1.COMMENTS FROM AND RESPONSE TO ANONYMOUS REFEREE #1

Referee Comments: This paper reports some interesting results which demonstrates the potential of stable carbon and nitrogen isotopes to gain insight into biogeochemistry of Indian reservoirs where the monsoons play an important role in controlling vertical mixing and dynamics of carbon and nutrients. However, the quality of the text is not sufficient and the data interpretation needs improvements.

Authors' Response: We thank the anonymous referee for her/his contructive comments that have been taken into consideration while revising the manuscript as described below.

Referee Comments: 1- Abstract: Please explain the " POC", " PON", " SPOM" and " DIN"

Authors' Response: The abbreviations POC and PON have been expanded in the abstract. In the revised version SPOM has been replaced by POM that is explained in the first line of the abstract.

Referee Comments: 2- Introduction: It is not clear that why did the authors carry out the study? What is the current research progress?

Authors' Response: The purpose of this study has been more clearly stated in the revision. The study was undertaken to gain insights into biogeochemical cycling in Indian freshwater reservoirs from which very little information is available so far. The Tillari Reservoir has been selected for detailed investigation that included measurements of natural abundance of nitrogen and oxygen isotopes in nitrate, and nitrogen and carbon isotopes in POM. These data, first of their kind generated from any Indian freshwater body, facilitate an understanding of biogeochemical processes (especially involving nitrogen) that should be typical of any relatively pristine, tropical, monsoon-affected freshwater body.
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Referee Comments: 3- Site Description: The description of the study area was not clearly mentioned in this section, such as, land use, evaporation, water quality.

Authors' Response: The information sought by the referee has been added under "Site description".

Referee Comments: 4- Sampling and field measurements: Please show the distribution of 51 samples in Figure (horizontal and longitudinal).

Authors' Response: The referee mistook "51 samples" as fifty one samples. We meant 5 litre volume. This has now been clarified.

Referee Comments: 5- Figure 2, Figure 4 and Figure 5: The data are only from one sample or are the average values?

Authors' Response: Each data point represents one sample. This has now been clarified in figure captions.

Referee Comments: 6- Figure 3: Please show the depth of Epilimnion and Hypolimion.

Authors' Response: Figure 3 is numbered as Figure 4 in the revised manuscript. Epilimnion : 0 – 10 m; Hypolimnion : 15 – 48m. This information has been added in the caption of Figure 4.

Referee Comments: 7- Isotopic and elemental composition of suspended particulate organic matter: The data of δ15N and δ13C should be shown in table or figure.

Authors' Response: A figure (figure 2) has now been included that shows mean annual variations of $\delta^{15}N$ and $\delta^{13}C$ of POM.

Referee Comments: 7- 4.2.2 Denitrification, L10: why did you get 0.95 and 0.85?

Authors' Response: In canonical denitrification, both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ increase linearly. The enrichment in isotopic value is ~1 in marine systems (Casciotti et al., 2002, Sigman et al., 2005, Granger et al., 2008). However, this value is reported to be lower (0.5-0.7) in freshwater systems (Lehmann et al., 2003 and references therein). The reasons for this difference are not fully understood. Also, studies in freshwater systems are sparse as compared to marine systems. In a batch of culture experiments, Granger et al. (2008) observed that nitrate-reducing enzymes play a role in altering the O to N isotopic enrichment, with periplasmic dissimilatory nitrate reductase (Nap) expressing a lower enrichment value (~0.62) than the membrane-bound dissimilatory nitrate reductase. Again, there is a lack of data on the isotopic expressions of these enzymes at the ecosystem level. Wenk et al. (2014) attributed the low O:N isotopic effect of ~0.89 to chemolithoautotrophic denitrification, rather than heterotrophic denitrification, in the northern basin of Lake Lugano.
Our data from the Tillari reservoir indicates the occurrence of denitrification in the suboxic hypolimnion under stratified conditions. However, this process is restricted to a narrow depth range of 10-20 m which limits the number of data points. There may be several factors responsible for the low (<1) isotopic enrichment factor in the Tillari but our data are not sufficient to identify the exact cause(s). This information is included in the revision.

Referee Comments: 8- I can't find the data from October, November, January, May, June, August and September. Why do you get the diagram to depict different biogeochemical processes taking place in the Tillari Reservoir over an annual cycle in Figure 5.

Authors' Response: Figure 5 (6 in the revised manuscript) schematically shows distinct seasonal variations and major biogeochemical processes occurring in the reservoir. This information in based on regular (monthly) monitoring of the reservoir that includes observations in the months mentioned by the referee. However, the isotope data presented here were not collected on the monthly basis. Shenoy et al. (manuscript under prep.) will provide a more detailed account of intra- and interannual variability in the reservoir based on monthly sampling. This has been clarified in the caption of the figure.

