

# ***Interactive comment on “Isotopic composition of nitrate and particulate organic matter in a pristine dam-reservoir of western India: Implications for biogeochemical processes” by Pratirupa Bardhan et al.***

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Referee Comments: Based on the isotopic technique, the paper identifies the biogeochemistry of Indian reservoir where monsoons play an important role in controlling vertical mixing and dynamic of carbon and nutrients. This is important for a better understanding of nutrient cycle in natural freshwater lakes. However, the results and analyses presented here are crude. Thus, a significant work has to be done to improve the overall quality of the manuscript. Authors' Response: We thank the anonymous referee for her/his constructive comments on the manuscript that have enabled us to

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improve the quality as detailed below.

Specific comments: Referee Comments: (1) Abstract, Line 22-24: The last sentence puts emphasis on the potential of stable carbon and nitrogen isotopes in the study reservoir. However, throughout the paper the major purpose appears to be identification of biogeochemical processes of the Tillari Reservoir using carbon and nitrogen isotopes. The major purpose of this paper should be made clearer. Authors' Response: We have modified this sentence to "Overall, this study, the first of its kind in the Indian subcontinent, provides an insight into biogeochemistry of Indian reservoirs, using stable carbon and nitrogen isotopes as a tool, where the monsoons play an important role in controlling vertical mixing and dynamics of carbon and nutrients."

Referee Comments: (2) The introduction section lacks sufficient overview of previous researches related to biogeochemistry of carbon and nutrients in the reservoirs. Authors' Response: We have now included a paragraph in the Introduction section that briefly discusses previous researches in some other reservoirs of the world.

Referee Comments: (3) The site description is inadequate. For examples, different vegetation types (C3 plants vs. C4 plants) have distinct values of carbon isotope, which may have an important influence on the carbon isotope of particulate organic matter of the reservoir. Authors' Response: The section on site description has been expanded in response to comments of both referees including vegetation types based on the available information.

Referee Comments: (4) The sampling section lacks a detailed sampling map, which makes it very hard for readers to understand the spatial variations of parameters. Authors' Response: Sampling was carried out only at one location at the deepest part of the reservoir. The station location is now included in the map (Figure 1).

Referee Comments:(5) Sampling and analyses. Overall, the sampling time and frequency are not clear. Also, the analyses time and frequency are not clear. For example, when the surface sediment is collected; when nitrogen isotope of NH<sub>4</sub> samples

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are measured. Authors' Response: We have tried to improve this section by providing more information. Sampling for isotopic analyses of POM commenced in March 2010 and continued on a monthly basis till 2012. From 2012 to 2015 samples were collected on a seasonal basis. This information is already mentioned in Section 2.4. Samples for nitrate isotopic measurements were collected from 2011. This sentence has been added now. The analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of POM were usually conducted within 1-2 months of collection. However, the water samples were filtered within 3-4 hrs of collection and the filter papers were frozen immediately. We have added this sentence in Section 2.4.3. The facility for nitrate isotope analysis was created in 2014 and samples from 2014 and 2015 were analysed immediately for natural abundance of N and O isotopes. Samples from 2011 and 2012 were also analysed on a selective basis. This information was present in Section 2.4. Surface sediment was collected during the May 2012 field trip. We have added this information. Samples for  $^{15}\text{N-NH}_4^+$  were collected in May 2012. We have added this information in the Methodology Section (Section 2.4.2.). The sample bottles were kept in incubator-shaker immediately on returning to the laboratory following the protocol for the ammonia diffusion method, as mentioned in the Methodology Section. After two weeks' incubation, the measurements were done.

Referee Comments: (6) Results. For this paper, isotopic variations are critical to identify the biogeochemical processes of carbon and nitrogen. Thus, the related isotopic data are needed to present in tables or figures. Authors' Response: A figure (tentatively titled Figure A) has now been included that shows mean annual variations of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of POM.

Referee Comments: (7) Line 10, Page 8: "We observed a nearly 1:1 trend for. . . ." Please display the related variations in figures. The figures can be uploaded as supplementary information. Authors' Response: We have now added a figure to be included under Supplementary Information (Supplementary Figure 1).

