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Comment

## ***Interactive comment on “Sources, fate and geochemical dynamics of nitrate in an oligotrophic lake” by U. Tsunogai et al.***

**U. Tsunogai et al.**

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Thank you for your comments on our manuscript. Prior to read our answers to your questions/criticisms, would you please reconfirm following two points concerning to our paper ?

(1) This paper was discussing “sources, fate and geochemical dynamics of NITRATE in a lake” as presented in the title, as well as in Introduction etc. Please note that our paper is not for those of fixed-N.

(2) The nitrate other than that supplied directly through atmospheric deposition was classified into remineralized nitrate (showing Detal17O values of zero), irrespective to the original supplying process as fixed-N onto the lake. That is to say, fixed-N that had

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originally supplied into the lake through atmospheric deposition but had become org-N through assimilation and then remineralized to nitrate was classified into remineralized nitrate, and not into atmospheric nitrate.

Thank you for your cooperation.

We would like to reply to you by citing each of your comment/question.

> (1) The study suffers from the confusion of interchanging concepts between source and sink terms to the entire lake system vs. internal N cycling in the water column: Atmospheric deposition consists of a source of fixed-N to the lake, in the form of nitrate, ammonium, or organic N. Assimilation is not a net sink of fixed-N but rather part of the lake's internal cycle, as fixed-N is neither created or loss but rather recompartimentalized by assimilation. Assimilation could be construed as an internal sink for nitrate, atmospheric or other, not to be confused with a net sink of fixed-N from the lake. Denitrification represents a net a sink of fixed-N to the lake system, as fixed-N is literally lost as N<sub>2</sub> gas. Input of newly nitrified NO<sub>3</sub> from the catchment is a net source of fixed-N to the lake, whereas nitrification of N remineralized within the lake is NOT a net source of fixed-N, but rather an internal source of NO<sub>3</sub>. These important distinctions are never considered in the text and result in flawed interpretation of the inherent isotope dynamics.

In response to your criticism, we checked throughout our manuscript. However, we could not find any sentences confusing the concepts as you stated. Throughout the Results and Discussion of this paper, we used the terms of “source” and “sink” only for those of NITRATE. Rather, we rarely commented on fixed-N during the Results and Discussion in this paper (limited to the sections 3.5 and 3.7) so that it was imcomprehensible for us that you persisted in the difference between internal/external source of fixed-N. If you insist that we are confusing, would you please clarify the position (page and line number) that we are confusing ?

Concerning to the catchment, we assumed that atmospheric nitrate deposited onto the

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whole catchment entered into the lake water column without reduction, including those deposited onto the area other than lake surface. Besides, contribution of remineralized nitrate from catchment to the lake was disregarded during the flux calculation. The reason will be presented to you later. Thus, atmospheric deposition was the only source of “external” NITRATE in this lake.

> (2) How did the concentration of NO<sub>3</sub> decrease below the pycnocline due to assimilation by phytoplankton? To which depth does light penetrate given a Secchi depth of 45 m? Penetration to 200 m seems unlikely.

The nitrate decrease below the pycnocline can be explained by the combination of both primary production (nitrate assimilation) below the pycnocline and vertical mixing of water, as presented in P7240 (lines 17-25). As you can see in the vertical profile of chl-a (Fig. 3(b)), the primary production was active even for the depths until 100 m in the highly transparent lake. Because the stratification below 50 m is unstable even during the summer in the lake as you can infer from the uniform temperature profile in Fig. 3, we can anticipate active vertical mixing of nitrate through both vertical advection and eddy diffusion in the water column. The detailed discussions for the vertical mixing within the lake water column have been made in Nojiri et al. (1990) in the reference list, by calculating density profiles in the lake, including their temporal variations.

> (3) How come there is no O<sub>2</sub> peak associated with the chlorophyll maximum in August? How come there is no O<sub>2</sub> depletion due to remineralization at depth, which is evidenced by the decrease in the d18O of nitrate and the  $\Delta^{17}\text{O}$ ?