2.COMMENTS FROM AND RESPONSE TO ANONYMOUS REFEREE #2

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 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 NH4 samples 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $^{15}N\text{-}NH_4^+$ 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 (figure 2) has now been included that shows mean annual variations of $\delta^{15}N$ and $\delta^{13}C$ 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 CO2 uptake and elevated δ13C-POM". This statement is wrong. Values of δ13C-POM are excepted to get more depleted due to the preferential uptake of 12C.

Authors' Response: We argue that higher productivity would result in enrichment of residual DIC with ^{13}C, and the organic matter synthesized would also become increasingly more enriched with this isotope. Of course, the $\delta^{13}C$ of POM would still be lower than that of DIC, as stated by the referee, but that is not the point. Similar enrichment of $\delta^{13}C$-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 δ13C-POM for surface-water? What is the typical range of lacustrine autochthonous organic matter?

Authors' Response: The range of $\delta^{13}C$-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 "µg l-1" should be uniformly expressed as "ng/l" and "µg/l", or "ng l-1" and "µg l-1"

Authors' Response: Accepted.

Referee Comments: (11) Line 20-22, Page 8: in addition to the reasons mentioned, the lower δ15N-POM values may be related to the atmospheric input, which have a low value of δ15N (-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 δ18O 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 δ18O.

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 $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$. Linear regression of $\delta^{18}O$ versus $\delta^{15}N$ 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 ÉZ15 and É ˙ Z18 computed? The values ˙ of ÉZ15 and É ˙ Z18 presented here are wrong. They should be corrected to be -8.7‰ ˙ and -10.7‰ respectively.

Authors' Response: We have followed Lehmann et al. (2003) where the calculations for ε^{15} and ε^{18} are explained in detail. Using the Rayleigh "closed-system" equation, the ε^{15} (and ε^{18}) were computed from the slopes of $\delta^{15}N\text{-}NO_3^-$ (and $\delta^{18}O\text{-}NO_3^-$) versus natural logarithm of f_{NO3-}, the fraction of remaining nitrate. The observed maximum $[NO_3^-]$ was considered as the $[NO_3^-]_{initial}$.

$$f_{NO3-} = [NO_3^-] / [NO_3^-]_{initial}$$

We have corrected the ε^{15} and ε^{18} values in the text to -8.7‰ and -10.7‰.

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 (Table 1 in the revised manuscript) is important as we are comparing the ε^{15} and ε^{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) The depth-wise variations of ammonium concentration and $\delta^{15}N\text{-}NH_4^+$ in May 2012. (b) Plot of $\delta^{15}N$-PON versus $\ln(NH_4^+)$. The negative linear correlation yields a fractionation factor (ε) of -2.4‰.

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 after the comparison?

Authors' Response: We have added a concluding sentence "For the low to moderate ammonium concentrations recorded (maximum ~12 μM 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 SO4.

Authors' Response: H_2S 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 $\delta^{13}C$ and $\delta^{15}N$ of POM during the period H_2S 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 NH4+. Hence, I am wondering whether there is any competitive uptake between NO3 and NH4.

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 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. In the revised manuscript, these are figures 4 and 7.

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 $\delta^{18}O$ and $\delta^{15}N$ values of nitrate in the epilimnion were high, a signature of assimilation: phytoplankton prefer nitrate containing ^{14}N and ^{16}O leaving residual nitrate enriched with $\delta^{15}N$ and $\delta^{18}O$ (Casciotti et al., 2002)"

Line 9, Page 10: "Dissimilatory nitrate reduction is known to be associated with 1:1 increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (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, Page9: "This is because, while the oxygen atoms in atmospheric nitrate are derived from interactions between NO_x and O_3 in the atmosphere, those in nitrate produced by nitrification come from dissolved oxygen and water (Kendall, 1998, Finlay et al., 2007)"

3.AUTHOR'S CHANGES IN MANUSCRIPT

Isotopic composition of nitrate and particulate organic matter in a pristine dam-reservoir of western India: Implications for biogeochemical processes

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Abstract:

