Dear 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.

It is difficult to identify a single driver for the differences in the proportion of atmospheric NO3- export between the two sites given that they differ both in terms of the amount of N deposition and their climate (the low deposition site receives significantly less rainfall and is significantly cooler than the high deposition site; L120-121). Differences in hydrology are not accounted for, but should be (e.g., both surface water – groundwater interactions and slope, both of which could impact N attenuation and the degree of stream water mixing with microbial NO3- sources).

Thank you for your comment and advice. Our conclusion was derived from FK, MY, and the past data ever reported in forested streams through continuous monitoring on $\Delta^{17}O$, where the data of amount of precipitation, average [NO_3^-], temperature, amount of discharge, and the M_{atm}/D_{atm} ratio were included (Table 1 in this file). 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. 1a), the amount of precipitation, temperature, and amount of discharge showed no significant relationship with the M_{atm}/D_{atm} ratio ($P > 0.12$; Figs. 1b, 1c, and 1d). Past studies have used the concentration of stream nitrate as one of the important indexes to evaluate the stage of nitrogen saturation in each forest (Aber, 1992; Huang et al., 2020; Rose et al., 2015; Stoddard, 1994). As a result, we concluded the M_{atm}/D_{atm} ratio was mainly controlled by the progress of the nitrogen saturation, rather than the amount of precipitation, temperature, and hydrology. We would like to mention these in the revised MS.

Table 1. The annual amount of precipitation (P), the average concentration of stream nitrate ($[NO_3^-]_{avg}$), amount of precipitation, average temperature, amount of discharge, and M_{atm}/D_{atm} ratio in the FK1, FK2, and MY, along with those in the catchments studied in past studies.

	$[NO_3^-]_{avg}$ μM	Precipitation mm	Temperature °C	Discharge mm	M_{atm}/D_{atm} %
FK1[a]	109.5	1769	15.9	894	13.9
FK2[a]	94.2	1769	15.9	894	7.9
My[a]	7.1	3837	10.8	3122	1.2
KJ[b]	58.4	2500	13	1276	9.4
IJ1[b]	24.4	3300	13	2057	6.5
IJ2[b]	17.1	3300	13	2057	2.6
Fellow1[c]	17.9	1450	9.3	567	3.6
Fellow2[c]	34.3	1450	9.3	450	6.3
Fellow3[c]	60.0	1450	9.3	578	10.3
Uryu[d]	0.7	1170	5.6	500	0.7
Qingyuan[e]	150.0	709	4.5	309	5.8