That's because the lake was highly oligotrophic. Even if all the nitrate in a water mass (ca. 1.5  $\mu\text{mol/L}$  in June) would have been assimilated through photosynthesis (primary production), DO increase is only +11  $\mu\text{mol/L}$  (calculated assuming the Redfield ratio), which corresponds to ca. +3% of air saturation of 4 °C water. And vice versa for the respiration (remineralization). Even if nitrate would increase from zero to 1.5  $\mu\text{mol/L}$ , DO decrease is -3%. Besides, DO level could become more closer to air saturation

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through gas exchange if the water mass is located closer to surface. In conclusion, the maximum extent of variation from air saturation is around 3% for DO in the lake, so that we can find the almost stable values (i.e. close to air saturation level at the temperature of hypolimnion) over the depth.

> (4) How do the authors calculate an isotope effect for assimilation in the lake? First, which assumptions are made? Is the lake treated as a closed system or an open system?

Assuming closed system. Besides, co-incident biological transformations other than assimilation were disregarded. We would like to clarify this in the revised manuscript.

> Second, how do the authors account for co-incident biological transformations that can alter the d15N and d18O of nitrate? While assimilation enriches the d18O of NO<sub>3</sub>, in situ nitrification in the deeper layers will tend to decrease the d18O of NO<sub>3</sub> toward the d18O of ambient water (what is the d18O of ambient water?). Denitrification occurring in sediment will tend to reduce the concentration of NO<sub>3</sub><sup>-</sup> without necessarily altering the d15N and d18O of NO<sub>3</sub> in the water column. Addition of atmospheric NO<sub>3</sub><sup>-</sup> will increase the d18O substantially in the surface layer, and will modify the d15N in the direction of the d15N of atmospheric NO<sub>3</sub> (What is the d15N of atmospheric NO<sub>3</sub>-deposited on the lake??). Similarly, nitrification of NH<sub>4</sub> deposited on the lake from the atmosphere will lower of the d18O and of the d15N relative to the enrichment from assimilation (assuming a low d15N of atmospheric NH<sub>4</sub><sup>+</sup>). Drainage of NO<sub>3</sub><sup>-</sup> from the catchment can also modify the d15N and d18O of water column NO<sub>3</sub> in a direction that cannot be anticipated without actually measuring it. In short: the reviewer cannot see how the 15ε and 18ε estimates put for th are meaningful or accurate in any way.

All the answers to your questions have been presented in sections 3.1, 3.3, and 3.5 of the submitted MS.

Our purpose to calculate the kinetic isotope effects (15ε and 18ε) is NOT to estimate accurate values for those during assimilation. Rather, we want to discuss our hypothe-

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sis that the MAJOR process to reduce nitrate from June to August was assimilation by phytoplankton/periphyton in the lake (P7238/L2-8).

We calculated kinetic isotope effects (15e and 18e) for the observed nitrate decrease between June and August (P7240/L2), assuming closed system for the lake and found that the kinetic isotope effects were reasonable values for those during assimilation of nitrate. Thus, we concluded that the MAJOR process to reduce nitrate from June to August as assimilation. This was also supported by the uniform average  $\Delta^{17}\text{O}$  of nitrate in the lake.

Of course the other minor processes which you persisted (nitrification and atmospheric deposition) should also control nitrate distribution in the lake, so that we discussed them in section 3.1 (P7240/L12-P7241/L10) and then quantified them in sections 3.3. Through these quantification, we further verified that the major process that controlled nitrate was assimilation during the observations.

> (5) Why is the  $\delta^{15}\text{N}$  of PN substantially more elevated than the  $\delta^{15}\text{N}$  of the nitrate from which the PN derives? The authors make no mention of this ĩŃagrnt disconnect.

All these had been already discussed in cited references (McCusker et al., 1999; Finlay et al., 2007 etc.) so that we didn't want to make further detailed discussions in this paper, because  $^{15}\text{N}$ -depleted  $\delta^{15}\text{N}$  data of nitrate in oligotrophic environments were not so novel in these days. In short, the  $\delta^{15}\text{N}$  of PN was different from phytoplankton that assimilate nitrate. Rather, PN included highly fractionated N that enriched in  $^{15}\text{N}$  (PN from  $^{15}\text{N}$ -enriched ammonium and/or residual N during remineralization). We would like to add a few sentences in the revised MS for those who are not familiar with this.