Referee Comments: (8) Line 15, Page 8: "As the summer progressed, productivity increased resulting in increased  $\text{CO}_2$  uptake and elevated  $\delta^{13}\text{C-POM}$ ". This statement

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is wrong. Values of  $\delta^{13}\text{C}$ -POM are expected to get more depleted due to the preferential uptake of  $^{12}\text{C}$ . Authors' Response: We argue that higher productivity would result in enrichment of residual DIC with  $^{13}\text{C}$ , and the organic matter synthesized would also become increasingly more enriched with this isotope. Of course, the  $\delta^{13}\text{C}$  of POM would still be lower than that of DIC, as stated by the referee, but that is not the point. Similar enrichment of  $\delta^{13}\text{C}$ -POM during periods of high productivity has also been observed in other lakes, for e.g., in Lake Lugano (Lehmann et al., 2004) and in Lake Wauberg (Gu et al., 2006). We have addressed this issue in the revised manuscript.

Referee Comments: (9) Line 14, Page 8: what is the range of  $\delta^{13}\text{C}$ -POM for surface-water? What is the typical range of lacustrine autochthonous organic matter? Authors' Response: The range of  $\delta^{13}\text{C}$ -POM for surface-water is -32 to -26‰. The typical range of lacustrine autochthonous organic matter is -42 to -23‰ (Kendall et al., 2001 and references therein). This has been included in the revision.

Referee Comments: (10) Line 18 and 19, Page 8: the units of "ng/l" and " $\mu\text{g l}^{-1}$ " should be uniformly expressed as "ng/l" and " $\mu\text{g/l}$ ", or "ng l<sup>-1</sup>" and " $\mu\text{g l}^{-1}$ ". Authors' Response: Accepted.

Referee Comments: (11) Line 20-22, Page 8: in addition to the reasons mentioned, the lower  $\delta^{15}\text{N}$ -POM values may be related to the atmospheric input, which have a low value of  $\delta^{15}\text{N}$  (-2.9‰ Line 1, Page 13) in the study area. Authors' Response: This possibility has also been included.

Referee Comments: (12) Line 21, Page 9: According to the authors, the decrease of  $\delta^{18}\text{O}$  is due to nitrification. How could you exclude the vertical variations of atmospheric contributions when considering the plentiful rainfall (3000 mm, Line 19, Page 2) in the study area? Authors' Response: The observation referred to in Line 21, Page 9 is from April when the rainfall is negligible and water column is strongly thermally stratified. Dry atmospheric deposition is highly unlikely to cause such a large vertical gradient in  $\delta^{18}\text{O}$ .

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Referee Comments: (13) Line 10, Page 10: How is “the slope values of 0.95 and 0.85” obtained? Authors’ Response: We considered samples within the suboxic hypolimnion where there was a decrease in nitrate concentration accompanied by an increase in  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ . Linear regression of  $\delta^{18}\text{O}$  versus  $\delta^{15}\text{N}$  yielded slope values of 0.95 in 2014 and 0.85 in 2012.

Referee Comments: (14) Line 15, Page 10: How are the values of  $\epsilon^{15}$  and  $\epsilon^{18}$  computed? The values of  $\epsilon^{15}$  and  $\epsilon^{18}$  presented here are wrong. They should be corrected to be  $-8.7\text{‰}$  and  $-10.7\text{‰}$  respectively. Authors’ Response: We have followed Lehmann et al. (2003) where the calculations for  $\epsilon^{15}$  and  $\epsilon^{18}$  are explained in detail. Using the Rayleigh “closed-system” equation, the  $\epsilon^{15}$  (and  $\epsilon^{18}$ ) were computed from the slopes of  $\delta^{15}\text{N-NO}_3^-$  (and  $\delta^{18}\text{O-NO}_3^-$ ) versus natural logarithm of  $f \text{NO}_3^-$ , the fraction of remaining nitrate. The observed maximum  $[\text{NO}_3^-]$  was considered as the  $[\text{NO}_3^-]_{\text{initial}}$ .  $f \text{NO}_3^- = [\text{NO}_3^-] / [\text{NO}_3^-]_{\text{initial}}$  We have corrected the  $\epsilon^{15}$  and  $\epsilon^{18}$  values in the text to  $-8.7\text{‰}$  and  $-10.7\text{‰}$ .

