

Dear Referee #1

Thank you very much for your valuable comments on our manuscript. We would like to respond to each of your comments and questions one by one.

We definitely can observed low M_{atm}/D_{atm} ratio if a forest is N limited and almost all precipitation nitrate is biologically processed. However, there are two exceptions. One is high precipitation may cause high M_{atm}/D_{atm} ratio due to limited contact time of precipitation nitrate with soil microbes and roots.

Thank you for your comment. Our conclusion was derived from FK, MY, and the past data ever reported in forested streams through continuous monitoring on $\Delta^{17}O$ (Table 3 in the Manuscript; Table 1 in this file), where the data of precipitation up to 3837 mm per year, average $[NO_3^-]$, and M_{atm}/D_{atm} ratio were included. While the stream nitrate concentration showed the strong linear relationship ($R^2 = 0.81$; $P < 0.0001$) with the M_{atm}/D_{atm} ratio (Fig. 3d in the Manuscript; Fig. 1a in this file), the amount of precipitation showed no linear relationship ($R^2 = 0.06$; $P = 0.48$) with the M_{atm}/D_{atm} ratio (Fig. 1b). Past studies have used the concentration of stream nitrate as one of the important indexes to evaluate the progress of nitrogen saturation in each forest (Aber, 1992; Huang et al., 2020; Rose et al., 2015; Stoddard, 1994). As a result, we concluded that the M_{atm}/D_{atm} ratio was mainly controlled by the progress of the nitrogen saturation, rather than the amount of the precipitation. We would like to mention this in the revised MS.

Table 1. The annual amount of precipitation (P), the average concentration of stream nitrate ($[\text{NO}_3^-]_{\text{avg}}$), the $M_{\text{atm}}/D_{\text{atm}}$ ratio, the $\Delta^{17}\text{O}$ of atmospheric nitrate, the $\Delta^{17}\text{O}$ of stream nitrate, and the gross nitrification rate (GNR) in the FK1, FK2, and MY, along with those in the catchments studied in past studies.

	Precipitation	$[\text{NO}_3^-]_{\text{avg}}$	$M_{\text{atm}}/D_{\text{atm}}$	D_{atm}	$\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{atm}}$	$\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{stream}}$	GNR
	mm	μM	%	$\text{mmol m}^{-2} \text{yr}^{-1}$	‰	‰	$\text{mmol m}^{-2} \text{yr}^{-1}$
FK1 ^a	1769	109.5	13.9	69.3	26.3	2.6	631.7
FK2 ^a	1769	94.2	7.9	69.3	26.3	1.7	1002.8
My ^a	3837	7.1	1.2	40.1	26.3	0.6	1718.3
KJ ^b	2500	58.4	9.4	45.6	26.3	1.5	759.3
IJ1 ^b	3300	24.4	6.5	44.5	26.3	1.5	735.7
IJ2 ^b	3300	17.1	2.6	44.5	26.3	0.9	1332.4
Fellow1 ^c	1450	17.9	3.6	23.4	21.3	1.9	236.6
Fellow2 ^c	1450	34.3	6.3	23.4	21.3	2.1	210.6
Fellow3 ^c	1450	60.0	10.3	23.4	21.3	1.5	311.1
Uryu ^d	1170	0.7	0.7	18.6	26.3	8.8	36.9
Qingyuan ^e	709	150.0	5.8	50	27.0	1.6	793.8