Isotopic composition of nitrate ($\delta^{15}N$ and $\delta^{18}O$) and particulate organic matter (POM) ($\delta^{15}N$ and $\delta^{13}C$) were measured in Tillari Reservoir, located at the foothills of the Western Ghats, Maharashtra, western India. The reservoir that is stratified during spring-summer and autumn seasons but gets vertically mixed during the Southwest Monsoon (SWM) and winter is characterized by diverse redox nitrogen transformations in space and time. The $\delta^{15}N$ and $\delta^{18}O$ values of nitrate were low ($\delta^{15}N$ = 2-10‰, $\delta^{18}O$ = 5-8‰) during normoxic conditions but increased gradually (highest $\delta^{15}N$=27‰, $\delta^{18}O$=29‰) when anoxic conditions facilitated denitrification in the hypolimnion during spring-early summer. Once nitrate was fully utilized and sulphidic conditions set in, NH_4^+ became the dominant inorganic N species, with $\delta^{15}N$ ranging from 1.3 to 2.6‰. Low $\delta^{15}N$ (~−5‰) and $\delta^{13}C$ (−37‰ to −32‰) of POM co-occurring with high NH_4^+ and CH_4 in sulphidic bottom waters were probably the consequence of microbial chemosynthesis. Assimilation of nitrate in the epilimnion was the major controlling process on the N-isotopic composition of POM ($\delta^{15}N$ = 2 - 6 ‰). Episodic low $\delta^{15}N$ values of POM (−2 to 0‰) during early summer coinciding with the absence of nitrate might arise from N-fixation, although further work is required to confirm the hypothesis. $\delta^{13}C$-POM in the photic zone ranged between −29‰ and −27‰ for most parts of the year. The periods of mixing were characterized by uniform $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- at all depths. Higher POM (particulate organic carbon (POC) as well as particulate organic nitrogen (PON)) contents and C/N values with lower $\delta^{13}C$-POM during the SWM point to allochthonous inputs. Overall, this study, the first of its kind in the Indian subcontinent, ~~demonstrates the potential of stable carbon and nitrogen isotopes to gain insight into biogeochemistry of Indian reservoirs~~ 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.

1.Introduction:

Nitrogen is an essential macronutrient the availability of which often limits primary production in aquatic ecosystems. It is a polyvalent element that undergoes redox transformation between the terminal oxidation states of +5 and −3. These transformations involve isotopic fractionation to varying degrees, and so natural abundance of stable isotopes (^{15}N and ^{14}N) in various N species provides useful insight into nitrogen cycling besides its sources/sinks in the oceanic (Altabet, 1988; Sigman et al., 2005), coastal (Thunell et al., 2004; Hu et al., 2015) and estuarine (Cifuentes et al, 1988; Savoye et al., 2012) water-bodies and sediments. Studies have also been undertaken in freshwater systems like lakes (Pang and Nriagu, 1977; Chen et al., 2014) and reservoirs (Chen and Jia, 2009; Junet et al., 2009). Some of the best studied freshwater ecosystems in this regard are Lake Lugano at the Swiss-Italian border, Lake Kinneret in Israel and Lake Superior in the USA.

In the eutrophic Lake Lugano, the highly depleted $\delta^{13}C$ and $\delta^{15}N$ of the near-bottom POM established the active presence of methanotrophic bacteria during suboxic conditions (Lehmann et al., 2004). Seasonal changes in nitrogen species were reflected in the isotopic composition of particulate organic matter (POM) and dissolved inorganic nitrogen (DIN) compounds in Lake Kinneret (Hadas et al., 2009). Various processes like nitrification, denitrification and N_2-fixation were identified with the help of the N isotopes. In Lake Superior, based on nitrate isotopic studies it was possible to identify the increasing inputs of reduced N to the lake and its subsequent nitrification to be the cause behind a century-long increase in the nitrate inventory of the lake, ruling out atmospheric deposition as the other probable cause (Finlay et al., 2007).

There are a large number of natural freshwater lakes as well as man-made reservoirs in India. In fact, India has the third-highest number of dams (around 4300) in the world, after China

and USA. However, these systems have not been well investigated for biogeochemical cycling.. In the very first study of its kind, Narvenkar et al. (2013) sampled eight dam-reservoirs spread across India and observed strong thermal stratification during summer in all reservoirs. Six of these reservoirs were found to experience varying degrees of oxygen depletion in the hypolimnia, ranging from hypoxia to complete anoxia, in spring-summer. Anoxia has been found to greatly affect the distribution of nitrogen species in these systems. ~~One of these reservoirs – the Tillari Reservoir – has been selected for detailed biogeochemical studies including stable isotope abundance in nitrate and POM. We report here results of this study, which to our knowledge are the first ever from any Indian freshwater body.~~ In order to gain insights into biogeochemical cycling in these poorly investigated water bodies, we selected the Tillari Reservoir for detailed studies. These included measurements of natural abundance of nitrogen and oxygen isotopes in nitrate, and nitrogen and carbon isotopes in POM. These data, first of their kind generated from any Indian freshwater body, facilitate an understanding of biogeochemical processes (especially involving nitrogen) that should be typical of any relatively pristine, tropical, monsoon-affected freshwater body.