> (6) p. 7244, 1st: Very confusing. The authors make no distinction here between net and gross, internal vs. external sources of nitrate from the catchment vs. in situ nitrification. For instance, the authors state that "0.52 Mmoles of remineralized nitrate

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had been fed into the lake water through nitrification.” First of all, “remineralized” nitrate means “newly nitrified,” such that nitrification of remineralized nitrate is nonsensical.

All the values of flux presented in this page were gross. These have been clearly stated in the previous page (P7243/L10). Besides, all the results of both net and gross have been compiled in Table 2, so that your criticism was a puzzle to us. I would like to beg you to check all the pages have been included in your downloaded file.

In this paper, the words “remineralized nitrate” is used for nitrate produced through nitrification, irrespective to new (in situ) or old (the other), because the equivalent Japanese word have such meaning. If this was not adequate in English, would you please recommend us more appropriate word?

> Second, does this statement mean that remineralized nitrate enters the lake from the catchment, in which case it is an external source of fixed N to the lake, or does that mean that it is remineralized in situ from organic material (PN), in which case it is an internal source of NO<sub>3</sub> but NOT necessarily a source of fixed N to the lake mass balance (depending on whether PN is allochthonous or autochthonous, which is unclear)?

Concerning to the catchment, we assumed that atmospheric nitrate deposited onto the whole catchment entered directly into the lake water column without reduction during the mass balance calculation (P7244/L6), including those deposited onto the area other than lake surface (most of which was occupied by the caldera wall in this lake), because more than 60% of the total catchment was lake surface (P7232/L2). Besides, most of the caldera wall have rocky ground, where active assimilation/denitrification were difficult to assume.

Eventhough, some of remineralized nitrate could be added to the lake from the catchment (= caldera wall), as you suggested. Thus, in strict speaking, remineralized nitrate in the lake water could be supplied not only from in situ nitrification in the lake (i.e. nitrification in the water column and the sedimentary layer) but also from nitrification in the caldera wall area. This was the reason we stated “production of NO<sub>3</sub>-(re) through

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nitrification in and AROUND the lake” (P7241/L8).

If this contribution of remineralized nitrate from catchment could be significant, we must reduce this contribution from the estimated total gross flux of nitrification ( $\Delta N_{nit}$ ) to estimate accurate flux of those within the lake. Actually, however, we disregarded the contribution of remineralized nitrate from the catchment during calculation due to the reasons shown below:

The outflux of fixed-N from a natural pristine ecosystem such as the caldera wall area must balance with influx of fixed-N under steady state condition. Concerning to the influx of fixed-N in a natural ecosystem in these area, deposition of atmospheric nitrate to the caldera wall area ( $0.047 \times 0.6$  Mmol in two months) occupy ca. 40% of total fixed-N influx (Tsunogai et al., ACP, 2010). Thus, even if all of the outflux would be eluted as nitrate, the maximum flux corresponds to ca. 0.07 Mmol ( $=0.047 \times 0.6 / 0.4$ ) in two months.

The possibly maximum outflux of remineralized nitrate from the catchment (0.07 Mmol) was less than 15% of that of the total gross flux of nitrification in and around the lake ( $(\Delta N_{nit} = 0.52 \pm 0.34$  Mmol; P7244) and even less than the error of the estimation. Furthermore, both ammonium and org-N usually occupied substantial portion (more than 70%) of fixed-N outflux from a natural pristine ecosystem. Thus, we disregarded the remineralized nitrate supplied from the catchment in our calculation and concluded that the estimated fluxes ( $\Delta N_{nit}$ ) corresponded to those in the lake.

We would like to add several sentences to clarify these in the revised manuscript.

> (7) The authors contend that: “2.6 Mmol of nitrate has been removed from the lake water through assimilation during the period between sampling events.” First of all, the authors do not have the  $\Delta PN$  and  $\Delta d_{15}N$  PN to justify this claim, but apparently forgot to sample PN in June.