Referee Comments: (15) I don’t think Table 1 and Table 2 are necessary. Table 1 is not even cited in the paper. They can be provided as supplementary information. Authors’ Response: We agree that Table 1 can be provided as supplementary information. But we feel Table 2 is important as we are comparing the  $\epsilon^{15}$  and  $\epsilon^{18}$  from our study site to those from other systems and also demonstrating the limited data available from freshwater systems in general.

Referee Comments: (16) It is not clear about the description of the data in the title of Fig. 4. Authors’ Response: Caption of Fig 4 has been modified as follows: Figure 4: (a) Depth-wise variations of ammonium concentration and  $\delta^{15}\text{N-NH}_4^+$  in May 2012. (b) Plot of  $\delta^{15}\text{N-PON}$  versus  $\ln(\text{NH}_4^+)$ . The negative linear correlation yields a fractionation factor ( $\epsilon^{15}$ ) of  $-2.4\text{‰}$ .

Referee Comments: (17) Line 11-19, Page 11: This paragraph compare the fractionation factor of the Tillari reservoir with previous studies. However, what is the conclusion

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after the comparison? Authors' Response: We have added a concluding sentence "For the low to moderate ammonium concentrations recorded (maximum  $\sim 12 \mu\text{M}$  in Figure 4) the fractionation factor computed by us compares well with previously reported values."

Referee Comments: (18) Line 20, Page 11: the subtitle is "Sulphate reduction and evidence for chemosynthesis". However, there is not any table or figure about the variations of  $\text{SO}_4$ . Authors' Response:  $\text{H}_2\text{S}$  is formed during sulphate reduction, and its presence indicates the prevalence of this process. We did not make measurements of sulphate concentration as we thought that these were not needed. In our manuscript, we only discuss this process to interpret the observed variations in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of POM during the period  $\text{H}_2\text{S}$  accumulates in the water column.

Referee Comments: (19) Line 21, Page 11: "microbial degradation of organic matter by sulphate. . . ." what does it mean? Does it mean sulphates act as electron acceptor? Please make it clear. Authors' Response: Yes. We have made appropriate changes in the text.

Referee Comments: (20) Line 21, Page 12: How can the nitrate isotopic data prove the nitrate uptake? However, the Fig. 4b indicates the uptake of  $\text{NH}_4^+$ . Hence, I am wondering whether there is any competitive uptake between  $\text{NO}_3$  and  $\text{NH}_4$ . Authors' Response: Line 21, Page 12 : Accommodating the referee's comments we have thoroughly revised the text.

Referee Comments: (21) Line 23, Page 12: Only a precipitation sample was collected. It is not enough. Is there any other research about the nitrate isotope of wet deposition in the nearby area? Author Comments: We agree that a single sample is not sufficient to reach any meaningful conclusion, and we show that the available data do not explain the observed nitrate concentration and its isotopic composition. In fact, our conclusion is that the composition of end members itself may be highly variable which underlines the need for more measurements. Unfortunately, there are no other data on isotopic

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composition of wet deposition in this area.

Referee Comments: (22) Line 4-6, Page 13: Where is the data of POM in the Tillari river? How could the POM data prove the input of Tillari river to the reservoir? Authors' Response: The POM data of the Tillari river was not measured during this study. We hypothesize input of Tillari river by the distinct thermal (colder) signature of the water mass at intermediate depths. As mentioned in the text, this water parcel had higher nitrate, lower DO and chlorophyll-a.

Referee Comments: (23) Line 12-13, Page 13: "atmospheric wet deposition seems to be the dominant nitrate source to the water column during the monsoon season". However, the related discussion about the atmospheric inputs is extremely scarce throughout the paper. Authors' Response: We have modified the text substantially in response to referee's comment.

Referee Comments: (24) For Figs. 3 and 6, it is clearer to change symbols in different shapes. Author Comments: We have modified the figures with different symbols.