2.Methods:

2.1 Site Description:

The Tillari Reservoir is situated in the Dodamarg *taluka* in the Sindhudurg district of Maharashtra (15°76'N, 74°12'E, Fig. 1). Created by damming the Tillari River, the reservoir has a maximum depth of ~50 m and a storage capacity of 0.45 x 10^9 m^3 (Kurian et al. 2012). ~~The rainfall in this area, averaging around 3000 mm, almost entirely occurs between June and September. The water from the reservoir is mainly used for irrigation.~~ The reservoir is located close to the foothills of the Western Ghats, with the drainage basin having evergreen forests

(C3 plant type) as well as grasslands (C3 or C4 plant types) (Sukumar et al., 1995). The drainage basin of Tillari has low population density, and so the river water is not much impacted by human activities such as municipal and industrial discharges, and agriculture. This is reflected by high water quality (Shenoy et al., manuscript in preparation). The region receives rainfall averaging around 3000 mm annually, almost entirely between June and September. The evaporation rate in Tillari Reservoir is not known, but for other Indian reservoirs the evaporative loss is reported to average around 0.2 m (Subramanya,2013) per month. Water from Tillari Reservoir is mainly used for irrigation. Some watershed characteristics of the Tillari Reservoir have been listed in Supplementary Table 1.

The Tillari Reservoir is a dimictic water body. Relatively low air temperatures and cool winds descending from the Western Ghats, located immediately to the east of the reservoir, result in convective mixing and well oxygenated conditions in winter. The water column gets thermally stratified in spring and remains so until the strong SWM winds and supply of relatively cold water homogenize the water column again. The water column gets stratified after the SWM. Stratification during spring-summer leads to anoxic condition that is most intense (sulphidic in most years) just before the onset of mixing in June-July. A previous study (Kurian et al, 2012) showed that the occurrence of sulphidic conditions within the euphotic zone supports anoxygenic photosynthesis by brown sulphur bacteria in this reservoir. Methane has been found to accumulate in high concentrations below the thermocline during this period; however, its emissions to the atmosphere are not very high (Narvenkar et al., 2013). Direct human impacts on nutrient inventory of the reservoir are relatively minor, as the basin is located amidst thick forests with low human population density and minimum agricultural activities.

2.2 Sampling and field measurements:

Sampling was conducted at one station located at the deepest part of the reservoir. Water samples from pre-fixed depths were collected with 5-litre Niskin samplers ~~(5 l)~~ attached to nylon ropes and equipped with reversing thermometers to measure temperature. Subsamples for dissolved oxygen (DO) and hydrogen sulfide (H_2S) were collected carefully avoiding air exchange. Subsamples for nutrients (nitrate and ammonium) were collected in clean 60-ml HDPE bottles and frozen immediately. Subsamples for stable isotopic analyses were collected in 5-litre acid-cleaned plastic carboys and transported to the laboratory within 3-4 hours.

2.3 Laboratory analyses:

Dissolved O_2 was estimated by the Winkler method (Grasshoff et al., 1983) with a precision of <1 µM. NO_3^- and NH_4^+ were measured using a SKALAR segmented flow analyzer following standard procedures (Grasshoff et al., 1983) with a precision of <0.1 µM. Dissolved H_2S concentration was determined colorimetrically (Cline, 1969).

2.4 Isotopic analyses :

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. Samples for nitrate isotopic measurements were collected from 2011. 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. Samples (upto 3l) for isotopic analyses of POM and DIN (dissolved inorganic nitrogen i.e. NO_3^- and NH_4^+) were filtered through precombusted (450° C for 4 hours) 47mm GF/F filters (pore size = 0.7 µm). The filtrate was used for DIN isotopic measurements and the filter papers were placed in petriplates and frozen immediately.

2.4.1 Analyses of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-:

Samples for isotopic analysis of nitrate were preserved in two ways. While samples collected in 2011 and 2012 were acidified with HCl to pH 2.5, those taken in 2014 and 2015 were frozen immediately and analysed within a week. Prior to the isotopic analyses, nitrate and nitrite concentrations were measured colorimetrically. Isotopic analyses of nitrogen and oxygen in NO_3^- were carried out following the "chemical method" (McIlvin and Altabet, 2005) involving reduction of NO_3^- to NO_2^- by cadmium and further reduction to N_2O by sodium azide in an acetic acid buffer. The resulting N_2O gas in the headspace was purged into a GasBench II (Thermo Finnigan) and analysed in a Delta V isotope ratio mass spectrometer.