a: This study

b: Nakagawa et al., 2018

c: Rose et al., 2015

d: Tsunogai et al., 2014

e: Huang et al., 2020

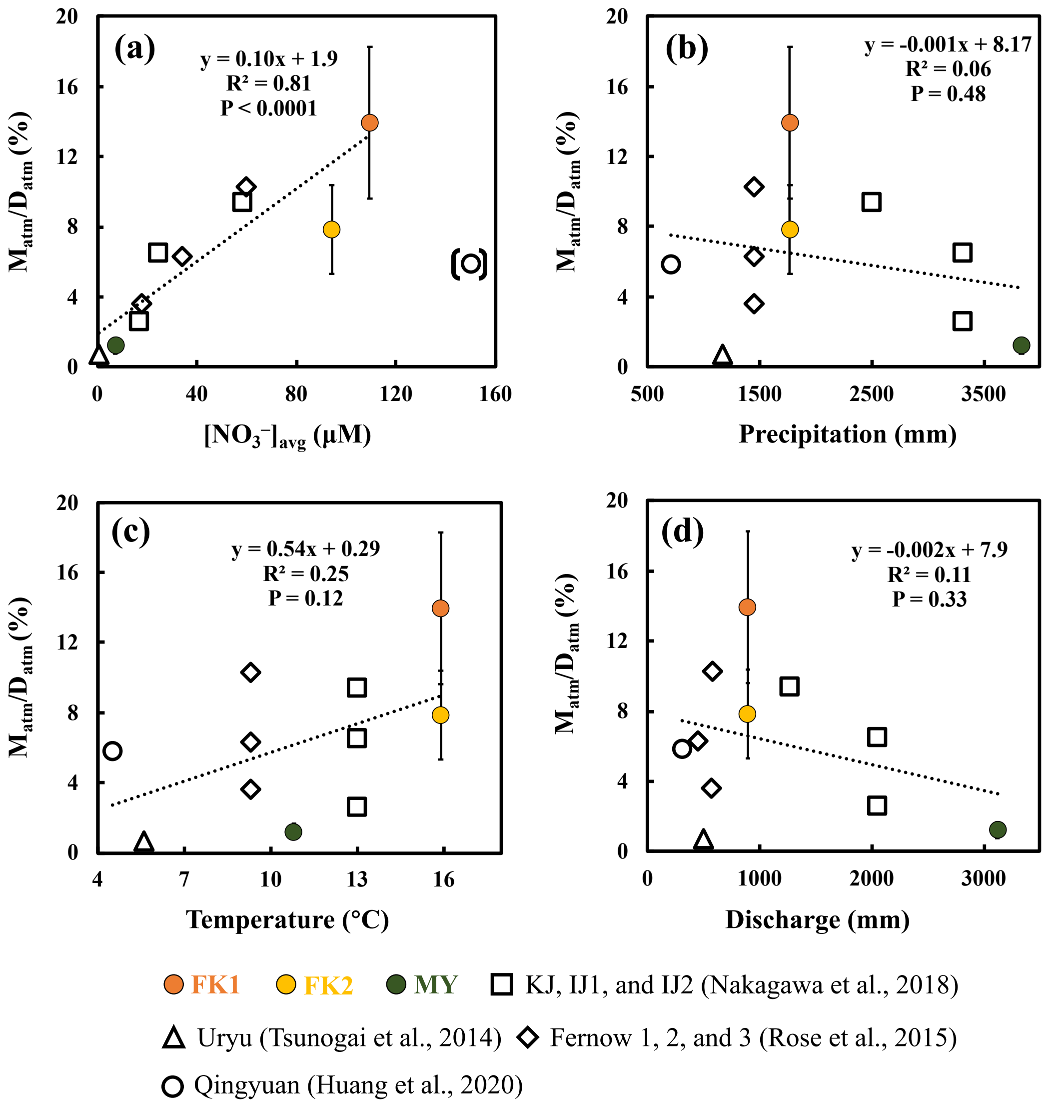


Figure 1. the M_{atm}/D_{atm} ratio plotted as a function of the average concentration of nitrate ($[NO_3^-]_{avg}$) (a), the M_{atm}/D_{atm} ratio plotted as a function of the amount of precipitation (b), the M_{atm}/D_{atm} ratio plotted as a function of the temperature, and the M_{atm}/D_{atm} ratio plotted as a function of the amount of discharge.

These also led to differences in vegetation between the two sites (L114-119).

Thank you for your comment. By compare the type and the age of plantations in FK1, FK2, and MY catchments, we concluded that the age and type of plantations caused the reduction in N uptake rates and thus increased of the nitrogen saturation and the M_{atm}/D_{atm} ratio in 4.2 section of manuscript.

The fact that FK has lower concentrations of atmospheric NO3- at the upstream site than the downstream does indicate that there is unaccounted for hydrologic mixing (or loss) occurring along the stream, which could significantly bias M/D estimates based on a single sampling point (as in the MY catchment).

Thank you for your comment. FK has higher concentrations of atmospheric nitrate at the upstream site than the downstream, insteadly (Table 3 in manuscript). The higher concentrations of atmospheric nitrate (or higher M_{atm}/D_{atm} ratio) in FK1 catchment than FK2 catchment indicated that progress of the nitrogen saturation was heterogeneities, even in a small area (< 100 ha). As a result, we only discussed the M_{atm}/D_{atm} ratio that the area can be covered by the ridgeline and sampling points in MY catchment (43 ha) and other forested catchments.

