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Discussions

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Interactive comment on "Accurate and precise quantification of atmospheric nitrate in streams draining land of various uses by using triple oxygen isotopes as tracers" *by* Urumu Tsunogai et al.

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Reply to your comment (Referee #1).

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

> The presentation quality (except for the figures) is mostly good, but there are a number of grammatical errors. Language copy-editing will be required.

The English of the manuscript was thoroughly edited by Editage English editing service

(http:// www.editage.jp/) prior to initial submission. We intend to have them edit the English again prior to submission of the revised manuscript.

> In line with the Biogeosciences data policy, all data (water fluxes, nitrate and nitrite concentrations, isotope values, etc.) should be publicly available, preferably by deposition in a data repository, or alternatively as electronic supplementary information. Please add a section on "Data availability".

We would like to present all the data in supplementary tables of the revised MS.

> The figures are somewhat antediluvian in their appearance due to the lack of colours. While the stated aim to make them compatible with black and white printer is commendable, most readers will either view them on a colour display or printed in colour. The figures should be redrawn in colour. The current use of different shapes to distinguish time series can be retained, for the benefit of the small number of readers without access to colour displays and printers, and for those with impaired colour vision.

Although, we cannot agree with you on this point, we would like to add colour to the figures during revision in response to your request.

> The figures in the supplementary information are discussed at crucial points and called out in the text. Fragmentation of the text into different documents is undesirable. They should be merged with the main text. The supplementary information should be used instead to present the full dataset in a table (water fluxes, nitrate and nitrite concentrations, isotope values, etc.), unless the authors can give a URL or DOI at a repository, at which they have lodged the data.

We would like to move Fig. S1 from the supplementary information to the main text in the revised manuscript, in response to your request.

> The treatment of systematic uncertainties and propagation of measurement uncertainties to derived properties is insufficient and/or has been insufficiently well presented. In particular, variations in the isotopic composition of the atmospheric nitrate

end member (delta17O, delta15N and delta18O) should be better documented and listed in a table. The resultant uncertainties in the calculation of the atmospheric nitrate fraction and the delta15N and delta18O values of the remineralised nitrate should be included in the text and figures of the paper.

The measurement uncertainties in the isotopic composition of nitrate (delta15N, delta18O, and Delta17O) in each sample were presented in Section 3.1 (L24–25/P8). The uncertainties in the isotopic composition of the atmospheric nitrate end member (delta15Natm, delta18Oatm, and Delta17Oatm) were presented and discussed in detail in Section 3.1 (from L17/P11 to L2/P13) and 3.3 (L14–18/P14).

The uncertainties in both absolute concentration of atmospheric nitrate (Catm) and the isotopic composition of the remineralized nitrate end member (delta15Nre, and delta18Ore) were simply calculated based on the propagation law of the errors, mostly derived from the errors in the values of delta15Natm, delta18Oatm, and Delta17Oatm in equations (2), (6) and (7). We would like to emphasize this in the revised MS.

The uncertainties in the data points were presented in the figures as well. The data points without error bars corresponded to errors smaller than the symbols. The only exception was Fig. 5, simply because the figure would be too complicated if we added error bars. We would like to emphasize this in the figure caption. Besides, we would like to add the errors in the tables (especially for those in supplement) of the revised MS in response to your request.

> A major systematic uncertainty arises from the current disregard of nitrate sinks and associated isotopic fractionation (section 3.5). Specifically, the delta15N and delta18O values of so-called "remineralised nitrate" may have undergone large modifications due to nitrate assimilation or denitrification. These processes have been currently ignored. While their effect on delta17O is most likely negligible, they cannot be discounted for delta15N and delta18O. In other words, assimilation and denitrification could have enriched "remineralised nitrate" in both 15N and 18O isotopes. The possibility of this

should be explored further, perhaps in the first instance using delta15N and delta18O scatter plots of the "remineralised nitrate" component. If there is a noticeable potential influence, this should be quantified as good as possible.

First of all, we did not disregard isotopic fractionations owing to partial removal through either nitrate assimilation or denitrification. Rather, we found significant isotopic fractionations owing to partial removal in a few streams in June and August, as presented in Section 3.5.

On the annual average, however, it was difficult to assume significant isotopic fractionations through assimilation or denitrification for the major portion of nitrate eluted from the watershed (see our discussions from L19/P14 to L19/P15), due to the low and uniform delta18O values of remineralized nitrate (i.e., delta18Ore) as presented in Section 3.3.

> The authors may also want to consider renaming the "remineralised nitrate" component as "residual nitrate".