Nitrite concentration was insignificant in most of the samples; sulphamic acid was added in a few samples that contained nitrite in concentrations exceeding 0.1 µM. Working standards were prepared in low-nutrient surface seawater (LNSW) collected from the Arabian Sea. Calibration was done using international nitrate isotope standards USGS-32, USGS-34 and USGS-35. For further quality assurance, an internal potassium nitrate standard (spanning the range of nitrate concentration in the samples) was run with each batch of samples. Magnesium oxide (MgO, Fisher; precombusted for 4 hours at 450˚C) was added to each sample to raise the pH close to 9 which was followed by addition of cadmium. We used cadmium powder (Alfa Aesar, −325 mesh, 99.5%) instead of spongy cadmium as mentioned in McIlvin and Altabet (2005). Each vial was wrapped in aluminium foil and placed on a horizontal shaker at low speed for 17 hours. After the stipulated time, samples were removed from the shaker, centrifuged and decanted into clean vials. The nitrite concentrations in the decanted samples were measured to check the extent of reduction.

Sodium azide (2M solution) and 20% acetic acid were mixed in 1:1 proportion (by volume) to yield the azide-acetic acid buffer (A-AA buffer) solution. In 20 ml crimp vials, samples

454 and standards were diluted with LNSW for a final concentration of 20 nmoles and a final
455 volume of 15 ml. Two international nitrite standards (N23 and N20) were added in this step
456 to check the efficiency of N_2O production by the buffer. After addition of the A-AA buffer,
457 the vials were allowed to stand for 1 hour and then the reaction was stopped by adding 0.5ml
458 of 10M NaOH.
459 The "chemical" method yielded a very low blank (~ 0.5 µM) and worked well for the low
460 concentration samples. The international standards were run before and after each batch of
461 samples, while the internal nitrate standards were run after every 5 samples. Analytical
462 precision (one standard deviation) was better than 0.3‰ for $\delta^{15}N$ and better than 0.7‰ for
463 $\delta^{18}O$. Results are expressed in δ notation ($\delta^{15}N$ and $\delta^{18}O$), as per mil (‰) deviation from
464 atmospheric nitrogen and Vienna Standard Mean Ocean Water (VSMOW), respectively.

465 ***2.4.2 Analyses of $\delta^{15}N$ of NH_4^+:***

466 Samples for measurements of $\delta^{15}N$- NH_4^+ was collected during May 2012 from the anaerobic
467 hypolimnetic waters.The $\delta^{15}N$ of NH_4^+ was measured by the "ammonia diffusion" method
468 (Holmes et al., 1998). Briefly, 500 ml of sample was collected in duplicates to which 1.5g of
469 MgO was added to elevate the pH. The diffused NH_4^+ was trapped onto acidified glass-fiber
470 filter sealed between two porous Teflon membranes. The sample bottles were kept in an
471 incubator-shaker (20˚C, 80 rpm) for two weeks for complete diffusion of NH_4^+. After two
472 weeks, the GF filters were removed from each sample, dried in a NH_4^+-free environment,
473 packed into tin cups and immediately analysed using CF-EA-IRMS. Results were corrected
474 for blank, percent recovery and fractionation. Analytical precision was better than 0.6‰.

475 ***2.4.3 Analyses of $\delta^{13}C$ and $\delta^{15}N$ of POM and surface sediment:***

476 The analyses of $\delta^{13}C$ and $\delta^{15}N$ of POM were usually conducted within 1-2 months of
477 collection. The frozen filters were acid-fumed with 36% HCl to eliminate carbonates and air
478 dried in a clean laminar flow. Two aliquots (each of 12 mm diameter) were sub-sectioned

from each filter and packed into tin cups for analysis. Detailed methodology is given in Maya et al. (2011). The $\delta^{13}C$ and $\delta^{15}N$ of POM along with particulate C and N contents were analyzed in the same sample using a stable isotope ratio mass spectrometer (Thermo Finnigan Delta V) connected to an elemental analyser (EURO3000 Eurovector). Results are expressed as per mil (‰) deviation with respect to PDB (Pee Dee Belemnite) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Analytical precision was better than ±0.2‰ as determined from repeated measurements (after every 5 samples) of a working standard, ε-Amino-n-Caproic Acid (ACA) having $\delta^{13}C = -25.3‰$ and $\delta^{15}N = 4.6‰$, and a laboratory sediment standard having $\delta^{13}C = -21‰$ and $\delta^{15}N = 7.5‰$.

Surface sediment collected from the reservoir during the May 2012 field trip was analysed on only one occasion to investigate its role as an ammonium source. The freeze-dried, homogenized sample was analyzed following similar protocol.

3.Results

3.1 Water column observations

Based on the vertical temperature distribution it appears that the reservoir gets vertically mixed through convective overturning in winter (December to February, with the exact duration of mixing depending upon meteorological conditions prevailing in a given year). In spring stratification sets in and is the most intense from April to June/July (with a surface-to-bottom temperature difference of 7-8°C). The water column is again homogenized following SWM induced mixing and flow of relatively cold water, followed by weaker stratification in autumn/early winter. A detailed discussion on the physico-chemical parameters is provided in Shenoy et al. (manuscript under preparation).