Even if we had sampled PN and analyzed  $d_{15}N$  in June, it was impossible to make mass  $^{15}N$  balance because some PN could be removed to sedimentary layer, or be

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changed to dissolved N (including ammonium), all of which had been difficult to make sampling and/or d15N analysis.

> Second, is that NET or GROSS PN production?

Because the flux was gross removal flux of nitrate, it must be GROSS PN production from NITRATE.

> One could imagine that much of the fixed N assimilated into particles at the surface since June had been remineralized since, thus contributing to the “newly nitrified” NO<sub>3</sub>, and no longer sequestered as particles. Because there is no estimate of the ΔPN or alternatively, the [NO<sub>3</sub>] delivered from the catchment, it is IMPOSSIBLE to construct an accurate mass balance here.

In case of fixed-N in the lake, it might be difficult to construct an accurate mass balance as you stated. As for NITRATE as presented in this manuscript, however, it was POSSIBLE to construct accurate mass balance using Delta17O, as presented in the manuscript. We don't want to repeat the detail here. But please note that, while total nitrate in the lake had the observed 17O-anomalies (showing Delta17O values around +2.5 per mil), the “newly nitrified” NO<sub>3</sub> had the Delta17O values equal to zero, so that we can distinguish them using Delta17O. As far as using Delta17O, ΔPN data is useless in the calculation.

If you insist that our mass balance calculation using Delta17O was not correct, would you please clarify the positions (page and line number) where we made wrong treatments ?

> (8) p. 7245, line 24: The contention that the relatively low d15N NO<sub>3</sub> found in the lake is 15N-deplete due to nitrification is absurd. First of all, the reader is provided with no constraint of the d15N of atmospheric N (NO<sub>3</sub> and NH<sub>4</sub>), which determines the d15N of the dominant reactive N source to the lake assuming no other sources of fixed-N from the catchment.

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The annual average d15N value of atmospheric nitrate have been presented in the text to be -1.1 per mil (P7248/L6). Without the data, however, we can explain the 15N-deletion of nitrate, as presented in the MS.

> Second, given that the lake is highly oligotrophic, there is no residual NH<sub>4</sub><sup>+</sup> that is fractionated by nitrification. Nitrification is COMPLETE such that the d15N of NO<sub>3</sub> will be like that of the original NH<sub>4</sub><sup>+</sup> substrate. The d15N of NH<sub>4</sub><sup>+</sup>, in turn, derives from the “reactive” PN, and will have nearly the SAME d15N as the PN from which it derives, because the ammonification of organic material does NOT implicate significant N isotope discrimination (< 3%.

As you pointed out, ammonium was trace in the oligotrophic lake water, less than the detection limit in most of recent studies (CGER NIES et al., 2004). However, the detection limit in these studies were 3 umol/L. Because both nitrate and PN were also trace in the lake (around 1 umol/L; Table 1), ammonium could be an important reservoir of fixed-N in the lake water.

High sensitivity analysis done in June 1981 in the lake water, for instance, reported ca. 1 umol/L of ammonium at surface and ca. 0.5 umol/L in the water column (CGER NIES et al., 2004). Because nitrification accompanies large fraction in N isotopes as presented in the text, your hypothesis that nitrification achieved complete and that ammonium have nearly the same d15N as the PN is difficult to assume in the lake.

> An important caveat here is that it is unclear why the d15N of PN is 15N-enriched relative to its NO<sub>3</sub> source, which evokes either (a) the contribution of allochthonous PN to the lake and/or (b) the existence of a highly recalcitrant PN pool that has been 15N-enriched through time, much like the suspended PN pool in the deep ocean.

We did not reject the possibilities (a) and (b) in the submitted MS. Either could be possible in the lake.

> Thus, the d15N of the “reactive” PN pool is not necessarily reflected by the measure-

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ments of the d15N of PN.

We can agree with you.

> However, a reasonable assumption that the authors could make is that the d15N of the labile PN derives from incomplete consumption of NO<sub>3</sub> in the lake, and will therefore be 15N-deplete relative to the d15N of NO<sub>3</sub>. In turn, the NO<sub>3</sub> becomes 15N-enriched by assimilation, yet this is countered by remineralization of the 15N-deplete PN.

