 3 ‰ in Japan (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 the partial metabolism through denitrification or assimilation after deposition in a water column, $\Delta^{17}O$ can be used as a conserved tracer of $NO_3^-{}_{atm}$ to calculate the mixing

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

ratio of $NO_3^-_{atm}$ to total NO_3^- ($NO_3^-_{atm}/NO_3^-_{total}$) in a water column using the following equation:

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

where $\Delta^{17}O_{atm}$ and $\Delta^{17}O$ 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 $NO_3^-_{atm}$ into the lake, the GNR has been successfully estimated (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 GNR determination (Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020). 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 $NO_3^-_{re}$ from the stream, which can be determined from the $\Delta^{17}O$ values of NO_3^- in stream water eluted from the catchment, the GNR in each forested catchment has been estimated in a manner similar to the estimation for the water environments (Fang et al., 2015). Contrary to water environments, where the $\Delta^{17}O$ values of NO_3^- within the water layers are homogeneous and can be measured easily, it is often difficult to determine the $\Delta^{17}O$ values of the NO_3^- metabolized in soil layers. Consequently, past studies approximated the values to be equal to the $\Delta^{17}O$ value of stream NO_3^- eluted from each forested catchment without actual observation (Fang et al., 2015, Hattori et al., 2019, Huang et al., 2020). However, such an approximation should be conducted with

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

extreme caution, as the $\Delta^{17}O$ values of soil NO_3^- are not always equal to those of the stream (Hattori et al., 2019, Rose, 2014, Osaka et al., 2010). To clarify the details of the approximation along with its impact on the final estimated GNR, we present an accurate relationship between the $\Delta^{17}O$ of soil NO_3^- and GNR, starting from the basic isotope mass balance equations. Then, we present the GNR estimated for a forested catchment in which the $\Delta^{17}O$ values of NO_3^- in soil are measured. Finally, we compare 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 catchment 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 each catchment, gross nitrification rate in each catchment, leaching flux of NO_3^- from each catchment, uptake rate of NO_3^- in each catchment, and 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:

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

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

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 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^- in the forested soil layers, where the NO_3^- was metabolized through either assimilation (by plants and other organisms) or denitrification, are equal to the $\Delta^{17}O$ value of NO_3^- in the stream, 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 $NO_3^-{}_{re}$ produced through nitrification ($\Delta^{17}O(NO_3^-)_{nitrification}$) to be 0 (Michalski et al., 2003; Tsunogai et al., 2010):

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

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

Eq. 6 corresponds to the equation used in previous studies for quantifying the GNR in each forested catchment (Eq. 4 in Fang et al., 2015; Eq. 8 in Hattori et al., 2019; Eq. 4 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 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), the limited observations show that the oxygen isotopic ratios of soil NO_3^- are mostly higher than those of stream NO_3^-. For example, Hattori et al. (2019) reported that more than 60 % of the soil exhibited $\Delta^{17}O$ values significantly higher than those of stream NO_3^- determined simultaneously ($\Delta^{17}O$ =+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 in $\delta^{18}O$ in another forested catchment, from greater than +35 ‰ at the surface soil to less than +10 ‰ at depths of 30–50 cm from the soil surface (Osaka et al., 2010). Besides, most of the soil NO_3^- also exhibited $\delta^{18}O$ values higher than those in the stream (Osaka et al., 2010). Furthermore, Rose (2014) monitored the horizontal distribution of $\Delta^{17}O$ of soil NO_3^- by randomly setting 15 tension-free lysimeters at depths of 0–10 cm in a 39 ha

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

forested catchment, reporting $\Delta^{17}O$ values significantly higher in soil NO_3^- (+9.1 ± 5.8 ‰ on average) than in the stream NO_3^- (+0.5 ‰ on average) eluted from the forested catchment. As most of the root biomass is concentrated in the top 10 cm of the soil in forested catchments (Jackson et al., 1996), 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, imply that the estimated GNRs in the forested catchment obtained from Eq. 6 were inaccurate.

To demonstrate the impact of the differences in $\Delta^{17}O$ between soil NO_3^- and stream NO_3^- on the GNR, along with presenting the problems associated with the approximation to obtain Eq. 6, we estimated the GNR for two simulated forested soils—one with NO_3^- showing a decreasing trend in $\Delta^{17}O$ down to the $\Delta^{17}O$ of stream NO_3^- (heterogeneous soil) (Fig. 1a and 1b) and one with NO_3^- showing the same $\Delta^{17}O$ values as those of stream NO_3^- (homogeneous soil) (Fig. 2a and 2b). With Hattori et al. (2019) reporting 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 their forested catchment study, we adopted the same values in our calculation.

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), in which the $\Delta^{17}O$ values of NO_3^- gradually decreased with increasing depth, varying from +28.0 to +2.2 ‰ with a rate of decrease of +2.58 ‰ for each step (Fig. 1b). Similarly, we

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

assumed a gradual decrease with increasing depth in the leaching flux of NO_3^-, i.e., from 7 to 2.6 kg of N ha^{-1} y^{-1} with a rate of decrease 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 change with depth in the leaching flux of NO_3^- was the same as that in the heterogeneous soils (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 metabolic rate of NO_3^- (GDR + uptake) (Figs. 1d and 2d) in each layer assuming the following: (1) $\Delta^{17}O$ values of NO_3^- are constant in each layer, (2) vertical flow of NO_3^- in the soil layers proceed downward from the 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) is zero in the water layer (layers beyond the no. 10 soil layer). Then, by integrating the GNR determined for each layer, we estimated the total GNR in each forested catchment.