We have been using "remineralized nitrate" since 2010 (Tsunogai et al., Atmos. Chem. Phys., 2010) to indicate nitrate produced through biogeochemical processing (including artificial processing) at surface (such as nitrification) showing Delta17O values almost 0‰. While it was very difficult for us non-native speakers to choose an appropriate English term, we discussed your recommendation for more than a week. Finally, we concluded that "remineralized nitrate" would be better to use, for the following reasons: (1) nitrate other than unprocessed atmospheric nitrate (such as those in soils, agricultural land, manure, and sewage), are mostly "remineralized nitrate" produced through microbial nitrification. (2) Almost the only exception [i.e. not (1)] within "remineralized nitrate" was fertilizer nitrate, which is produced in chemical plants through inorganic oxidation of ammonium and eluted from agricultural land without processing. This cannot be a major portion of "remineralized nitrate" in the streams, at least in Japan, where the rate of fertilizer application to agricultural land is strictly controlled. (3) While the

production reactions of fertilizer nitrate in chemical plants are different from the exact microbial nitrification, oxidation of ammonium can be classified as "nitrification" and the "nitrification" of N2-derived ammonium can be classified as "remineralization": producing inorganic nutrients from the other components. (4) Because we used "remineralized nitrate" again and again to indicate nitrate produced through biogeochemical processing (including artificial processing) at the surface (such as nitrification) showing Delta17O values of almost 0‰ in past studies (Tsunogai et al., 2011; 2014, Nakagawa et al., 2013), we would like to avoid confusion by using a different one here. (5) Because "residual nitrate" has often been used to indicate the residue of denitrification/assimilation reactions in a system, the term "residual nitrate" would be confusing. With all this in mind, we have continued use of the term "remineralized nitrate" with added clarification in the definition presented above.

> The term "17O anomalies" has been criticised for its subjectivity and biased nuances. A more neutral term, which has been adopted by many authors, is "17O excess" (similar to deuterium excess and thermodynamic "excess" properties; a negative "17O excess" reflects a deficit). Alternatively, "delta17O values" would also be acceptable.

We would like to use either "17O excess" or "delta17O values" in the revised MS.

> The term "mixing ratios" is ambiguous and should be replaced by "mole fractions" or "mass fractions".

We would like to use "mole fractions" in the revised MS.

> Averages and uncertainties should be enclosed in round brackets so that the unit applies to both, e.g. p. 2/3 "(2.3±1.1) μmol L-1", in line with standard practice in natural sciences.

We would like to enclose averages and uncertainties in round brackets in the revised MS, as suggested.

> In line with international conventions, chemical phase information should be added

in round brackets after the chemical formula, i.e. NO3-(atm) and NO3-(re), not as subscript index. Alternatively, text abbreviations should be used, e.g. AN for atmospheric nitrate and RN for remineralised / residual nitrate.

We would like to use NO3-(atm) and NO3-(re) in the revised MS, as suggested.

> Title: The words "accurate and precise" should be dropped from the title. The claimed "accuracy" would require an absolute measurement method and/or validation by an independent laboratory, neither fo which have been performed. "Precision" is not a meaningful metrological term. In any case, the quantification method has known significant systematic uncertainties, e.g. the isotopic composition of atmospheric nitrate deposition, temporal undersampling, etc., so neither the qualifications "accurate" nor "precise" apply.

Traditional quantification of atmospheric nitrate using the d18O values of nitrate has been done in many of the past studies. Compared with the traditional method using d18O values of nitrate only, our quantification of atmospheric nitrate using Delta17O was more accurate and more precise. Ohte et al. (2010) studied the same watershed using d18O values of nitrate, for instance, but could not quantify the concentrations of atmospheric nitrate in the streams. We used the words "accurate and precise" so as to differentiate our results from those of past studies.

> Abstract: A sentence should be inserted at the beginning of the abstract that explains the motivation for the present study. The current first sentence of the abstract is too long and should be split into two. Abstract p. 2, lines 14 to 18 should be moved up to before "We conclude ..." in line 4.

We would like to revise the abstract as suggested.

> Introduction: The discussion misses fertiliser inputs and groundwater recharge as nitrate sources - see also p. 12.

The inputs of fertilizer are included in "In addition to natural processes, anthropogenic

processes could have a significant impact on the nitrate dynamics within each catchment area, particularly for those eluted from urban or agricultural catchment zones (L29–31/P2)". We would like to revise this part to avoid misleading readers.