We can agree with you.

> Therefore in June, if one assumes that the “reactive” PN pool is negligible compared to the total NO<sub>3</sub> pool, the low d15N of NO<sub>3</sub> in the lake likely reflects the d15N of the fixed N sources to the lake, which appears to be dominated by atmospheric deposition of NO<sub>3</sub> and NH<sub>4</sub> – nothing to do with nitrification. If atmospherically derived fixed-N were 15N-enriched, then so would the NO<sub>3</sub> in lake water, regardless of nitrification.

Within this paper, we never rejected the possibility that the 15N-depleted fixed-N in the lake (including those dissolved in the lake as nitrate) had been ORIGINALY supplied through deposition from atmosphere (such as atmospheric ammonium, atmospheric nitrate, atmospheric org-N etc.). What we rejected here in this paper is that direct NITRATE feeding through atmospheric deposition (proposed by Ostrom et al. (1998) for the origin of 15N-depleted nitrate in Lake Superior) as the major source of NITRATE dissolved in the lake. Instead, nitrate produced through nitrification in and around the lake (occupy ca. 90 % of total nitrate) must be responsible for the geochemical characteristics of nitrate in the lake, including the absolute 15N-depletion, as well as the relative 15N-depletion in comparison with PN.

As for the absolute value of d15N, we also expected 15N-enrichment of NO<sub>3</sub> in the lake, if total fixed-N would be 15N-enriched, as you stated. That was the reason we determined d15N of PN in the lake in August. We succeeded to verify that d15N of PN

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(on behalf of total fixed-N) was depleted in 15N as well (showing d15N values less than 0‰).

The atmospheric deposition could be the ultimate (original) source of total fixed-N showing 15N-depletion in the lake but we didn't want to discuss this in this paper because we didn't have enough evidences to prove this. Irrespective to the original source, however, low potential for the denitrification process must be needed to maintain the 15N-depletion of fixed-N in the lake, as presented in the manuscript.

As for the relative 15N-depletion in comparison with PN, your hypothesis (dividing PN to "reactive" and "stable" and assuming 15N-depletion for the "reactive" PN while 15N-enrichment for the "stable" PN) could explain the observed 15N-depletion of nitrate relative to PN, without assuming some isotope fractionation processes within N cycle in the lake. If so, however, you must assume infinite long residence time for "stable" PN which is difficult to assume for labile PN within water column. Furthermore, how you will explain the 15N difference between "reactive" PN pool and "stable" PN pool without considering isotope fractionation? Taking into account that ammonium can be an important reservoir of fixed N in the lake as already presented, the isotope fractionation during nitrification is a more plausible reason for the isotopic discrimination between the two pools of PN (nitrate-derived 15N-depleted PN and ammonium-derived 15N-enriched PN).

> P7229/Line 27: nitrate is not technically "decomposed" by denitrification but it is respired/reduced.

We would like to revise it.

> p. 7233, line 13: why was PON refrigerated rather than frozen?

PON was FROZEN in a DEEP FREEZER (p7233/L13), as presented in the submitted manuscript. Do you use a deep freezer just for refrigeration in your country? If so, I must revise the text to clarify the deep freezer was used to freeze samples.

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> How long after collection? Could there have been decomposition of PN while refrigerated, which would fractionate PN due to NH<sub>3</sub> volatilization?

Storage was around 6 months under FROZEN condition. No decomposition was observed in our experiments on oceanic PON.

> P. 7234, line 5: what is “an original automatic reaction line?”

The processes of (1) evacuation of 15 samples (simultaneously), (2) HN<sub>3</sub> synthesis from NaN<sub>3</sub> solution and acetic acid, (3) evacuation of HN<sub>3</sub>, (4) addition of HN<sub>3</sub> solution to each sample, and (5) addition of NaOH solution to each sample, have been done in a automatic reaction line made by one of the authors (U.T.).

> p. 7235, line 20: Were the nitrate isotope standards used in the same low concentration and in similar matrix as the samples?