Although the GNR estimated for the catchment with the homogeneous $\Delta^{17}O$ values in soil NO_3^- was 83.6 kg of N ha^{-1} y^{-1}, exactly equal to that estimated by Hattori et al. (2019) using Eq. 6 (Fig. 2e), the total GNR was a much smaller 13.0 kg of N ha^{-1} y^{-1}, simulated for the catchment with the heterogeneous $\Delta^{17}O$ values in soil NO_3^- (Fig. 1e). Consequently, we conclude the following: (1) past studies estimating the GNR using Eq. 6 approximated the $\Delta^{17}O$ value of soil NO_3^- was homogeneous and always

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Preprint. Discussion started: 12 December 2023

equal to that of stream NO_3^- mathematically and (2) the differences between the $\Delta^{17}O$ values of the soil NO_3^- metabolized 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.

Note that 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 the forested catchments. It is impossible to decide whether the linear variation was realistic until the downward water flux, along with the concentration and $\Delta^{17}O$ values of NO_3^-, is 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) imply 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 $\Delta^{17}O$ value of stream NO_3^-, shown in Fig. 2b, is 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

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

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 using 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. Then, applying Eq. (7) in each layer, we can more accurately estimate the GNR for the forested catchment by integrating the GNR estimated for each soil layer together with a more accurate metabolic rate of NO_3^- (GDR + uptake) of the forested catchment. However, without such an observation of the distribution of the $\Delta^{17}O$ value of NO_3^-, 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 should be reported with 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 the GNR in each forested catchment. The equations used in the calculation presuppose that the $\Delta^{17}O$ values of NO_3^- in forested soils are homogeneous and equal to those of NO_3^- in the stream; however, in reality, the values are often heterogeneous and do not always equal to those corresponding to the stream. It is essential to clarify/verify the $\Delta^{17}O$ values of

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

NO_3^- in the forested soils and stream before applying the stream NO_3^- $\Delta^{17}O$ values 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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Preprint. Discussion started: 12 December 2023

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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.

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

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

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.

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.

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

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.

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023

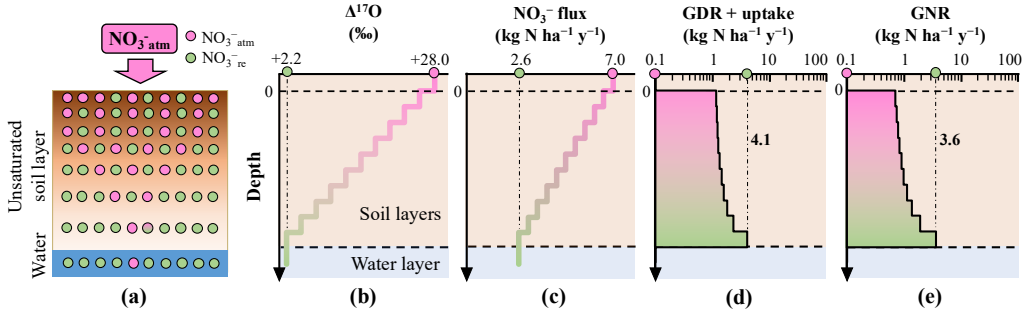
Tsunogai, U., Daita, S., Komatsu, D. D., Nakagawa, F. and Tanaka, A.: Quantifying nitrate dynamics in an oligotrophic lake using $\Delta^{17}O$, Biogeosciences, 8(3), 687–702, doi:10.5194/bg-8-687-2011, 2011.

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.

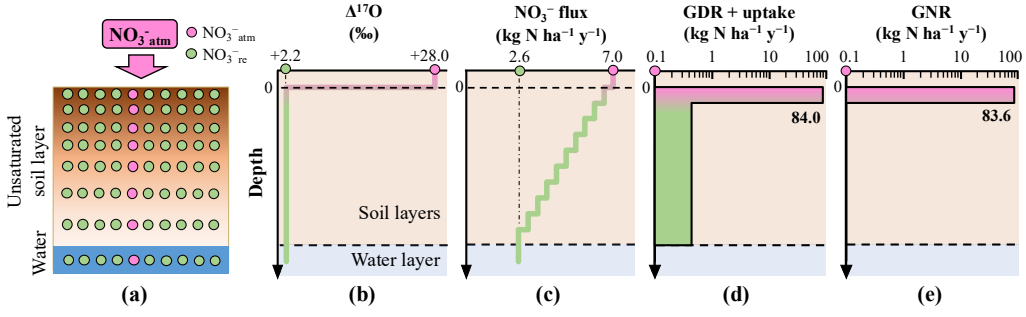
Verchot, L. V., Holmes, Z., Mulon, L., Groffman, P. M. and Lovett, G. M.: Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types, Soil Biol. Biochem., 33(14), 1889–1901, doi:10.1016/S0038-0717(01)00095-5, 2001.

https://doi.org/10.5194/egusphere-2023-2753
Preprint. Discussion started: 12 December 2023



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

309



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