Referee Comments: (25) Throughout the paper, some statements lack the related references and some statements lack original references. For example, related references should be added for Line 7, Page 8 and Line 9, Page 10; the original references should be added for Line 8 and Line 12 on Page 9. Author Comments: We have now added the required references. Line 7, Page 8: "The  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values of nitrate in the epilimnion were high, a signature of assimilation: phytoplankton prefer nitrate containing  $^{14}\text{N}$  and  $^{16}\text{O}$  leaving residual nitrate enriched with  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  (Casciotti et al., 2002)" Line 9, Page 10: "Dissimilatory nitrate reduction is known to be associated with 1:1 increase in  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  (Granger et al., 2008)" Line 8, Page 9: "Ammonium, the primary N source, undergoes strong fractionation producing isotopically light nitrate (Delwiche and Stein, 1970, Casciotti et al., 2003)." Line 12, Page 9: "This is because, while the oxygen atoms in atmospheric nitrate are derived from interactions between  $\text{NO}_x$  and  $\text{O}_3$  in the atmosphere, those in nitrate produced by

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nitrification come from dissolved oxygen and water ( Kendall, 1998, Finlay et al., 2007)”

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-270/bg-2016-270-AC2-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-270, 2016.

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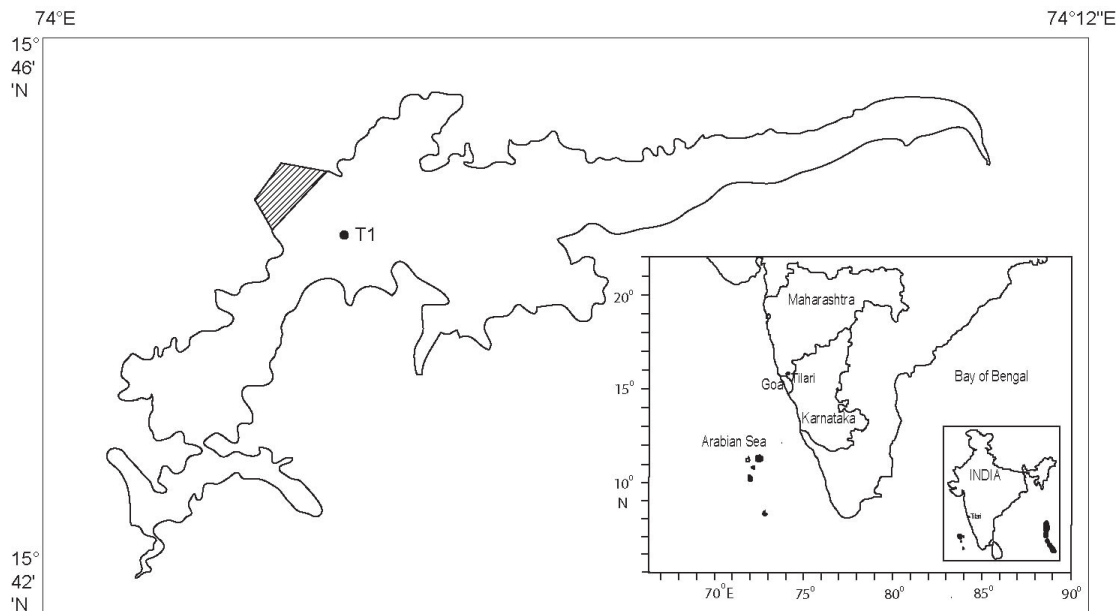
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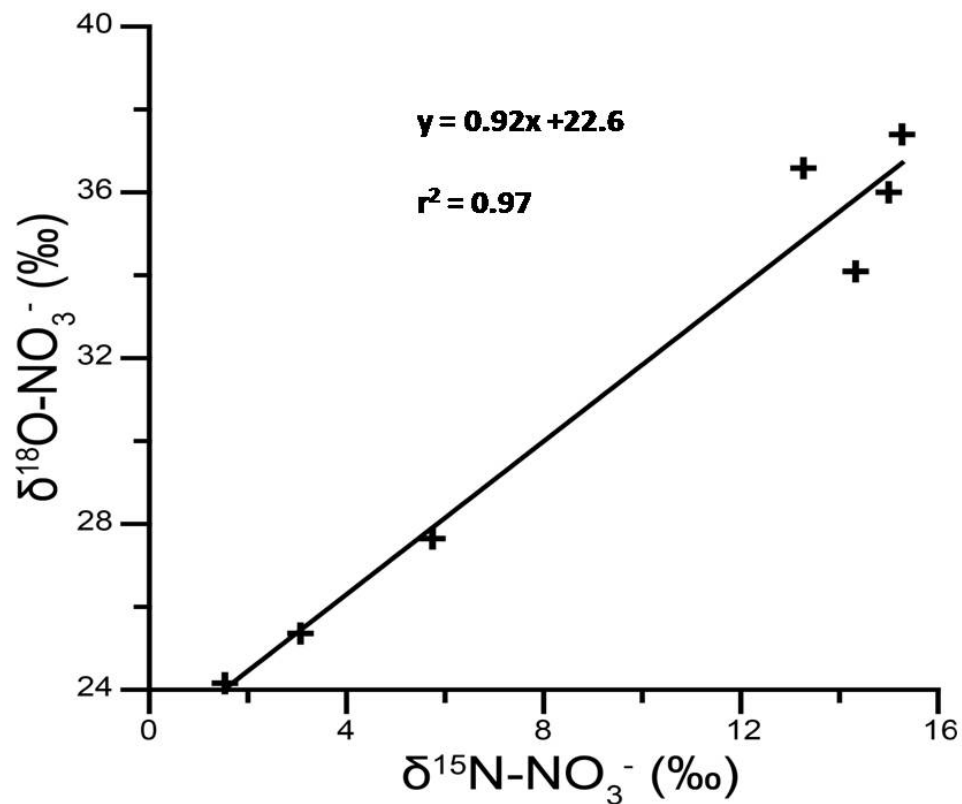