<u>The atmospheric deposition info used to calculate M/D (the crux of the study) were collected over 10 years, but these measurements ended prior to the stream water sampling that is the primary data here. This is a major limitation, given how much atmospheric N deposition can vary month to month and year to year. A robust approach to constrain the uncertainty created by relying on this 'mean' data is required.</u>

Thank you for your comment. Chiwa (2020) reported the bulk deposition rate of atmospheric nitrate (D_{atm}) as 4.7 and 3.4 kg ha^{-1} yr^{-1} for 2009 to 2018 in FK and MY catchments, respectively, which all the D_{atm} showed no temporal variation (decreased or increased trend during 2009 to 2018). The standard deviation (SD) and coefficient of variation (CV) were 0.9 kg ha^{-1} yr^{-1} and 16 % for FK catchments, 0.5 kg ha^{-1} yr^{-1} and 15% for MY catchment, respectively. Besides, 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). Thus, seasonal variation of D_{atm} in the forested catchments in Japan will be buffered by groundwater. In this study, we assumed the uncertainty of the D_{atm} as 20% (large than 16 % and 15 %) in FK and MY catchments, which is enough for the temporal variation in each forested catchment. We would like to mention this in the revised MS.

<u>Information is also needed on the exact location of the atmospheric sample collection relative to the streamwater collection sites (in particular for helping to assess whether there might be differences in atmospheric inputs at sites FK1 v FK2)</u>

Thank you for your advice. We would like to add the information in the revised MS. The GPS of the monitored sites of the atmospheric sample deposition were 33.638155, 130.516719 and 32.372358, 131.144182 for FK and MY forested catchments, respectively. The distance between the atmospheric monitored sites and stream sampling points were 3.9, 2.9, and 4.5 km for FK1, FK2, and MY forested catchments (Calculated from google map).

<u>L4: The abstract should be revised to start with establishing the 'big picture' issue addressed and aim of the study, rather than jumping straight in to site differences.</u>

Thank you for your advice. We would like to revise as suggested.

<u>L4-6: Here and elsewhere, I suggest referring to the sites by name rather than using acronyms, as this will make it easier to connect this to other work on the sites and more intuitive to follow within the manuscript.</u>

Thank you for your advice. We would like to add the name after the acronym in here in revise as suggested.

<u>L50: This line suggests that groundwater inputs are greater in humid temperate forests than other biomes, which is as far as I know not true.</u>

Thank you for your advice. We would like to revise this in revised manuscript.

<u>L66: Word missing after 'recent'</u>

Thank you for your advice. We would like to revise this in revised manuscript.

<u>L93-95: How could the validity of the approach be tested with the collected data?</u>

Past studies have reported that the forested catchments under the nitrogen saturated exported the elevated levels of nitrate, together with the high concentration of nitrate (Aber et al., 1989; Mitchell et al., 1997; Peterjohn et al., 1996). The higher concentration of nitrate and export flux of nitrate (M_{total}) in FK catchments compare to the KJ forested catchment, the maximum value of the M_{atm}/D_{atm} ratio before this study, implied progress of nitrogen saturation in FK catchments were sever. The higher M_{atm}/D_{atm} ratio in FK catchments supported the implication.

<u>Why is there reason to think that this method wouldn't work in catchments with higher rates of N deposition?</u>

Because concentration of nitrate and export flux of nitrate of FK forested catchments higher than the KJ forested catchment, where the M_{atm}/D_{atm} ratio was the highest prior to this study. While we expected high M_{atm}/D_{atm} ratio in FK forested catchments, we conducted this study to verify this.

<u>A clear hypothesis about how and why catchment retain v export atmospheric NO3- will be important for setting up a stronger discussion section.</u>

Thank you for your advice. We would like to add this in revised manuscript.

L96: Word missing after 'recent'

Thank you for your advice. We would like to revise this in revised manuscript.

L105-107: As above, it is not clear how the reliability of the M/D ratio can be evaluated using these methods. What results would show that it's unreliable?

If the M_{atm}/D_{atm} ratio would be lower in FK catchments than the other low export flux of nitrate (M_{total}) catchments, it was difficult to conclude that the M_{atm}/D_{atm} ratio is reliable as an index of nitrogen saturation.

L126: How were the boundaries between the FK1 and FK2 catchments determined? Fig. 1 indicates that these sites are both located along the same stream in the same catchment.

