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Dr. Perran Cook
Editor of Biogeosciences

Title: Stable isotopic evidence for the excess leaching of unprocessed atmospheric nitrate from forested catchments under high nitrogen saturation
Authors: Weitian Ding et al.
MS No.: egusphere-2022-717

Dear Dr. Cook:

Thank you very much for handling our manuscript. We would like to thank the Referee #2 as well for the constructive comments on our manuscript. We have carefully studied the comments and revised the manuscript accordingly. We include below point-by-point responses to the comments, and detailed descriptions of the modifications we made to the manuscript. Besides, we also uploaded the revised manuscript in MS Word, in which all the revisions were recorded. We hope that with these changes you will find our revised manuscript appropriate for publication in your journal.

Sincerely yours,
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Response to the referee #2:

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.

1. **Reviewer #1 raises a significant point about the fact that high atmospheric N leaching rates can also be caused by hydrology (fast leaching rates) rather than biology (slow production rates). These two scenarios can be thought of as 'kinetic limitation' (not enough time for atmospheric N processing) v 'capacity limitation' (not enough biology to process all received atmospheric N), *sensu* (Lovett and Goodale, 2011). These two competing explanations could not be distinguished based solely on correlations with rainfall amounts. This is because transit time of NO3- through the canopy, soils, and vadose zone will depend on multiple factors, which include rainfall amount as well as soil types, vegetation root structures, and antecedent moisture conditions. The site descriptions, data analysis, and discussion need to be expanded to adequately address the kinetic limitation hypothesis for Matm/Datm dynamics.**

It is difficult to explain the high concentration of stream nitrate ([NO_3^-]) and the high export flux of nitrate (M_{total}) by "kinetic limitation" alone, even though high M_{atm}/D_{atm} ratios can be explained by "kinetic limitation" such as a rapid leaching rate, since the majority of nitrate eluted from the catchments was $NO_3^-{}_{re}$ that had been produced by microbial nitrification. Alternatively, "capacity limitation" can explain both high [NO_3^-] and high M_{atm}/D_{atm} ratios, simultaneously. Significant correlations ($P < 0.0001$) between M_{total} and M_{atm}/D_{atm} ratios in the eleven catchments supported "capacity limitation" as the leading cause of the high M_{total} in FK1 catchment.

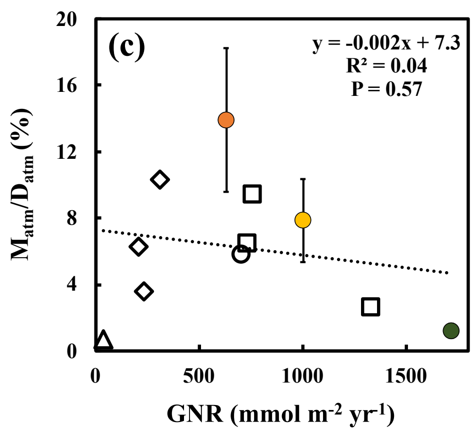
In addition, Chiwa (2020) reported the bulk deposition rate of atmospheric NO_3^- and NH_4^+ for recent ten years observation was 4.7 and 5.6 kg ha^{-1} yr^{-1} at FK catchments, respectively, and was 3.4 and 4.3 kg ha^{-1} yr^{-1} at MY catchment, respectively. On the other hand, the export flux of total nitrate (M_{total}) from FK1 and MY catchment was 13.8 and 3.3 kg ha^{-1} yr^{-1}, respectively. As a result, compared to MY catchment, FK1 catchment was a net source for N, which also suggest that FK1 catchment was 'capacity limitation' rather than 'kinetic limitation'.

Furthermore, the old age of the plantation in the FK1 catchment also supported that the catchment exhibited "capacity limitation" as opposed to "kinetic limitation".