The epilimnion was always oxic. During the stratification periods, the DO concentrations dropped rapidly within the thermocline. The water column became well-oxygenated

following the onset of the southwest monsoon. H_2S was detected below 20 m during the period of intense stratification (Kurian et al., 2012), with the highest concentration recorded being 9.88 µM. The occurrence of H_2S was accompanied by the appearance of CH_4 and NH_4^+. Upto 160 µM of CH_4 and 30 µM of NH_4^+ were observed in the anoxic bottom waters during peak summer (Narvenkar et al., 2013).

A thorough analysis of nutrient dynamics in Tillari Reservoir is provided by Naik et al. (manuscript under preparation). Here we provide a brief description of nitrate profiles during the study period. Surface water nitrate concentrations were typically low throughout the year ranging from below detection limit to 0.7 µM. However, the surface nitrate concentrations were as high as ~10 µM (Fig. 3~~2~~a) during the SW Monsoon. Nitrate concentrations gradually increased below the epilimnion during the period of weak stratification. However, with the depletion of DO, nitrate concentrations in the hypolimnion decreased from 3.6 µM (at 20m) to 0.3 µM (at 35m), indicating N-loss. Reoxygenation of hypolimnion during the SW monsoon was accompanied by increase in nitrate concentrations (5-10 µM).

3.2 Isotopic composition of nitrate and ammonium

Large variations in the isotopic composition of nitrate and ammonium were observed in space and time. Isotopic composition of nitrate in the epilimnion could not be measured on several occasions due to low concentrations. However, when the measurements could be made it was observed that the $\delta^{15}N$ and $\delta^{18}O$ values of epilimnetic (0-10 m) NO_3^- were high ($\delta^{15}N$ = 8-25‰, $\delta^{18}O$ = 24-29‰) (Fig 3~~2~~b) during the summer stratification presumably due to autotrophic assimilation whereas relatively lower values ($\delta^{15}N$ = 5-8‰, $\delta^{18}O$ = 12-15‰) were observed during the monsoon mixing events. Increasing $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-, coupled to decreasing [NO_3^-], were also observed in the suboxic hypolimnion during April and May, when the water column was strongly stratified. The highest $\delta^{15}N$ values observed

were 27.7‰ (in 2014) and 22.4‰ (in 2012) while the corresponding highest $\delta^{18}O$ values were 29.5‰ and 28.8‰, respectively.

The water column remains weakly stratified for a large part of the year, usually from October to March. A trend of increasing concentrations of isotopically light ($\delta^{15}N$ = 2-8‰ and $\delta^{18}O$ = 5-8‰) nitrate was observed in the hypolimnion along with gradually decreasing levels of oxygen and ammonium implying the occurrence of nitrification. As the stratification intensified, this phenomenon was restricted only to the metalimnion. After nitrate was exhausted, high ammonium build up was observed in the bottom waters. In May 2012, NH_4^+ concentrations increased from 0.6 µM at 20m to nearly 12 µM at 40m with a corresponding decrease in $\delta^{15}N$–NH_4^+ from 2.6‰ at 20m to 1.3‰ at 40m (Fig. 54a).

Elevated nitrate concentrations occur throughout the water column during the SW monsoon. The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- showed little vertical variations at this time. However, interannual variability was seen in the $\delta^{15}N$ of nitrate (3.94±2.4‰ in 2011, 11.38±1.6‰ in 2014, and 5.47±1.8‰ in 2015), the cause of which will be examined. By contrast, the $\delta^{18}O$-NO_3^- values were relatively less variable (13.01±4.8‰ in 2011, 15.41±2.3‰ in 2014, and 12.46±4.9‰ in 2015).

3.3 Isotopic and elemental composition of suspended particulate organic matter

The suspended particulate organic matter in the Tillari Reservoir showed distinct seasonal and depth-wise variations in its isotopic and elemental compositions (Fig. 2). Primary productivity in the epilimnion led to higher $\delta^{15}N$ (2‰ to 6‰) and $\delta^{13}C$ (−28‰ to −26‰) in POM and higher POC (35-60 µM) and PON (4-6 µM) contents as compared to the bottom water. The molar C/N ratios in the surface waters ranged between 7 and 10. Depleted $\delta^{15}N$

(~−1.4‰) in the epilimnion was observed during the early stratification period (February and March). As the stratification intensified, the $\delta^{15}N$ and $\delta^{13}C$ of the epilimnetic POM became heavier, presumably reflecting a gradual enrichment of heavier isotopes in the dissolved inorganic N and C pools. Both $\delta^{15}N$ and $\delta^{13}C$ decreased with depth with the lowest values occurring in the anoxic bottom water during peak stratification period. The C/N values in these waters were in the range of 4-7. In terms of seasonal variability, $\delta^{13}C$ values of POM were lower during monsoon mixing and became more enriched as the stratification intensified. The $\delta^{15}N$ values, however, did not depict any distinct seasonal pattern. High POC (upto 80 µM) and PON (upto 9 µM) along with high C/N (>10) were recorded during the monsoon season apparently reflecting allochthonous inputs.

