30 September, 2013

Dear Dr. Liu and anonymous reviewers,

We are submitting our reply to the Reviewers' comments. We appreciate your valuable comments and amendments to our manuscript. We will specify our revisions in boldface, following the reviewers' specific comments.

We hope that you appreciate our revisions. Thank you very much.

Sincerely yours,
Yu UMEZAWA

Faculty of Fisheries, Nagasaki University
1-14 Bunkyo, Nagasaki-city, Nagasaki, 852-8521, Japan
e-mail: umezawa@nagasaki-u.ac.jp
Tel: +81-95-851-2849
Fax: +81-95-851-2799

Responses to Reviewer#1

Specific comments:

1) In $\delta^{15}N$-$\delta^{18}O$ plots (Fig. 4 and 5), most data seem to deviate from the line of $\Delta\delta^{18}O/\Delta\delta^{15}N$ = 1:1, suggesting that other processes (e.g. N_2 fixation, N deposition) control the isotopic composition of nitrate in the surface and subsurface layers. However, the authors put emphases on estimating N isotope fractionation during assimilation using $\delta^{15}N$-ln[NO_3] diagram and they suggest that only a few data out of the fractionation lines were probably attributed to other processes. Explain this inconsistency.

→Chen et al. (2013) reported various $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values in seawater collected around Changjiang Estuary and adjacent ECS. When we consider the variation of $\delta^{15}N$ and $\delta^{18}O$ in NO_3 derived from Changjiang River, most of the values observed at surface water at ECS continental shelf in our study seemed to be plotted on or near the lines of $\Delta\delta^{18}O/\Delta\delta^{15}N$=1:1 starting from several potential

NO_3 sources especially in summer. This is correspondence with the fact that these water masses had lower salinity. Furthermore, heavier $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values in subsurface water were often observed at chlorophyll maximum layer. Therefore, in our idea, it would be reasonable to have discussion based on the fractionation associated with NO_3-uptake by phytoplankton (i.e., $\Delta\delta^{18}O/\Delta\delta^{15}N = 1:1$)

2) Authors have missed the very recent publication (Chen et al., 2013, Acta Oceanol. Sin., 32: 11-17) which deals with similar topic in nearby region. Please ascertain what additional insights and findings been brought to the body of literatures for the ECS.

→Thank you for advising important paper. Because the area studied by Chen et al. (2013) is located between Changjiang River mouth and our study area, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values reported in their study gives us important information on the signal of Changjiang River-derived NO_3^-, which underwent the modification by phytoplankton uptake. Chen et al. (2013) reported that $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values collected in northern transect showed the active assimilation by phytoplankton. Therefore, we also plotted these $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values in Figures 4, 5, 8 and 9 in our paper, as an indicator of Changjiang River-derived NO_3^-, which underwent the modification by phytoplankton uptake. Thanks to their study, we confirmed that most of the values observed at surface and subsurface water at ECS continental shelf in our study seemed to be plotted on or near the lines of $\Delta\delta^{18}O/\Delta\delta^{15}N=1:1$ starting from several potential NO_3 sources especially in summer.

3) A recent study (Zhang et al., 2011, GBC, 25, GB3020) showed much higher N deposition in winter than that in summer. So, why the signal is not significant in winter?

→There are several possibilities that the signal of atmospheric deposition was not detected in our study during winter. First, the atmospheric depositions are high at the Yellow Sea and along the China coast, but dramatically decrease with a distance from the coast. Furthermore, during winter when the water is well mixed from the bottom to the surface, higher contribution of nitrate supplied from the bottom layer likely masked the impact of atmospheric N deposition.

4) Page 10150, Line 11-12: add the detection limit and standard deviations of [NO_3] and

[NO_2] measurements.

→The detection limits and standard deviation of the measurements were about 0.05 μM and 0.02 μM for NO_3+NO_2, which were calculated based on the variation of concentrations in several blank samples. In this study, however, we didn't focus on nano molar level nutrient condition. Instead, only the water samples, which had NO_3 of more than 1.5 μM, were used for the stable isotope analyses. All data of [NO_3+NO_2] in the tables was listed in the order of 0.1 or more. Therefore, we thought that it is not necessary to add the detection limit and S.D. of [NO_3] and [NO_2] measurements in this paper. On this decision, we also referred to other published papers, in which similar topics ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) were discussed.

5) Page 10151, Line 1-3: what are the $\delta^{15}N$ and $\delta^{18}O$ values of the laboratory working standard? Since many of $\delta^{15}N$ values of samples largely deviate from the N isotopic values of international isotope standards, could you evaluate the offset of calibration?