**Fig. 1.** Map of the sampling location (Tillari Reservoir). T1 represents the sampling point at the deepest part of the reservoir.

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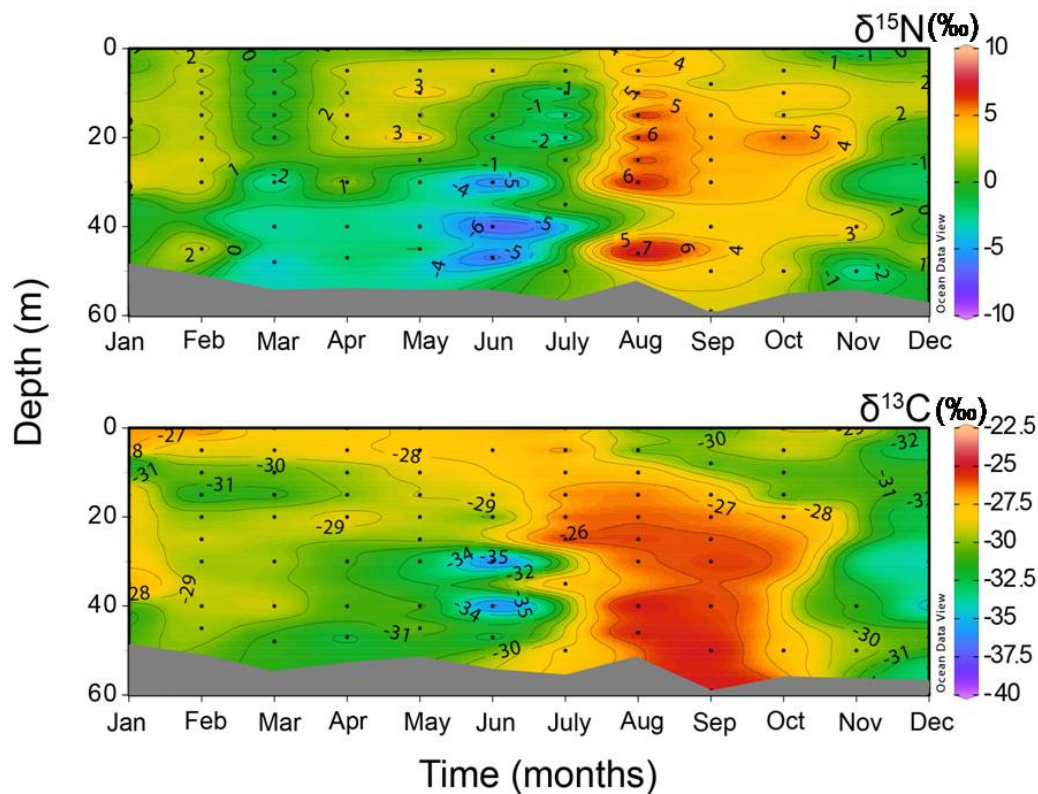
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**Fig. 2.** Supplementary Figure 1 Scatter plot between  $\delta^{18}\text{O-NO}_3^-$  vs.  $\delta^{15}\text{N-NO}_3^-$  of the surface samples during summer. A nearly 1:1 line indicates occurrence of nitrate assimilation.