We would like to add this discussion to the revised manuscript as follows (P24/L474-L486):

The differences in the residence time of water in each catchment could also impact the M_{atm}/D_{atm} ratio, as the residence time of water in forested catchments ranges from one month to more than one year (Asano et al., 2002; Farrick and Branfireun, 2015; Kabeya et al., 2008; Rodgers et al., 2005; Soulsby et al., 2006; Tetzlaff et al., 2007). It is difficult to explain high [NO_3^-] and high M_{total} eluted from the catchment by the residence time of water alone, while the M_{atm}/D_{atm} ratio could be higher in catchments with a shorter water residence time, as the majority of nitrate eluted from the catchment with a high M_{atm}/D_{atm} ratio was $NO_3^-{}_{re}$ produced by microbial nitrification. The significant correlation between M_{total} and M_{atm}/D_{atm} ratios ($P < 0.0001$; Fig. 3a) supported nitrogen saturation as the leading cause of high M_{total} in catchments with a high M_{atm}/D_{atm} ratio. Additionally, the high loading of atmospheric nitrogen, the type of plantation, and the old age of plantation in the FK1 catchment all supported the conclusion that the FK1 catchment was under the nitrogen saturation.]

2. **Based on Fig. 1 supplied in the response to reviewer comments there is a strong inverse relationship between gross nitrification rate and Matm/Datm (i.e., more nitrification means lower export of atmospheric N). It is only the inclusion of literature values that breaks down the relationship. So why is this? A robust discussion that addresses how (or how not) the high gross nitrification rates fit, or don't, the interpretation that Matm/Datm represents differences in catchment N saturation status.**



The figure was derived in response to a request from Reviewer #1, who concerned the obseved low demand on atmospheric nitrate, thus the high M_{atm}/D_{atm} ratio could be caused by high gross nitrification rate (GNR) in the catchments, FK1 in particular. We have discussed the hypothesis in our reply to referee 1.

First of all, the GNR and M_{atm}/D_{atm} ratios exhibited an inverse correlation instead of a positive correlation, which indicates that the hypothesis was not supported. In addition, the GNR in each catchment estimated from the $\Delta^{17}O$ of stream nitrate eluted from each catchment was generally inaccurate, as explained below. (Ding et al., 2023).

The GNR had been estimated by applying Eq. (1) (Riha et al., 2014; Fang et al., 2015; Hattori et al., 2019; Huang et al., 2020):

$$GNR = D_{atm} \times (\Delta^{17}O(NO_3^-)_{atm} - \Delta^{17}O(NO_3^-)_{stream}) / \Delta^{17}O(NO_3^-)_{stream} \quad (1)$$

where D_{atm} denote the deposition flux of atmospheric nitrate ($NO_3^-{}_{atm}$) into the catchments, $\Delta^{17}O(NO_3^-)_{atm}$ and $\Delta^{17}O(NO_3^-)_{stream}$ denote the $\Delta^{17}O$ value of $NO_3^-{}_{atm}$ and stream nitrate, respectively.

To obtain Eq. (1), $\Delta^{17}O(NO_3^-)_{stream}$ must be equal to $\Delta^{17}O$ of NO_3^- consumed in each catchment. The actual $\Delta^{17}O$ of NO_3^- consumed in each catchment (soil NO_3^-), however, is always higher than $\Delta^{17}O(NO_3^-)_{stream}$ in forested catchments (Hattori et al., 2019), so Eq. (1) always overestimates GNR (Ding et al., 2023). Almost all $NO_3^-{}_{atm}$ deposited onto MY catchment was consumed within the catchment contrary to the FK1 and FK2 catchments. As a result, the differences between $\Delta^{17}O(NO_3^-)_{stream}$ and $\Delta^{17}O$ of NO_3^- consumed in MY catchment should be larger than those in FK1 and FK2 catchments. Thus, Eq. (1) particularly overestimated GNR in the MY catchment.

Ding, W., Tsunogai, U., and Nakagawa, F.: 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, Biogeosciences Discuss. [preprint], https://doi.org/10.5194/bg-2022-236, in review, 2023.