4. Discussion:

4.1 Epilimnetic processes:

Nitrate concentrations in surface waters of the Tillari Reservoir varied from below detection limit during the premonsoon period to 10.7 µM during the SW monsoon. The $\delta^{18}O$ and $\delta^{15}N$ values of nitrate in the epilimnion were high, a signature of assimilation: phytoplankton prefer nitrate containing ^{14}N and ^{16}O leaving residual nitrate enriched with $\delta^{15}N$ and $\delta^{18}O$ (Casciotti et al.,2002). We examined the slopes of the $\delta^{18}O$ vs. $\delta^{15}N$ regression in the surface water. While a 1:1 line would represent assimilation of epilimnetic nitrate, a steeper slope would imply assimilation along with the regeneration of nitrate via nitrification (Wankel et al., 2007). We observed a nearly 1:1 trend for most of the surface water samples during the summer stratification implying that assimilation exerts the major control on surface NO_3^- isotopic composition (Supplementary Fig. 1).

573 The isotopic composition of the DIN source exerts the key control on the $\delta^{15}N$ of POM
574 (Altabet, 2006). The epilimnetic POM in the Tillari Reservoir is expected to have $\delta^{15}N$ less
575 than or equal to the $\delta^{15}N\text{-}NO_3^-$. Indeed, the $\delta^{15}N$-POM was always lower than the $\delta^{15}N$ of the
576 source nitrate (Fig. 3~~2~~b). The range of $\delta^{13}C$ values of surface-water POM (-32 to -26‰) was
577 typical of lacustrine autochthonous organic matter (-42 to -24‰, Kendall et al.,2001 and
578 references therein). As the summer progressed, productivity increased resulting in increased
579 CO_2 uptake and elevated $\delta^{13}C$-POM. During photosynthesis, phytoplankton preferentially
580 uptake ^{12}C leaving the DIC (dissolved inorganic carbon) pool enriched in ^{13}C. However,
581 when dissolved C is scarce and/or growth rate is high, the phytoplankton would consume the
582 available DIC with reduced or no isotopic discrimination. As the summer progressed at the
583 study location, increased water temperature and low dissolved inorganic nutrient and DIC
584 concentrations would cause the phytoplankton to express reduced isotopic discrimination.
585 This would result in enriched $\delta^{13}C$ of POM. Similar enrichment of $\delta^{13}C$-POM during periods
586 of high productivity have also been observed in other lakes, for e.g., Lake Lugano (Lehmann
587 et al., 2004) and Lake Wauberg (Gu et al., 2006).

588 In March, when nitrate was close to detection limit, surface $\delta^{15}N$-POM was −1.4‰. The
589 POM resulting from nitrogen fixation by cyanobacteria usually has a $\delta^{15}N$ of 0 to −2‰
590 (Carpenter et al., 1997). Zeaxanthin, marker pigment of cyanobacteria, was present in
591 significant concentrations (305.1±21 ng~~4~~ l^{-1}) within the epilimnion, whereas Chl-*a*
592 concentration was ~1.7 µg l^{-1} (S. Kurian, unpublished data). However, measurements of
593 nitrogen fixation rates in the Tillari Reservoir have yielded very low values during summer
594 (unpublished data). Alternatively, the lower $\delta^{15}N$ values may also result from isotopically
595 light nitrate that is produced in the hypolimnion and diffuses upward into surface waters.
596 Another possible source of isotopically lighter N could be atmospheric deposition, although

the magnitude of atmospheric inputs is not expected to be very large during early summer. Further work is required to understand the episodic occurrence of low δ^{15}N-POM.