We would like to answer this question later.

L161-163: More information on internal standards needed (number, delta values, etc). Information on calibration for del17O also needed.

In this study, we used three kinds of the local laboratory nitrate standards, which were named to be GG01 ($\delta^{15}N = -3.07$ ‰, $\delta^{18}O = +1.10$ ‰, and $\Delta^{17}O = 0$ ‰), HDLW02 ($\delta^{15}N = +16.11$ ‰, $\delta^{18}O = +22.20$ ‰), and NF ($\Delta^{17}O = +19.16$ ‰), which the GG01 and the HDLW02 were used to determine the $\delta^{15}N$ and $\delta^{18}O$ of stream nitrate, and the GG01 and the NF was used to determine the $\Delta^{17}O$ of stream nitrate (Tsunogai et al., 2016; Nakagawa et al., 2018; Ding et al., 2022). The oxygen exchange rate between nitrate and water during the chemical conversion was calculated through Eq. (1):

$$\text{Oxygen exchange rate (\%)} = \Delta^{17}O(N_2O)_{NF} / \Delta^{17}O(NO_3^-)_{NF} \quad (1)$$

where the $\Delta^{17}O(N_2O)_{NF}$ denote the $\Delta^{17}O$ value of N_2O that convert from the NF nitrate, the $\Delta^{17}O(NO_3^-)_{NF}$ denote the $\Delta^{17}O$ value of NF nitrate ($\Delta^{17}O = +19.16$ ‰) (Tsunogai et al., 2016; Nakagawa et al., 2018; Ding et al., 2022). Thank you for your advising. We would like to clarify this in the revised manuscript.

L226-229: Were climate conditions (rainfall, stream flow, temperature) significantly different between the years where atmospheric N was measured v the years where stream N was measured?

We could not find significant differences in both rainfall and temperature between 2009-2018 (the years when atmospheric N was measured) and 2019-2021 (the years when stream N was measured). We compiled the rainfall and temperature during 2009 to 2021 based on the Japan Meteorological Agency at the nearest Fukuoka station (33°34′N, 130°22′E) and Miyazaki station (31°56′N, 131°24′E) (Fig. 2). There are no significant different of rainfall and temperature between 2009-2018 and 2019-2021 (t-test; all the $P > 0.21$). Because the stream flow was mainly controlled by the rainfall and temperature, we think the stream flow also have no significant different between 2009-2018 and 2019-2021. We would like to use the average value of them during 2009-2021 in the revised manuscript.

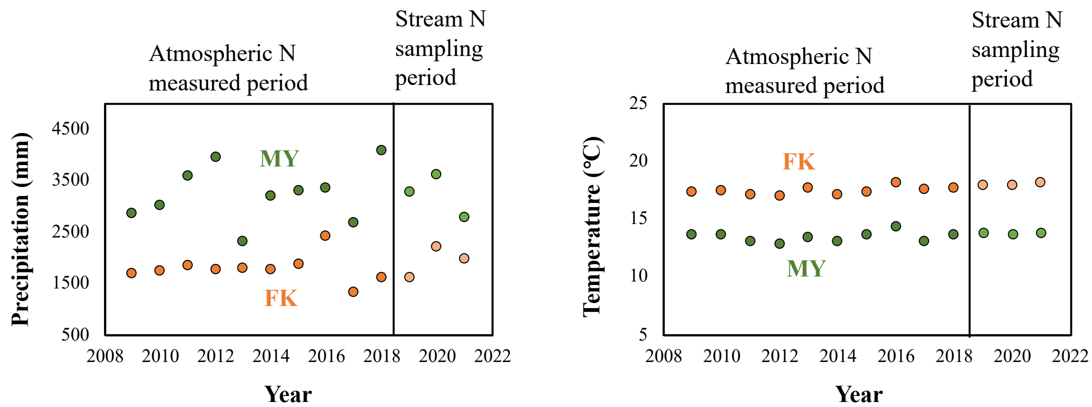


Figure 2. Temporal variations in the precipitation and temperature during 2009 to 2021 at Fukuoka province (orange) and Miyazaki province (green).