3. **More details are needed in the methods section about how uncertainties were incorporated into the findings. The Matm/Datm calculations rely on several assumptions that needed to be made in order to account of lack of data (streamflow) or overlapping measurement periods (atmospheric sampling did not occur on the same years as stream water sampling). There are accordingly a number of significant sources of uncertainty incorporated into the Matm/Datm calculations: the relationship between precip amount and streamflow (which itself incorporates a number of uncertainties: the relationship between temperature and evapotranspiration, potential rate of loss to groundwater), the interannual consistency of 17O of atmospheric nitrate, and the spatial consistency in the amount of rainfall and the 17O of atmospheric nitrate. It is therefore essential to critically evaluate the potential magnitude of impact these assumptions have on the resultant Matm/Datm values. A sensitivity analysis needs to be performed for each parameter, and these ranges need to be clearly represented in the figures, tables, and text.**

We would like to include an appendix detailing the calculation of these uncertainties, as shown below (P19/L365-L367; P26-P28/L525-L555):

Appendix A: Calculating of uncertainties in the values of [$NO_3^-{}_{atm}$], M_{atm}, and M_{atm}/D_{atm} ratio

The uncertainty in the values of [$NO_3^-{}_{atm}$] was estimated from the uncertainties in the $\Delta^{17}O$ values of stream nitrate ($\Delta^{17}O$) and $NO_3^-{}_{atm}$ ($\Delta^{17}O_{atm}$) according to the divisive equation of error propagation (A1):

$$\sigma_{[NO_3^-{}_{atm}]} = [NO_3^-] * \sqrt{\left(\frac{1}{\Delta^{17}O_{atm}} * \sigma_{\Delta^{17}O}\right)^2 + \left(\frac{\Delta^{17}O}{\Delta^{17}O_{atm}{}^2} * \sigma_{\Delta^{17}O_{atm}}\right)^2} \quad \text{(A1)}$$

where $\sigma_{[NO_3^-{}_{atm}]}$, $\sigma_{\Delta^{17}O}$, and $\sigma_{\Delta^{17}O_{atm}}$ denote the uncertainties in $[NO_3^-{}_{atm}]$, $\Delta^{17}O$ values of stream nitrate, and $\Delta^{17}O$ values of $NO_3^-{}_{atm}$, respectively. The standard error of the mean (SE) of ±0.1 ‰ and the areal/seasonal variations of ±3 ‰ was used in calculating $\sigma_{\Delta^{17}O}$ and $\sigma_{\Delta^{17}O_{atm}}$, respectively. As a result, the uncertainty in $[NO_3^-{}_{atm}]$ ($\sigma_{[NO_3^-{}_{atm}]}$) was ±1.30, ±0.67, and ±0.03 µM at FK1, FK2, and MY catchments, respectively.

The uncertainty in the values of M_{atm} was estimated from the uncertainties in $[NO_3^-{}_{atm}]$ and in F_{stream} according to the multiplicative equation of error propagation (A2):

$$\sigma_{M_{atm}} = \sqrt{(F_{stream} * \sigma_{[NO_3^-{}_{atm}]})^2 + ([NO_3^-{}_{atm}] * \sigma_{F_{stream}})^2} \quad \text{(A2)}$$

where $\sigma_{M_{atm}}$, $\sigma_{[NO_3^-{}_{atm}]}$, and $\sigma_{F_{stream}}$ denote the uncertainties in M_{atm}, $[NO_3^-{}_{atm}]$, and F_{stream}, respectively. Komatsu et al. (2008) proposed the uncertainty in F_{stream} to be ±162.3 mm when using the water balance method in estimating F_{stream}. Here, the uncertainty in M_{atm} ($\sigma_{M_{atm}}$) was ±2.1, ±1.0, and ±0.1 mmol m^{-2} yr^{-1} at FK1, FK2, and MY catchments, respectively.