Matrix was similar because we added the standards to some of the alternative aliquot of the samples for internal standardization during the analyses. The lowest concentrations were 4 times higher than the lowest sample.

> p. 7236, line 18: “using another aliquot”. . . replicate samples?

Yes.

> Does that mean that the measurements were only replicated for low nitrate samples?

Yes.

> p. 7236, eq (3) is wrong. See Mcllvain and Altabet 2005. Rather than repeating all the methods, the authors could simply cite Mcllvain and Altabet.

The eq (3) (the formula to correct oxygen atom exchange with water) is NOT identical with that presented in Mcllvain and Altabet (2005) as you pointed out. However, this is NOT our mistake as you suspected. We calculated to derive the formula but could not obtain the same one with that presented in Mcllvain and Altabet (2005). We concluded

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our eq. (3) is the more accurate one for the correction and thus we used this formula instead of that in Mcllvain and Altabet (2005). Actually, however, the difference made little variation in the results in this study, within the error of the analytical precisions, because the  $d_{18O}\text{-H}_2\text{O}$  differences between samples and standards ( $\Delta_{18O\text{water}}$ ) were always small (less than 0.7 per mil).

Besides to above mentioned differences in data calculation, our method was more than somewhat different from those described in Mcllvain and Altabet (2005), such as treating much more larger sample sizes, using  $\text{NaHCO}_3$  reagent for the pH buffer, using automatic reaction line for producing/degassing/pipetting the hazardous  $\text{N}_3\text{H}$  solution, using purge and trap system to concentrate sample  $\text{N}_2\text{O}$ , measuring  $\Delta_{17O}$  in CF-IRMS, etc. Furthermore, we asserted that it must be impossible to determine the isotopic compositions of nitrate in the lake, if we analyzed the samples simply based on Mcllvain and Altabet (2005). It would be better to show our analytical procedures to justify our results.

> However, in such a case the reviewer would never have known that the  $d_{18O}$  data were calculated incorrectly.

If some significant errors would be included in geochemical data, you can often find some contradictions within the data. Thus, it is the responsibilities for you reviewers to make every efforts to find such errors, by devoting as much time as possible for reading. If you don't have enough time to do this, you should not undertake to review.

Besides, more ideal way to avoid such problem is to promote inter-laboratory calibration. In our case, we had participated GEOTRACES intercalibration program of nitrate  $d_{15N}$  and  $d_{18O}$  (organizer: Dr. Karen Casciotti in WHOI) and had evaluated the maximum extent of the difference were small.

> And what is the  $d_{18O}\text{ H}_2\text{O}$  of the lake samples? How were these measured?

We would like to add these in the revised MS. We would like to add a reference which

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presented the method for d18OH2O analyses in our laboratory.

Ijiri, A., Tsunogai, U., and Gamo, T.: Simple method for oxygen-18 determination of milligram quantities of water using NaHCO<sub>3</sub> reagent, Rapid Commun. Mass Spectrom., 17, 1472-1478, 2003.

> p. 7237, line20: 15N-depletion relative to what? Give d15N value range here.

That was fully based on the evaluation of d15N of nitrate in lakes presented in the cited reference (Ostrom et al., 1998). We would like to revise this to clarify the base.

> p. 7238, lines 1 – 8: refer to a figure. . .

We would like to refer figures as you suggested.

> p. 7238, line 1: the d15N, d18O, and  $\Delta 17O$  were NOT vertically uniform in June: d15N had a 2‰ range (which the authors never discuss), d18O was uniform to 25 m up from which it increased by 3‰ and  $\Delta 17O$ .

We would like to add a word “almost” before “uniform” in the revised manuscript. The slight heterogeneities can be explained by a slight progress of primary production and nitrification subsequent to active mixing until June.

> p. 7239 line 24: it should be the ratio of the square root of the REDUCED masses

We would like to revise this as suggested.

> p. 7242: The difference between eq(2

Because the concept of temporal variation was added to eq (2), we newly presented the equations (8) and (9).

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

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Cc: S. Daita, D. D. Komatsu, F. Nakagawa and A. Tanaka

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