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**Fig. 3.** Figure A: Mean annual variations of  $\delta^{15}\text{N}$ -POM and  $\delta^{13}\text{C}$ -POM at the main sampling location.

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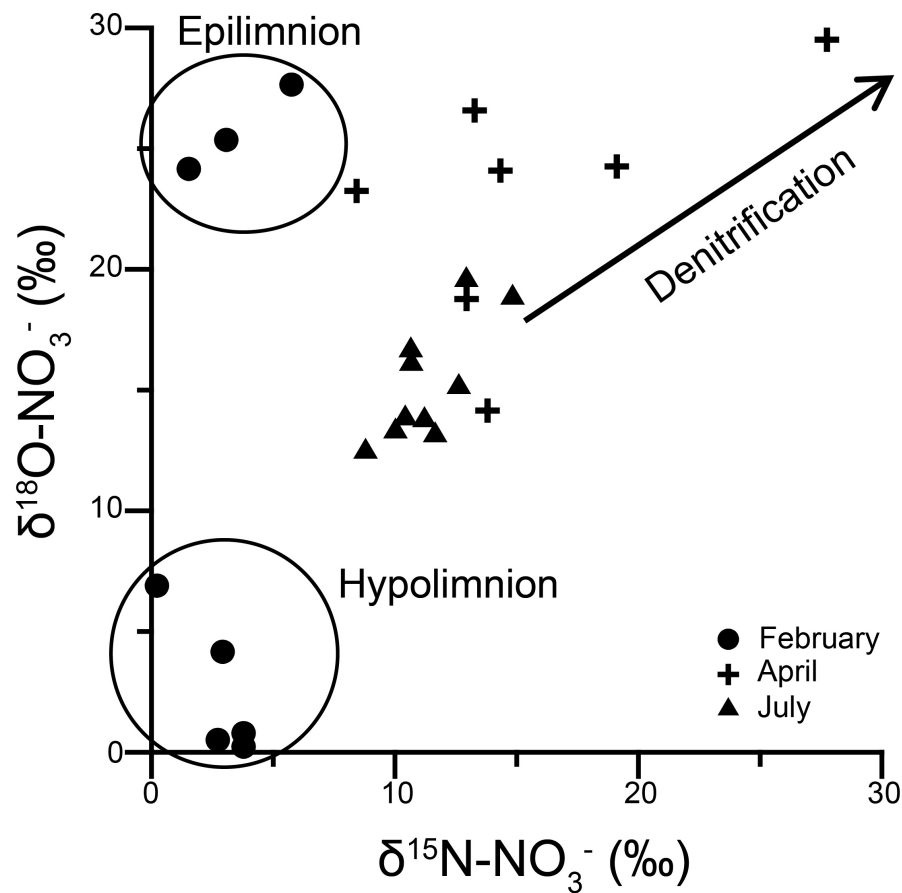


Fig. 4. Figure 3(edited)