The uncertainty in M_{atm}/D_{atm} ratio was estimated from the uncertainties in M_{atm} and in D_{atm} according to the divisive equation of error propagation (A3):

$$\sigma_{M_{atm}/D_{atm}\ ratio} = \sqrt{\left(\frac{1}{D_{atm}} * \sigma_{M_{atm}}\right)^2 + \left(\frac{M_{atm}}{D_{atm}{}^2} * \sigma_{D_{atm}}\right)^2} \quad \text{(A3)}$$

where $\sigma_{M_{atm}/D_{atm}\ ratio}$, $\sigma_{M_{atm}}$, and $\sigma_{D_{atm}}$ denote the uncertainty in M_{atm}/D_{atm} ratio, M_{atm}, and D_{atm}, respectively. Comparing the deposition rate of $NO_3^-{}_{atm}$ obtained at the other atmospheric monitoring stations nearby, the uncertainty of 20 % was adopted for those of D_{atm} in each catchment, which corresponds to the uncertainty in D_{atm} of ±13.9, ±13.9, ±8.0 mmol m^{-2} yr^{-1} at FK1, FK2, and MY catchments, respectively. As a result, the uncertainty in M_{atm}/D_{atm} ratio was ±4.1 %, ±2.0 %, and ±0.4 % at FK1, FK2, and MY catchments, respectively.

These uncertainties were shown in the figures, tables, and text in the revised manuscript.

4. **I am still worried about the reliance on, essentially, rainfall and average annual catchment temperature to calculate downstream NO3- discharge. The**

relationship between rainfall amounts and stream discharge is generally highly complex, and affected by a number of factors such as catchment slope, soils, vegetation, and groundwater connectivity. These factors need to be robustly and quantitatively addressed (i.e., a hydrodynamic model is needed) given how important Fstream is to Matm, and thus the interpretation of systems as N saturated.

First of all, the variation in the stream water flux (F_{stream}) has small effect on the calculation of M_{atm} and M_{total} as compared to the variations of $[NO_3^-{}_{atm}]$ and $[NO_3]$ in monsoon regions with high precipitation, where the majority of rainwater elutes as stream water. Komatsu et al. (2008) compiled the precipitation, F_{stream}, and evapotranspiration (E) determined in 43 forested catchments in Japan (Fig. 1). The evapotranspiration (E = precipitation − F_{stream}) in the 43 forested catchments ranged from 109 to 1267 mm, with an average E of 733 mm and standard deviation (SD) of 218 mm, which corresponds to a 30% coefficient of variation (CV). In contrast, the CVs of $[NO_3^-{}_{atm}]$ and $[NO_3^-]$ compiled for this study were 99% and 92%, respectively. Consequently, $[NO_3^-{}_{atm}]$ and $[NO_3^-]$ in the stream water, and not F_{stream}, are the primary determinants of M_{atm} and M_{total}.

In addition, the water balance method in forested catchments has been well-established in previous research (e.g., Komatsu et al., 2008; Zhang et al., 2001; Harder et al., 2007; Combalicer et al., 2008; Milly, 1994), and the method has been used in quantifying the flux of stream water (F_{stream}) and evapotranspiration flux of water in numerous past studies (e.g., Wang et al., 2022; Che et al., 2022; Clark et al., 2014; Kozii et al., 2020). Komatsu et al. (2008) confirmed that the estimated F_{stream} derived from the water balance method is consistent with the F_{stream} observed in three forested catchments (Fig. 2). As a result, we employed the water balance method proposed by Komatsu et al. (2008) in quantifying the F_{stream} in the catchments. We would like to add the following information to the revised manuscript (P14/L259-L262):

They also confirmed that the estimated F_{stream} using the model corresponded well with the observed F_{stream} in three forested catchments, with the estimated errors of less than 6 %. As a result, we utilized the water balance method proposed by Komatsu et al. (2008) to quantify the F_{stream} in each catchment.