While groundwater is an important reservoir for nitrate, the nitrate in the groundwater had been derived from either internal microbial nitrification or addition from external sources including anthropogenic (fertilizer, manure, sewage etc.). Because all these were taken into account as the sources of nitrate in streams, we did not rate "the groundwater recharge/discharge" as the source of nitrate in this text to avoid double counting the sources. We would like to revise this part to avoid misleading readers.

> 4/9: Use of beta = 0.5247 is unusual. Most other research groups use 0.528, based on the meteoric water relationship (e.g. Savarino, Hastings, Michalski). The references given by the authors either do not express a preference (Miller 2002) or also use a coefficient of nearly 0.528 (Kaiser et al. 2007).

We are very sorry but 0.5247 was a typographic error. We changed the beta of nitrate in our calculation from 0.516 to 0.5279 in 2008, but typed 0.5247 in text. Thank you for pointing this out. We would like to revise this part.

> 4/12: This statement only applies to mass-dependent fractionation processes following a slope of 0.5247. It also does not apply to mixing applications because mixing follows linear, not power-law relationships. As in the present case, the authors are primarily using delta17O as a mixing tracer, a linear definition of delta17O would be preferable.

We cannot agree with you on this point. First of all, if either definition would be used, the difference in the final results would be much smaller in the case of nitrate. As a result, we would prefer to use the same definition as used in past studies (Tsunogai et al., 2010; 2011; 2014; Nakagawa et al., 2013) to avoid confusion. Second, atmospheric nitrate in steam nitrate had been isotopically fractionated, around +7‰ on the average (Fig. 6), and much more in some of the samples (Fig. 8). The most important merit

in using Delta17O tracer of nitrate is that we can assume the same Delta17O values during the isotopic fractionation processes, such as partial removal through assimilation/denitrification. The definition of the linear approximation would have abandoned this merit in principle.

Of course we should take into account the non-linear variation during mixing and thus we should correct to that extent. Similar corrections would be needed for the linear definition as well, in principle, by extrapolating the extent of fractionations. We prefer the definition using the power law, assuming appropriate ranges of errors during mixing, as done in this paper.

> At the least, the potential systematic error due to fractionating processes following other mass-dependent relationships (e.g. beta = 0.5) and the effect of linear mixing on the non-linear delta17O definition used here should be quantified and carried forward to the subsequent discussion.

We would like to add this in the revised MS.

> Sections 2.1 and 2.2 should be merged. Sections 2.1.2 and the last paragraph of sections 2.2 are highly repetitive.

We would like to make the suggested revision.

> The use of Japanese "financial years" should be abandoned in favor of the use of calendar years as mandated by the manuscript submission guidelines for Biogeosciences. All dates should be revised accordingly, as well as the calculation of annual averages.

We cannot agree with you to change the starting month for calculating each annual average from April to January. First of all, we could not find such mandate to obligate us to use January as the starting month of statistics in any part of the manuscript submission guidelines for Biogeosciences. Although the reason we started sampling on April was related to management, not science, starting from January has no scientific basis either. Besides, if we started the calculation from January 2010 and ended at

December 2011, the other data (from April to December 2009 and from January to March 2012) would have been excluded from calculation of annual averages without scientific reason. We do not think such statistical treatment is scientifically appropriate. If you do not like the term Japanese "financial year (FY)" with the provided in our paper, please recommend a more appropriate term to express a one-year period from April to March.

> 7/7-14: This discussion is confusing. At the least, it needs to be made clear that there is no 5th sample and that the authors have instead used the data from the previous year (March 2013) to calculate changes over the so-called "winter period", i.e. the difference between October 2013 and March 2013. Rather than using "sampling" numbers, the calculation scheme could perhaps be better presented in a table.

We would like to make the suggested revision.

> 7/28-8/2: The H2O isotope measurements referenced here have not been presented in the manuscript. Please include the results as a figure or table and include the data in the supplementary information.

We would like to add the delta18OH2O data in the supplements of the revised MS.

> 8/3-11: There are too many references given here. Please give just the one that documents the method used here.

We would like to reduce the number of citations, as suggested.

> The azide method is known to require larger isotope exchange corrections for oxygen isotope ratios. How large was the required exchange correction applied to the raw measurements?

The exchange corrections were about 20% (Tsunogai et al., Biogeosciences, 2011).

> 8/12-17: It is not appropriate to use ion chromatography as a reference method to determine the efficacy of N2O conversion. Ion chromatography can also be affected

by measurement biases. Nitrate standards should be used instead to check the azide method is working properly.