a: This study

b: Nakagawa et al., 2018

c: Rose et al., 2015

d: Tsunogai et al., 2014

e: Huang et al., 2020

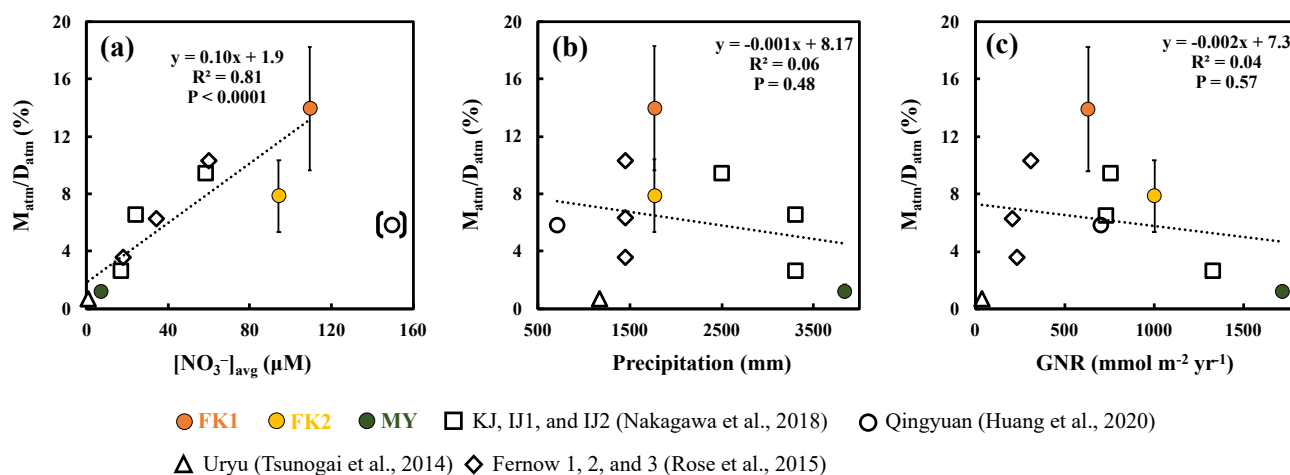


Figure 1. the $M_{\text{atm}}/D_{\text{atm}}$ ratio plotted as a function of the average concentration of nitrate ($[\text{NO}_3^-]_{\text{avg}}$) (a), the $M_{\text{atm}}/D_{\text{atm}}$ ratio plotted as a function of the precipitation (b) and the $M_{\text{atm}}/D_{\text{atm}}$ ratio plotted as a function of the gross nitrification rate (GNR).

The other is high soil nitrate production (gross nitrification rate), which can dilute of $\Delta^{17}\text{O}$ of precipitation nitrate that reaches the soil.

Thank you for your comment. For the aspect of calculating, the high or low gross nitrification rate (GNR) does not influence the annual export flux of NO_3^- (M_{atm}), and thus the $M_{\text{atm}}/D_{\text{atm}}$ ratio. For the aspect of the GNR influence the nitrogen saturation of forest and thus the $M_{\text{atm}}/D_{\text{atm}}$ ratio, we would like to discuss.

Past studies determined the gross nitrification rate (GNR) in the forested catchments based on the elution flux of unprocessed atmospheric nitrate and remineralized nitrate via stream, determined from the $\Delta^{17}\text{O}$ values of NO_3^- in stream water eluted from the catchment, and deposition flux of atmospheric nitrate into the catchment (Riha et al., 2014; Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020).

$$\text{GNR} = D_{\text{atm}} \times (\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{atm}} - \Delta^{17}\text{O}(\text{NO}_3^-)_{\text{stream}}) / \Delta^{17}\text{O}(\text{NO}_3^-)_{\text{stream}} \quad (1)$$

where D_{atm} denote the deposition flux of nitrate into the catchments, $\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{atm}}$ and $\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{stream}}$ denote the $\Delta^{17}\text{O}$ value of atmospheric nitrate and stream nitrate, respectively.

We compiled all past data ever reported in forested streams through continuous monitoring, where the data of D_{atm} , $\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{atm}}$, $\Delta^{17}\text{O}(\text{NO}_3^-)_{\text{stream}}$, GNR, and the $M_{\text{atm}}/D_{\text{atm}}$ ratio were included (Table 1 in this file). The GNR showed no linear relationship ($R^2 = 0.04$; $P = 0.57$) with the $M_{\text{atm}}/D_{\text{atm}}$ ratio (Fig. 1c). As a result, the GNR have no influence with the $M_{\text{atm}}/D_{\text{atm}}$ ratio.

The streamwater samples for the three forested catchments were collected in 2019 to 2021, while $\Delta^{17}\text{O}$ of precipitation nitrate used in the calculation was from the site Sado island in central Japan during 2009 to 2012. So the space and time both were mismatched between stream water sampling sites and precipitation sites. So it is better that authors justified the mismatch. In addition, the average of $\Delta^{17}\text{O}$ in precipitation nitrate were used. However, there are a number of studies reporting highly seasonal variation of $\Delta^{17}\text{O}$ in precipitation nitrate.