Additionally, Komatsu et al. (2008) proposed that the standard error when employing the method to estimate F_{stream} was 162.3 mm, which was factored into the uncertainty of M_{total}, M_{atm}, and M_{atm}/D_{atm} ratio in this study.

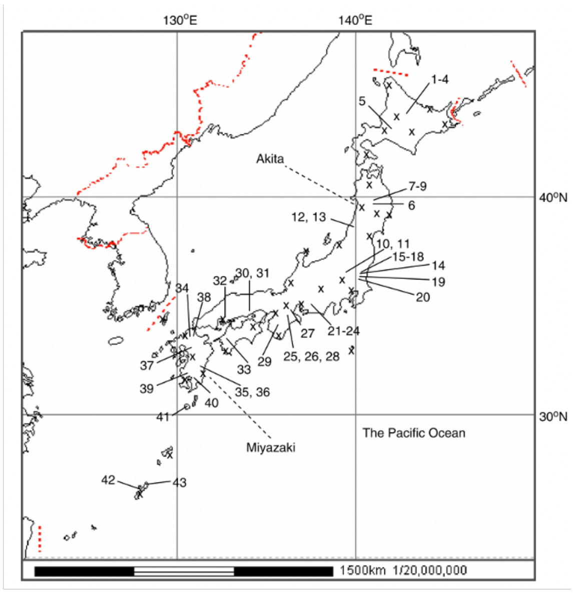


Figure 1. Locations of the 43 forest catchments compiled by Komatsu et al. (2008).

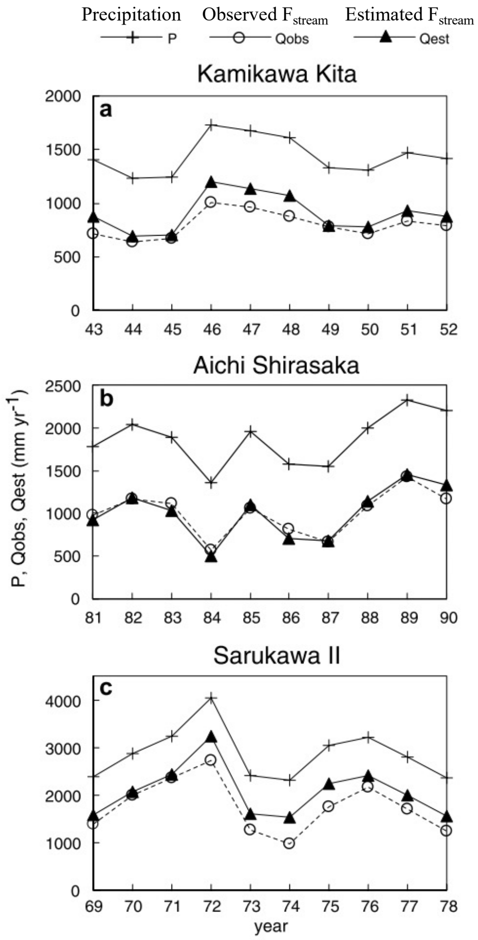


Figure 2. Comparisons between observed F_{stream} and estimated F_{stream} by applying the water balance method proposed by Komatsu et al. (2008) in three different forested catchments in Japan. Precipitation data P is also shown (Komatsu et al., 2008).

5. **As a consequence of the above (big) assumption that Fstream = precipitation – evapotransiration, the Matm/Datm ratio is essentially: $([NO3]stream*(P-E))/([NO3]bulk*P)$ (ignoring for a moment the calculations around dry and gaseous deposition). This really is then a almost directly a comparison of the concentration of 17O-NO3- measured in stream water over a few years relative to the concentrations of 17O-NO3- measured in the rain over the previous decade, with correction factor for the average annual temperature of the catchment (used to calculate E). Without a more robust approach to uncertainty and stream flow, and a more nuanced discussion of these uncertainties, it is hard to draw any conclusions about ecosystem N saturation from these values. It is also difficult to justify statistical analyses comparing temperature, precipitation, and discharge to Matm/Datm, given that all three parameters are directly used to calculate the ratio (and indeed that temperature and precipitation are themselves used to calculate discharge).**

The water balance method is well established in Japan, as was stated previously. In addition, the uncertainties associated with the estimated M_{atm}/D_{atm} ratios included all parameter-related uncertainties.