The measurement biases in nitrate concentrations using ion chromatography are estimated to be less than 5%, at least for the usual freshwater samples. (We would like to clarify this in the revised MS). Besides, because both the conversion efficiency from nitrate to N2O and the extent of fractionation could be a function of the sample matrix in some cases (Nakagawa et al., Biogeosciences, 2013), it must be better to use the nitrate concentration in each sample determined by Ion chromatography just after each sampling to check the conversion efficiencies, together with the other factors presented.

> Please explain how many samples have been excluded based on the ion chromatographically determined concentrations.

The samples excluded due to lower conversion efficiency were about 10% for all the analyses (measured by one of the graduate school students in our lab). Because this result is a function of the proficiency of the analyst, the value varied.

> 8/24: What "error" do these values represent? Are they the standard deviations of 3 replicate measurements?

They are the standard errors of the mean, in which the error of each single analysis was divided by the square root of the number of analyses. We have clarified this in the revised MS.

> 8/28: Please replace "approximation" by "definition". The definition of delta17O can be made in any arbitrary fashion; there is no approximation in a definition. However, of course, the interpretation of the resultant delta17O values may change, depending on the definition.

We would like to make the suggested revision.

> 9/4: A 5 % contribution of nitrite leads to a significant bias in the delta18O value

and cannot be neglected. The bias could be as large as -1.3 ‰ because the isotopic fractionation associated with conversion of nitrite to N2O is about 27 ‰ lower than for conversion of nitrate to N2O (e.g. Casciotti et al. 2007). All measurements need to be corrected for this bias, or re-analysed after NO2- removal (e.g. following the sulfamic acid protocol of Granger Sigman 2009).

More than 90% of the samples showed NO2- concentrations less than the detection limit (0.05 micro mol/L) and thus showing the NO2-/NO3- ratios less than 0.2%. The NO2- concentrations in the samples that could have NO2-/NO3- ratios more than 1% were also less than the detection limit. Because the NO3- concentrations also were low for the samples (as low as 1 micro mol/L), the possible maximum NO2-/NO3- ratios became 1–5%. As a result, we presented that "all samples showed NO2-/NO3- less than 5%".

Your suggestion to re-analyse after NO2 removal will not be successful because the NO2- was already less than the detection limit (0.05 micro mol/L) in the samples. It is also impossible to correct for NO2- contribution from the isotopic compositions as you suggested, because it is impossible to estimate the actual isotopic compositions of NO2- in the samples.

The NO3–exhausted samples showing nitrate concentrations < 5 micro mol/L were found only in summer (June or August) when the water flow rates were low. As a result, the values of delta15N and delta18O in these samples had little influence on quantifying the flow-weighted annual average isotopic compositions in each river in this study. As a result, we used the results without corrections, as was also done in most of the stable isotope studies of nitrate in freshwater/seawater in the past. We would like to add an explanation of this in the revised MS.

> 9/11-13: It is unclear how the monthly stream flow measurements are used together with the less frequent nitrate concentration and isotope measurements. Do you ignore the flow measurements from the months when no sampling has occurred?

Yes, we used the same flow rate, nitrate concentration, and isotopic compositions for the interval until the next observation. See equations (3) to (5).

> Do you interpolate the concentrations to match each flow measurement with a corresponding nitrate concentration or isotope delta value?

No. We use the same flow rate, nitrate concentration, and isotopic compositions for the interval until next observation. See equations (3) to (5).

> It seems that you ignore major parts of the flow (based on the counting index of 4).

Yes. This is the reason we used the correcting factor alpha in equation (10) when we estimated annual total flux.

> How much stream flow is "missed" due to this temporal under sampling?

Because we used 4 flow data out of 12 (annual), we missed 8. Again, this is the reason we used the correcting factor alpha in equation (10) when we estimated annual total flux.

> 10/13: Please replace "isotopic compositions" with "excess" - see above. "Composition" is not an extensive quantity.

We would like to make the suggested revision.

> 11/13-16: What are uncertainties / variability in the atmospheric end member values? Which values and which uncertainty did you actually use when you calculated the delta values of residual nitrate (see also p. 12, l. 26-31)?

We estimated the uncertainty derived from the difference in the locality as 1 per mil. This was based on the standard deviation between the annual average Delta17O values determined in four different monitoring stations located in the same mid-latitudes, in the past (La Jolla, Princeton, Rishiri, and Sado). Besides, we estimated the uncertainty derived from the seasonal difference in the Delta17O values of atmospheric nitrate as 1.8 per mil, based on the standard deviation of six-month moving averages

of atmospheric nitrate determined at the Sado monitoring station in this study (the six months corresponded to the minimum residence time of water in the watershed). Adding an additional 0.2 per mil as a margin, we adopted 3 per mil as the possible error for Delta17Oatm in the streams. We would like to add an explanation about this in the revised MS.