Thank you for your advice and question. We estimated the uncertainty derived from the difference in the locality as 1 ‰ (Nakagawa et al., 2018). This was based on the standard deviation between the annual average $\Delta^{17}\text{O}$ values determined in four different monitoring stations located in the same mid-latitudes, in the past studies such as La Jolla (33° N; Michalski et al., 2003), Princeton (40° N; Kaiser et al., 2007), Rishiri (45° N; Tsunogai et al., 2010), and Sado (38° N; Tsunogai et al., 2016). Besides, we estimated the uncertainty derived from the seasonal difference in the $\Delta^{17}\text{O}$ values of atmospheric nitrate as 1.8 ‰, based on the standard deviation of six-month moving averages of atmospheric nitrate determined at the Sado monitoring station. Adding an additional 0.2 ‰ as a margin, we adopted 3 ‰ as the possible error for $\Delta^{17}\text{O}$ atm in the streams (we mentioned that in Line 258-261 of manuscript). Additionally, the residence time of groundwater is longer than a few months for most forested catchments in Japan with a humid temperate climate (Takimoto et al., 1994; Kabeya et al., 2007). As a result, seasonal variation of the $\Delta^{17}\text{O}$ values of atmospheric

nitrate in the forested catchments in Japan will be buffered by groundwater and the uncertainty of 1.8 ‰ is enough for the seasonal difference in the $\Delta^{17}\text{O}$ values of atmospheric nitrate. In addition, Tsunogai et al. (2010) reported the $\Delta^{17}\text{O}$ values of atmospheric nitrate in Rishiri as +26.2 ‰ for 2006 to 2007. Tsunogai et al. (2016) reported the $\Delta^{17}\text{O}$ values of atmospheric nitrate in Sado island as +25.5 ‰ for 2009, +27.2 ‰ for 2010 and +25.7 ‰ for 2011. As a result, the temporal variation of the $\Delta^{17}\text{O}$ values of atmospheric nitrate can be negligible. We would like to clarify this in the revised MS.

We would like to thank you for the helpful comments and suggestions. We hope that our responses to your comments and questions are satisfactory.

Sincerely,
Weitian Ding
PhD student
Graduate School of Environmental Studies,
Nagoya University
Furo-cho, Chikusa-ku, Nagoya,
464-8601, JAPAN
Phone: +81-70-4436-3157
E-mail: ding.weitian.v2@s.mail.nagoya-u.ac.jp

Reference

- Aber, J. D.: Nitrogen cycling and nitrogen saturation in temperate forest ecosystems, *Trends Ecol. Evol.*, 7(7), 220–224, doi:10.1016/0169-5347(92)90048-G, 1992.
- 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.
- 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 $\Delta^{17}\text{O}$ 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.
- 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.

Kabeya, N., Katsuyama, M., Kawasaki, M., Ohte, N., and Sugimoto, A.: Estimation of mean residence times of subsurface waters using seasonal variation in deuterium excess in a small headwater catchment in Japan, *Hydrol. Process.*, 21, 308–322, 2007.

Kaiser, J., Hastings, M. G., Houlton, B. Z., Röckmann, T. and Sigman, D. M.: Triple oxygen isotope analysis of nitrate using the denitrifier method and thermal decomposition of N₂O, *Anal. Chem.*, 79(2), 599–607, doi:10.1021/ac061022s, 2007.

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

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.

Rose, L. A., Elliott, E. M. and Adams, M. B.: Triple Nitrate Isotopes Indicate Differing Nitrate Source Contributions to Streams Across a Nitrogen Saturation Gradient, *Ecosystems*, 18(7), 1209–1223, doi:10.1007/s10021-015-9891-8, 2015.

Riha, K. M., Michalski, G., Gallo, E. L., Lohse, K. A., Brooks, P. D. and Meixner, T.: High Atmospheric Nitrate Inputs and Nitrogen Turnover in Semi-arid Urban Catchments, *Ecosystems*, 17(8), 1309–1325, doi:10.1007/s10021-014-9797-x, 2014.

Stoddard, J. L.: Long-Term Changes in Watershed Retention of Nitrogen, , 223–284, doi:10.1021/ba-1994-0237.ch008, 1994.

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 $\Delta^{17}\text{O}$, *Atmos. Chem. Phys.*, 10(4), 1809–1820, doi:10.5194/acp-10-1809-2010, 2010.

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., Komatsu, D. D., Ohyama, T., Suzuki, A., Nakagawa, F., Noguchi, I., Takagi, K. and Nomura, M.: Quantifying the effects of clear-cutting and strip-cutting on nitrate dynamics in a forested watershed using triple oxygen isotopes as tracers, , (1), 5411–5424, doi:10.5194/bg-11-5411-2014, 2014.

Takimoto, H., Tanaka, T., and Horino, H.: Does forest conserve runoff discharge during drought?, *Transactions of The Japanese Society of Irrigation, Drainage and Reclamation Engineering*, 170, 75–81, https://doi.org/10.11408/jsidre1965.1994.170_75, 1994 (in Japanese with English abstract)