<u>**L234: Is this a reasonable explanation for the two sites? Some geologic / hydrologic information is needed to support this.**</u>

Yes. By using the water balance method ($E\ (mm) = 31.4T_{avg}\ (°C) + 376$), Komatsu et al. (2008) estimated the flux of stream water (F_{stream}) of three forested catchments in Japan for ten years. They found the estimated year-to-year F_{stream} were well corresponded to year-to-year observed F_{stream} variations in three forested catchments. The estimated errors were less than 6%, and R^2 values were higher than 0.91. Thus, the water balance method was reasonable.

<u>**L236: Given how important this value is for estimated M/D (L264), it would be illustrative to calculate stream flow based on a range rather than a single average value.**</u>

Komatsu et al. (2008) proposed the standard error when use the method to estimate the flux of stream water (F_{stream}). The standard error (range) was included in the calculated M_{atm}/D_{atm} ratio.

<u>**L273-275: Did rainfall differ between the two stream water sampled years? This would be useful information for helping interpret differences in NO3- over time.**</u>

No. We also could not find significant difference in rainfall of FK and MY catchments between 2009-2018 and 2019-2021 (Fig. 3) (t-test; all the $P > 0.16$). We would like to use the average value of rainfall during 2009-2021 in the revised manuscript.

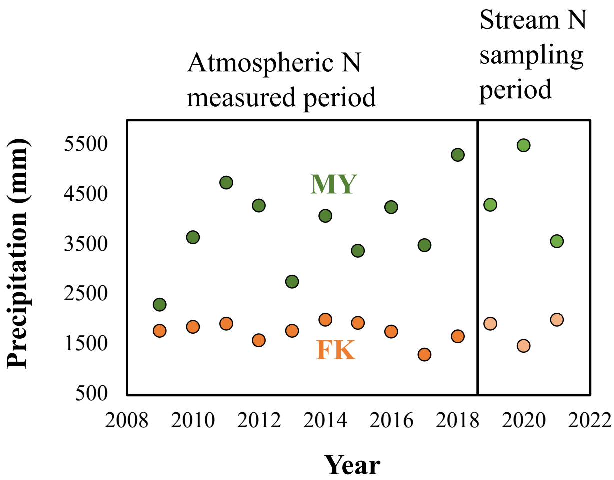


Figure 3. Temporal variations in the precipitation during 2009 to 2021 at FK catchments (orange) and MY catchment (green).

<u>L290: Report in more quantitative terms (what is 'little' variation?)</u>

Thank you for your advice. We would like to add the relationship information of the concentrations of stream nitrate and the time (month), together with the standard deviation (SD) and the coefficient of variation (CV) of them in the revised MS.

<u>L302-305: Move to Discussion.</u>

Thank you for your advice. We would like to revise as suggested.

<u>L325-329: What is the likely source of the 20% discrepancy? Is this due to differences in method (and if so how / what?) or genuine inter-annual differences in either N inputs or N retention? These points should be expanded on here.</u>

We think the environmental difference of observation site is likely source of the 20% discrepancy. The assumption should be verified by the observation. However, this is not the target in this study.

<u>L336-343: The collected data would need to be combined with more detailed meteorological information and/or isotopic modelling in order to determine the source of atmospheric N to the two sites. Consequently this explanation for the differences between the two sites is mostly speculation and does not have much baring on the overall aim of the study (to understand forest N saturation</u>

dynamics), so I suggest removing altogether or moving to the site description as part of the explanation for the known difference in N deposition rates between the two locations.

Thank you for your advice. We would like to revise as suggested.

L349: But how many locations has this been reported for? Given the relatively small dataset shown in Table 3 I wonder how surprising the relatively high M/D ratio is.

The average [$NO_3^-{}_{atm}$] of forested stream have reported by many past studies ((Bostic et al., 2021; Bourgeois et al., 2018b, 2018a; Hattori et al., 2019; Huang et al., 2020; Nakagawa et al., 2018; Rose et al., 2015; Sabo et al., 2016; Tsunogai et al., 2014, 2016). However, for calculating the M_{atm}/D_{atm} ratio, not only the average [$NO_3^-{}_{atm}$] was needed, the D_{atm} (deposition rate of atmospheric nitrate) and the flux of stream water were also needed. Some past studies have not reported the D_{atm} or the flux of stream water. Thus, the number of the forested catchments we compiled in the Table 3 of manuscript were smaller than the number of the forested catchments that reported the average [$NO_3^-{}_{atm}$] data we listed.