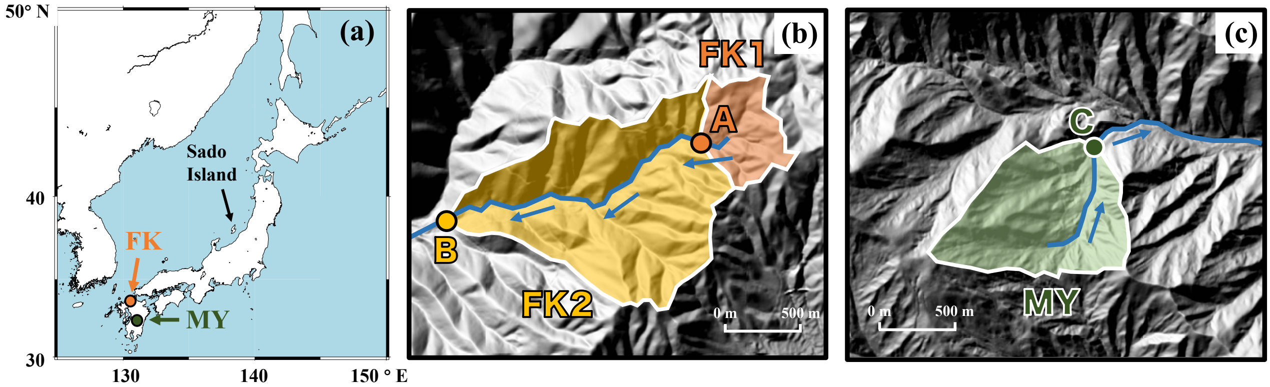
Significantly elevated $[NO_3^-{}_{atm}]$ and a high M_{atm}/D_{atm} ratio were found in stream water eluted from the FK1 catchment with significantly elevated $[NO_3^-]$ in this study. This discovery is without a doubt significant in elucidating the causes of the high $[NO_3^-]$ in the forested stream.

6. **I am still very confused about the relationship between FK1 and FK2. Are these in the same catchment or different catchments?**

Thank you for your questions. They are different catchments. Therefore, we have revised the manuscript to clarify this.

Does one flow into the other (referred to as upstream v downstream sites at some points), or do they flow off different sides of a ridge?

One flows into the other. We would like to add the stream flow direction to the revised map. The blue arrows indicate the flow direction of stream water.



If the latter, does this affect the amount of precipitation received at both sites? **If the former, should these really be considered as independent sites?**

Because there are significant differences between concentrations, $\delta^{18}O$ and $\Delta^{17}O$ of the stream nitrate in catchments FK1 and FK2 (all $P < 0.001$). Here, catchments FK1 and FK2 should be considered independent catchments.

It also seems the reliance on rain and temperature to determine flow would have a big impact here.

Because the central distance between FK1 and FK2 catchment was no more than 2 km, the differences in rain and temperature between FK1 and FK2 catchment can be ignored.

Are the streams actually the same size, as would presumably be determined by these calculations?

The flow rates measured at stations A and B on 2021/01/15 was 0.85 L/s (flow rate of FK1) and 4.75 L/s (flow rate of FK1+FK2), respectively. As a result, the stream flow rate of FK1 catchment was 0.85 L/s, and the stream flow rates of FK2 catchment can be calculated as 3.90 L/s, respectively. Because the relation between the measured flow rates was comparable with the relation between the catchment area of FK1 (14 ha) and that of FK2 (62 ha), we concluded that the measured flow rates on 2021/01/15 were reasonable. We have discussed this point in section 2.7 of the manuscript (P15-P16/L285-L313).

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

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