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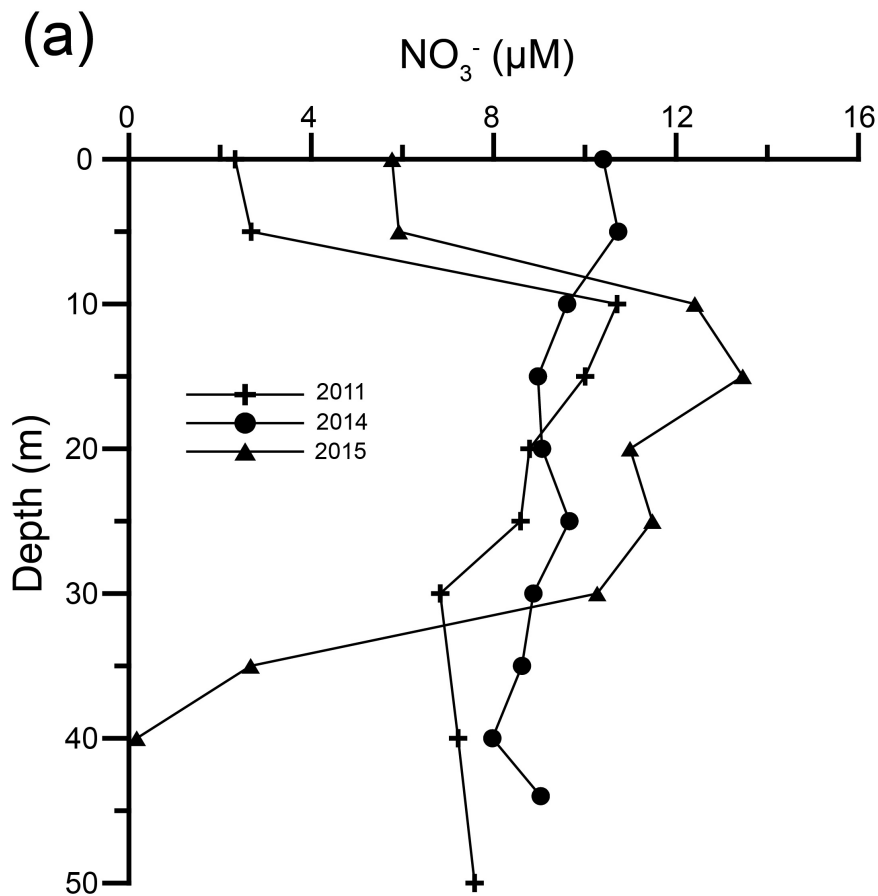


Fig. 5. Figure 6a (edited)

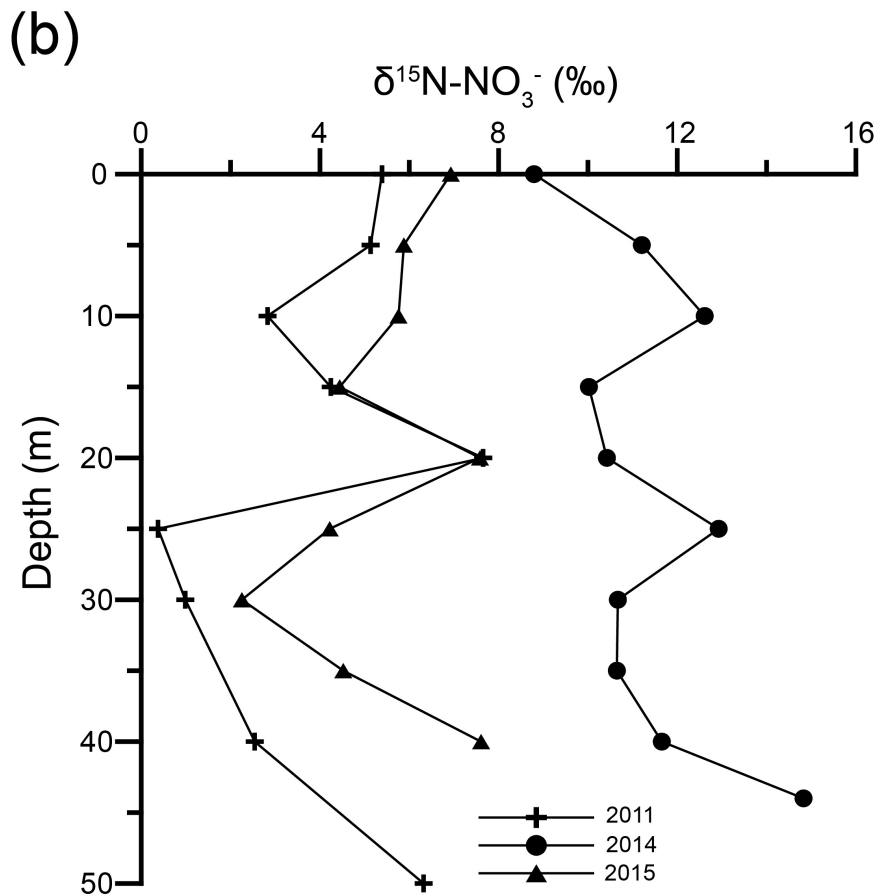


Fig. 6. Figure 6b (edited)

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