Is it likely that other sites around the world will have similar (or even higher!) ratios?

Yes. We expect the M_{atm}/D_{atm} ratios higher than the FK catchments in forested catchments where the progress of nitrogen saturation is more severe than the FK catchments. We would like to conduct the further observations in the future, when the COVID-19 become stable.

L353: What else besides Datm could cause the high concentration of NO3(atm) in the stream water? Alternative explanations (if they exist) should be discussed.

We assumed the happening of the snowmelt or storm events could also cause the high concentration of atmospheric nitrate in the stream water, because the happening of them could bring the atmospheric nitrate to the stream water directly. However, the number of happening of snowmelt in the FK and MY forested catchments can be negligible. Besides, the amount of the snowmelt is smaller than the amount of the precipitation significantly. Additionally, in our recent study, we concluded that the storm events have few impacts on the concentration of atmospheric nitrate in the stream (Ding et al., 2022). Thank you for your advice. We would like to add the information as suggested.

<u>L370-388: Beyond forest N uptake, what could cause catchment retention of N deposition? E.g., retention in soils or groundwater?</u>

In this study, the retention is included in uptake.

<u>L415-418: How does this finding compare to other parts of the world where precipitation is low but N deposition is high (e.g., parts of the southwestern US)?</u>

We compiled all past data ever reported in forested streams through continuous monitoring in Table 3, where the data of average [NO_3^-], average [$NO_3^-{}_{atm}$], M_{atm}, M_{total}, D_{atm}, and M_{atm}/D_{atm} ratio were included.

<u>L421-422: The relationship between precipitation and N losses really cannot be evaluated here given that the stream and precipitation data is decoupled (stream data collected after the precipitation sampling was concluded), and that dynamics are consequently evaluated only at a very broad timescale based on mean average annual precipitation and evapotranspiration for the two sites.</u>

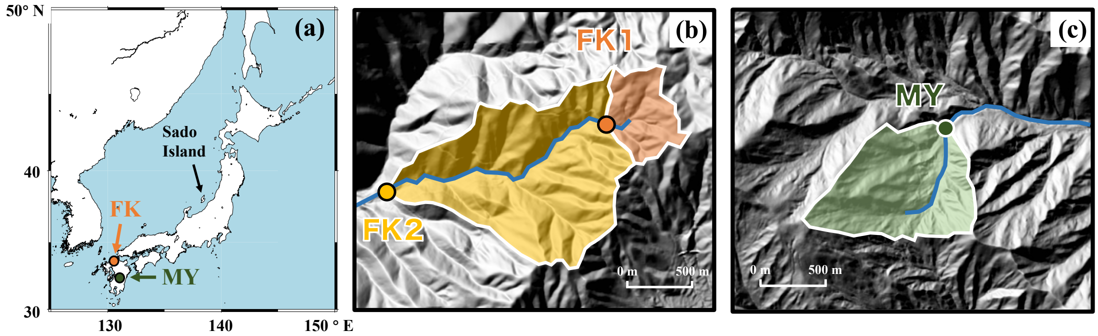
As we already presented, there was no significant difference in precipitation between 2009-2018 and 2019-2021 (t-test; $P > 0.21$) (Fig. 2 in this file). We would like to use the average value of precipitation during 2009-2021 in the revised manuscript. Besides, the uncertainty in D_{atm}, uncertainty in stream water flux, and uncertainty in concentration of unprocessed nitrate in the streams were included in the calculated M_{atm}/D_{atm} ratios. Because the M_{atm}/D_{atm} ratios in FK forested catchments were significantly large, even account for the uncertainties, the M_{atm}/D_{atm} ratios can be an index for evaluating nitrogen saturation.