→We use four standard, USGS34 (-1.8‰), USGS35 (2.7‰), -IAEAN3 (4.7‰) and other laboratory working standard (1.56‰) for the usual analyses of nitrate isotopes. But we also have the other standard (USGS32) with higher $\delta^{15}N$ values (180‰). At the step of the system performance test, we have already confirmed that there were linear relationships between the measured values and actual values using these standard samples including USGS32 (180‰). Although we don't use USGS32 having extremely high $\delta^{15}N$ values at usual analyses, we believe that $\delta^{15}N$ values are correctly determined.

6) Page 10160, Line 3-9: I cannot understand that the constant characteristics of water column from the bottom to middle layer at some sites could imply minor effect of sedimentary denitrification (SD) in this region. Actually, SD has little isotope fractionation on $\delta^{15}N$ and $\delta^{18}O$ in nitrate thus SD is not detectable by using isotopes unless there is a release of pore water containing mid-way SD.

→We agreed with your suggestion. We have not investigated the $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in porewater, and the resolution in vertical comparison (i.e., bottom, 10m and 30m above) was not appropriate to check the effect of sedimentary denitrification and the release of pore water into overlying water. Instead, we added another discussion (see below) based on N* to imply the relatively minor

contribution of denitrification at N cycling at bottom water. We consider about denitrification, but did not specify the contribution of sedimentary denitrification.

Added sentences----------

"Furthermore, N* (i.e., [DIN]-16 x [PO_4^{3-}] + 2.9) at the bottom layer on the continental shelf was positive (i.e., 0.6-6.4 in July 2009 and 2011), while N* in the water mass intruding from the deep layer of Okinawa Trough to the continental shelf were negative (i.e., -11.5- -2.1 below 200 m depth in July 2009 and 2011), implying that the effect of denitrification at the sedimentary boundary layer on the NO_3 characteristics of the ECS shelf water was minor during our observation period."

7) Page 10161, Line 1-10: I note that $\delta^{15}N$ of nitrate in surface and subsurface at some sites (e.g. D11, D13, C1) in July 2011 followed different fractionation lines. Could the authors give some interpretations for this phenomenon?

→Most probable explanation can be the temporal variation of original $\delta^{15}N$ in Changjiang River, as explained above. Actually, $\delta^{15}N_{NO3}$ collected near the Changjiang River mouth had variation from +2.0‰ to +8.0‰, and we modified our discussion based on this probable explanation. Furthermore, NO_3^- uptake by different species of phytoplankton with different fractionation factor may be another potential reason. However, it is difficult to consider the effect of phytoplankton species specific fractionation factor on the isotope values observed in natural fields.

8) Page 10161, Line 11- 15: "In July 2009, when: : :" This sentence is so ambiguity and difficult to understand, please rewrite it.

→We decided to delete this redundant sentence. Because there are variation in $\delta^{15}N_{NO3}$–ln[NO_3] values in CDW observed near Changjian River Estuary (cross symbols in Figs. 8 and 9; Chen et al. 2013), it is probable that the values plotted between different fractionation line can be also explained as remaining NO_3 after active uptake of Changjian River originated-NO_3 by phytoplankton, especially when the salinity of the water mass was relatively low.

9) Page 10162, Line 8-10: the authors mentioned Changjiang River plume goes northeasterly in the open ocean, which is basically towards the sampling locations. Moreover, based on T-S diagram the Changjiang freshwater had an apparent influence on the nearshore sites with lower salinity. I wonder why the river-derived nitrate was impossible to supply continuously to "CDW".

→**I'm sorry that our explanation was not enough. For example, in the stagnant waters such as lake and semi-closed bay, land-derived nutrients stay in the water column even after phytoplankton uptake them with some modifications to the stable isotope values. So the nutrients and their isotopic signatures in the water consist of the mixture of newly (and continuously) supplied nutrients and remnant of nutrients, which were previously supplied. This is defined as open system. On the other hand, Changjiang River plume in ECS may be similar to the situation of river waters, which unidirectionaly flow to lower streams. The terrestrial nutrients discharged at upper streams are carried to the middle stream, changing their concentration and isotopic signatures due to biotic and abiotic reactions. However these nutrients, which were carried to middle stream, no longer mix with original terrestrial nutrients supplied from upper region. This is kind of closed system. At the satellite image of ECS area, we can see the patch of higher Chlorophyll generally characterized by lower salinity, suggesting the water mass having Changjiang River originated-nutrients. The [NO_3] in this water mass could be reduced by phytoplankton uptake and dilution by nutrient-depleted KSW, but could not be rarely replenished by original Changjian River nutrients.**

10) Page 10153, Line 6-7: the range of 1-3 in winter?