> Which error do you estimate the systematic neglect of dry deposition has caused?

As already presented in previous papers (Tsunogai et al., 2010; 2014), we regarded the isotopic compositions of nitrate in the wet deposition as those of total deposition because dry deposition occupied less than 20% of the total.

> 14/4: What do you mean by "almost correlated"? Please give a quantitative measure of what "almost correlated" constitutes.

We would like to add to the description of statistical treatments in the revised MS.

> 15/28-31: The last sentence does not make sense at the end of a paragraph. It should appear at the beginning of a new paragraph. You should then explain how you verified that the "remineralised" portion was actually responsible for the positive correlation with delta15N and population density.

What past studies (e.g. Ohte et al., 2010; Mayer et al., 2002) found was a positive correlation between delta15N of total nitrate (= atmospheric + remineralized) and population density. As presented in the Figure 7(c), in which positive correlation (r^2 = 0.64) between delta15N values of remineralized nitrate (y-axis) and population density (x-axis) can be found, we verified that the "remineralized" portion was responsible for the positive correlation between delta15N and population density. We would like to add the r^2 value (0.64) in the revised MS.

> 16/14: It is unclear why a uniform Catm (atmospheric nitrate contribution to the total nitrate concentration) is indicative for low denitrification. This could be offset of atmospheric inputs of equal magnitude.

Catm is the absolute concentration of atmospheric nitrate in each sample (L20/P4), and may be different from the "atmospheric nitrate contribution to the total nitrate concentration".

In any case, if the denitrification in riverbed sediments were active in streams having high population densities, Catm should be smaller in the streams, while the observed Catm were almost uniform irrespective of the population densities. Of course, the initial Catm could vary between streams. To explain the observed uniform Catm, however, unrealistic assumptions are needed, such as that the initial Catm were higher in accordance with the increase in population densities. To explain both the low and uniform d18O values of remineralized nitrate and the low and uniform Catm, we concluded that nitrate removal through denitrification in riverbed sediments should be minor in the streams. We would like to add an explanation about this in the revised MS.

> A more sensitive approach would be to look for a correlation (or absence there of) between delta15N and delta18O of "remineralised" nitrate.

We trust our approach was adequately sensitive.

> 17/23: delta15N measurements should be used to verify this hypothesis, especially incombination with delta18O (scatter plots).

We also used delta15Nre to verify that assimilation/denitrification was active or not, as suggested by you in our previous paper studying bottled drinking water (Nakagawa et al., 2013). In this study, we did the same but eventually gave it up, because the result was not so clear (low correlation). This was probably because the major factor controlling delta15Nre was not assimilation/denitrification but addition of 15N-enriched nitrate, and that it was active or not in each catchment, especially for those with high population densities, as presented in the previous Section 3.4.

We think the present evidence is sufficient to prove that assimilation/denitrification was responsible for the observed 18O-enrichment in some of the streams. To reduce the

number of figures and length of the text as requested by the other reviewer, we would like to avoid using delta15Nre to verify assimilation/denitrification.

> 22/17: Which value was used for alpha?

The alpha, estimated from equation (9), was 1.9.

> 23/15: Which value was used for gamma?

The gamma, estimated from equation (9), was 1.1.

> 23/25: Please speculate on the fate of nitrogen. Are they lost due to gaseous emissions (denitrification, anammox?) Sedimentary deposition? Eutrophication (secular nitrate concentration increases)?

Of course gaseous emissions should be substantial; otherwise, we should assume an unrealistic non-steady state in the lake. However, I don't think the other reviewer (#2) will allow us to describe such speculations in our paper without data.

> 25/19-25/25: This is not a conclusion, this is the premise/rational of the present paper. These lines should be deleted.

We would like to delete the lines suggested.

> The section Conclusions is incomplete. Seasonal and inter annual changes in stream nitrate concentrations and isotopic composition are missing. Also, the substantive section on the lake nitrogen budget is not reflected by a corresponding conclusion. I would expect a statement on the apparent nitrogen sink and possible identification including any relevant past or future work.

We would like to make the revision suggested.

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

Sincerely, Urumu

Cc: Drs. Takanori Miyauchi, Takuya Ohyama, Daisuke D. Komatsu, Fumiko Nakagawa, Yusuke Obata, Keiichi Sato, and Tsuyoshi Ohizumi

BGD

Interactive comment