<u>Fig. 1: This indicates that sites FK1 and FK2 are just two points along the same stream, meaning that they represent the same catchment. Some clarification is needed in the Methods and here to describe the hydrologic connection between the two locations and whether they should be considered upstream/downstream or two different sub-catchment (in which case this map should be updated to clearly show the catchments).</u>

On 2021/01/15, we estimated the flow rate of stream water (F_{stream}) at sampling point of FK1 and FK2 with the value as 0.85 L/s and 4.75 L/s, respectively, by using the salt dilution method (Sappa et al., 2015). According to the mass balance of water, we can estimate the F_{stream} of FK2 catchment as 3.90 L/s. The ration of F_{stream} (FK2) / F_{stream} (FK1) was 4.59. On the other hand, the ration of Area of FK2 (62 ha) / Area of FK1 (14 ha) was 4.43, which the value was comparable with the ration of F_{stream} (FK2) / F_{stream} (FK1). As a result, the increased F_{stream} (FK2) compared to the F_{stream}

(FK1) was origin from the FK2 forested catchment, and thus we think FK1 and FK2 are the different forested catchments.

Besides, we would like to update our map as follow as suggested:



In addition, we would like to update our data that relation to the FK2 by using Eq. (2):

$$X_{(FK2)} = (X_{(FK1+FK2)} * F_{stream(FK1+FK2)} - X_{(FK1)} * F_{stream(FK1)}) / F_{stream(FK2)} \quad (2)$$

where the $F_{stream(FK1)}$, $F_{stream(FK2)}$, and $F_{stream(FK1+FK2)}$ denote the flux of stream water of FK1, FK2, and FK1 + FK2, respectively. $X_{(FK1)}$, $X_{(FK2)}$, and $X_{(FK1+FK2)}$ denote the $[NO_3^-]_{avg}$, $[NO_3^-{}_{atm}]_{avg}$, M_{total}, M_{atm}, or the M_{atm}/D_{atm} ratio of FK1, FK2, and FK1 + FK2, respectively. The values of 0.85 L/s, 3.90 L/s, and 4.75 L/s were used as the $F_{stream(FK1)}$, $F_{stream(FK2)}$, and $F_{stream(FK1+FK2)}$, respectively. Thank you for your advising. We would like to add the new section of 2.7 to clarify these.

<u>L126: How were the boundaries between the FK1 and FK2 catchments determined? Fig. 1 indicates that these sites are both located along the same stream in the same catchment.</u>

Firstly, we determined the sampling point in the map by using the GPS data (33.39.31.2689, 130.32.55.0910 for FK1; 33.39.20.9586, 130.32.18.8808 for FK2) (Fig. 4a in this file). Then, we connected the ridge line and the upstream sampling point, which the area (orange) is the FK1 catchment (Fig. 4b in this file). Lastly, by using the same method, the FK2 catchment area was drawn in Fig 4c.

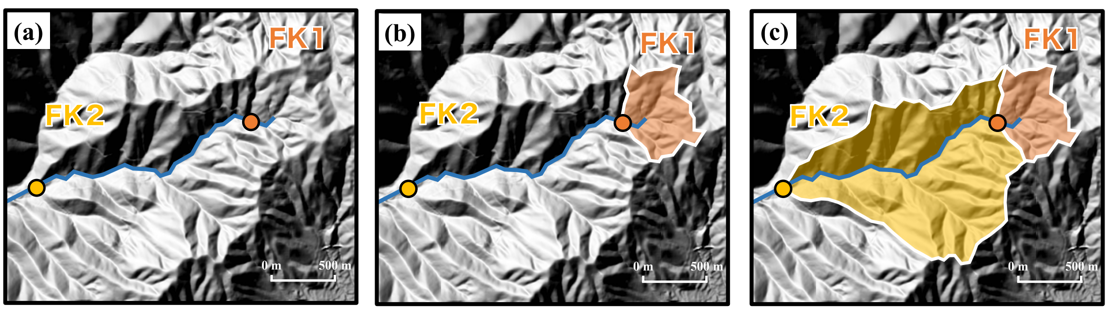


Figure 4. The maps showing how we determined the boundary line of the FK1 and FK2 forested catchments.

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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