→**Thank you for indicating our mistake. We corrected this part.**

On the other hand, most of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values in Changjian Diluted Water can be located on the line of $\Delta\delta^{18}O:\Delta\delta^{15}N = 1:1$ in summer, because Chen et al. (2013) have reported that Changjiang River originated-NO_3 had large variation in $\delta^{15}N$ and $\delta^{18}O$ values.

11) Page 10157, Line 24-29: add the unit (‰ of 15 ε)

→**Thank you for indicating our mistake. We corrected this part.**

12) Page 10162, Line 27: change "will" to "may". If the isotopic fractionation of NH_4^+ assimilation is high and/or the $\delta^{18}O$ of nitrate and nitrite produced by nitrification is low (e.g. Buchwald et al., 2012 L&O), $^{18}\varepsilon/^{15}\varepsilon$ may shift to below 1:1.

→I agree to your suggestions that $^{18}\varepsilon/^{15}\varepsilon$ may shift to below 1:1 because nitrification reaction generally lowers $\delta^{18}O$ and there is direct uptake of ammnoium. We deleted the words, "more positive $^{18}\varepsilon/^{15}\varepsilon$", and changed "will" to "may".

13) Fig. 4 and 5: I suggest changing "18ε/15ε" to "$\Delta\delta^{18}O/\Delta\delta^{15}N$" in the figures, same as that in figure captions.

→Thank you for your suggestions. We have changed $^{18}\varepsilon/^{15}\varepsilon$" to $\Delta\delta^{18}O/\Delta\delta^{15}N$ in the Figures 4 and 5.

Responses to Reviewer#2

Specific comments:

1) My main suggestion for this paper is to isolate the physical processes and biological processes more clearly. It is challenging, but may be achievable with some simple model/calculations. For instance, can the authors use the mixing curve based on their T-S diagram to calculate the end members of the nitrate concentration and isotopic signature? And then proceed with discussions of the biological processes. In order to achieve the above, it will be important to characterize the isotopic signatures of the water mass sources. The authors have referenced some random isotopic values, but recognizing that the values could be variable, except the Kuroshio, which seems to be well constrained. It may be better if a few more words being said about the selections of the values and the range of the observations.

→Thank you so much for your valuable comments. As you suggested, we are also feeling that the interpretation of stable isotope values are complicated, because many biological and physical factors affect $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in ECS.

Although we well-considered this topic, and tried to separate the effect of biological processes from that of physical process (mixing) by making mixing line, there was difficulty to do it, especially in summer when the plume of Changjian River discharge flows on the surface of ECS and mainly mix with nutrient-depleted KSW. When the water mass mix with NO_3-depleted water mass (e.g., KSSW mix with nutrient-depleted KSW), $\delta^{15}N$ values do not change, and the effect of physical mixing on NO_3 characteristics is just decrease of [NO_3] as indicated as dilution-line in Figures 4a-c.

On the other hand, levels of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in the northern ECS seemed to be higher in the chlorophyll maximum irrespective of season and year (Figs. 4 and 5). In addition, the ratio of enrichment of heavier O and N isotopes from the potential NO_3 sources (i.e., $\Delta\delta^{18}O$:$\Delta\delta^{15}N$) was about 1.0 in summer and close to 3.0 in winter. Therefore, isotopic fractionation during NO_3 uptake by phytoplankton was expected to be a major factor causing changes in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in the northern ECS at least during summer.

Therefore, we considered that the discussion primarily based on the diagram between $\delta^{15}N_{NO3}$ vs. ln[NO_3] emphasizing the effect of phytoplankton uptake on $\delta^{15}N$ in NO_3 can be better approach, rather than based on physical mixing.

As for the variation of isotopic values in Changjiang River derived NO_3, we added some more values cited from Chen et al. (2013) and indicated the range of the values.

2) In the top paragraph on page 10159, the authors seem to indicate that diatom or phytoplankton productions are limited by temperature and nitrate supply. But are there really evidences to support this?

→We had mistake on the description of this part. As we mentioned before (i.e., page 10158, L22-23), the major factor limiting phytoplankton growth on the continental shelf is light availability due to the convection of the shelf water during winter and nutrient supplies due to the developed stratification during other seasons (Ning et al., 1988, Hama et al.1997; Chiang et al. 2004). In this part, we have added "transparency increase" as one of reasons to cause phytoplankton bloom in winter.

3) Fig. 4 and 5 are just too busy. It seems that the symbol size on Fig. 4C has changed, but nothing has been said to explain what it means. Is this intended?

→I'm sorry that there was no intentional change for the symbol size on Figure 4A-C. We almost adjusted the symbols to the same size. However, we have left some symbols smaller than the others, because large symbols hide the others in case that the symbols are plotted nearby.

4) There are too many depth information on the plots. I would recommend that you first remove the DCM info, because it is not really needed on the plot. You could consider adding the information on your tables. The sampling depths for the water samples may instead be indicated by the color scale or symbol size scale.

→We agreed that the depth information on the Figures 4 and 5 is not necessary for the discussion. Therefore the depth info was deleted from the Figures 4 and 5. The information on each layer is available on the Tables A1, B1 and C1. On the other hand, we have left the depth information on the Figures 8 and 9, because we did many discussions on NO_3 dynamics at section 4.2 referring to the depth information.

5) It is an interesting finding that the nitrogen and oxygen isotopes increase close to the bottom of the shelf. Although it is less likely that water column denitrification has occurred, sedimentary denitrification coupled with nitrification has been observed to cause the upper water column nitrate $\delta^{15}N$ and $\delta^{18}O$ to increase, accompanied with a loss of N (Granger et al., Coupled nitrification-denitrification in sediment of the eastern Bering sea shelf leads to ^{15}N enrichment of fixed N in shelf waters, JGR, 116, C11006, doi:10.1029/2010JC006751, 2011). I wonder if the authors think this could explain their observation? Why and why not?

→Sedimentary denitrification coupled with nitrification can occur also in the sediment on ECS continental shelf. However, unfortunately we don't have enough data (e.g., $\delta^{15}N$ of NO_3 and NH_4 (or TDN) both in porewater and overlying water) to verify this possibility and contribution. In our understanding, $\delta^{15}N$ in NH_4 (or TDN-NO_3) will be elevated due to sedimentary nitrification, which produces isotopically lighter NO_3 and partially denitrified to N_2. The release of ^{15}N-elevated

NH_4 may affect $\delta^{15}N$ in phytoplankton and NO_3 through the NH_4 assimilation in the water column, but final level of $\delta^{15}N$ in NO_3 after nitrification in the water column of ECS is not certain because of isotopic fractionation.

In our study, actually, $\delta^{15}N$ values were nearly constant from the deep water to the bottom water on the shelf to a different extent in each year (i.e., from the continental slope to the mid-shelf area in 2009 (Fig. 4b), but limited to the edge of the outer shelf in 2011). Furthermore, a slight increase in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in the bottom water was observed in different zones of the continental shelf in different years (i.e., [Sta. D5, 6′] in 2009 (Fig. 4b) and [D4′–11] in 2011(Fig. 4c)) can be primarily attributed to intrusion of YSCWM based on the evidence from T-S diagram.

6) I agree that based on the current knowledge of the region and the proxy, it has a lot of uncertainties to interpret the low $\delta^{15}N$ and high $\Delta 18$: $\Delta 15$ ratio below the euphotic zone. It would be interesting to quantify the sinking flux at different depths and their $\delta^{15}N$ values.

→Recent paper reported that Changjiang River originated-NO_3 had large variation in $\delta^{15}N$ and $\delta^{18}O$ values. Although we reported high $\Delta\delta^{18}O/\Delta\delta^{15}N$ ratio below the euphotic zone in our original draft, most of the values may be simply located on the line of $\Delta\delta^{18}O:\Delta\delta^{15}N$ =1:1, suggesting active uptake by phytoplankton. However, higher $\Delta\delta^{18}O:\Delta\delta^{15}N$ ratio was still observed during winter. At next step, it would be interesting to check the sinking flux and their isotopic signatures based on isotope mass balance model in ECS.

On the continental shelf of ECS, the vertical mixing occurs and turbid water reaches to the surface during winter. As Granger et al. (2010) reported that a species of heterotrophic bacteria showed NO_3 uptake with $\Delta\delta^{18}O:\Delta\delta^{15}N$ = 2:1, other organisms originally inhabiting in the surface sediment rather than phytoplankton may contribute to enhance $\delta^{18}O_{NO3}$ relatively to $\delta^{15}N_{NO3}$ during winter.