4.2 Biogeochemistry of hypolimnion

4.2.1 Nitrification:

Stratification in the Tillari Reservoir sets in soon after the decline of the monsoon-fed inflow following which nitrate concentrations increased in oxygenated bottom waters with a concomitant decrease in ammonium concentrations, indicating the occurrence of nitrification. The nitrate concentrations ranged from below detection limit in the upper 10 m to nearly 10 µM close to the bottom. Nitrification occurs in two steps: ammonia oxidation to nitrite (performed by ammonia oxidising archaea and bacteria) and nitrite oxidation to nitrate (performed by nitrite oxidising bacteria). Ammonium, the primary N source, undergoes strong fractionation producing isotopically light nitrate (Delwiche and Stein, 1970, Casciotti et al., 2003). The δ^{15}N-NO_3^- values ranged from 2-10‰ and the δ^{18}O-NO_3^- ranged from 5-8‰ during this period. Nitrate accumulation due to atmospheric deposition and microbial nitrification will have distinct δ^{18}O-NO_3^- values. This is because, while the oxygen atoms in atmospheric nitrate are derived from interactions between NO_x and O_3 in the atmosphere, those in nitrate produced by nitrification come from dissolved oxygen and water (Kendall, 1998, Finlay et al., 2007). This is well reflected in the ^{15}N-^{18}O scatter plot where the δ^{18}O-NO_3^- data-points from the epilimnion and hypolimnion form completely distinct clusters in February (Fig 4~~3~~). As the ammonium pool gets used up, the nitrification rate decreases accompanied by a decrease in the extent of fractionation (Feigin et al., 1974).

Ammonium, oxygen and carbon dioxide are the major substrates needed for nitrification (Christofi et al., 1981). While ammonium largely comes from the sediments, oxygen is supplied from aerated surface waters. During the early stratification period, conducive

conditions exist for nitrifiers to grow within the hypolimnion. However, as the bottom waters turn increasingly more oxygen-depleted with the intensification of stratification the "ammonium-oxygen chemocline" (Christofi et al., 1981) moves upward in the water column and the metalimnion becomes more suitable for the occurrence of nitrification. In April 2014, $\delta^{18}O$ declined within the thermocline from 34‰ at 5m to 14‰ at 20m owing to nitrification. Epilimnetic nitrate isotope data are not available for 2012 due to very low nitrate concentrations. However, the $\delta^{18}O$ declined from 25‰ at 15m to 17‰ at 20m. The $\delta^{15}N$ values in both the years did not show a similar decline, but this is consistent with the results of several other studies (Böttcher et al., 1990; Burns and Kendall, 2002), where the $\delta^{18}O$ was found to be better suited for source and process identification than $\delta^{15}N$. It may be noted that this decoupling of $\delta^{15}N$ and $\delta^{18}O$ was only observed during the peak stratification period at the thermocline.

The $\delta^{15}N$ and $\delta^{13}C$ values for the POM were generally low during the nitrification period as also observed in Lake Kinneret (Hadas et al., 2009). The $\delta^{15}N$ varied from −4‰ to 3‰ while $\delta^{13}C$ varied from −31‰ to −29‰. Assimilation of newly nitrified NO_3^- may be a possible contributor to POM as indicated by the low $\delta^{15}N$ values.

4.2.2 Denitrification:

During the period of strong stratification, the water column loses oxygen below the thermocline, which apparently results in N loss. Along with a decrease in nitrate, there also occurs an increase in NH_4^+ concentration. Dissimilatory nitrate reduction is known to be associated with 1:1 increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Granger et al., 2008). Linear regression of $\delta^{18}O$ versus $\delta^{15}N$ yielded slope values of 0.95 and 0.85 in 2014 and 2012, respectively. In canonical denitrification, both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ increase linearly. The enrichment in isotopic value is ~1 in marine systems (Casciotti et al., 2002, Sigman et

al., 2005, Granger et al., 2008). However, this value is reported to be lower (0.5-0.7) in freshwater systems (Lehmann et al., 2003 and references therein). The reasons for this difference are not fully understood. Also, studies in freshwater systems are sparse as compared to marine systems. In a batch of culture experiments, Granger et al. (2008) observed that nitrate-reducing enzymes play a role in altering the O to N isotopic enrichment, with periplasmic dissimilatory nitrate reductase (Nap) expressing a lower enrichment value (~0.62) than the membrane-bound dissimilatory nitrate reductase. Again, there is a lack of data on the isotopic expressions of these enzymes at the ecosystem level. Wenk et al. (2014) attributed the low O:N isotopic effect of ~0.89 to chemolithoautotrophic denitrification, rather than heterotrophic denitrification, in the northern basin of Lake Lugano.

Our data from the Tillari reservoir indicates the occurrence of denitrification in the suboxic hypolimnion under stratified conditions. However, this process is restricted to a narrow depth range of 10-20 m which limits the number of data points. There may be several factors responsible for the low (<1) isotopic enrichment factor in the Tillari but our data are not sufficient to identify the exact cause(s).

Assuming the N loss was largely through denitrification, an attempt was made to compute the fractionation factor using a Rayleigh "closed-system" model (Lehmann et al., 2003). Although there have been several attempts to compute the nitrogen isotope enrichment factors in marine systems, ground waters and laboratory cultures (Table 1~~2~~); similar information is relatively scarce from freshwater lakes and reservoirs.

The available information on oxygen isotope fractionation is even scarcer. The values of ε^{15} and ε^{18} computed by us are –8.7‰ and –10.7‰, respectively. The ε^{15} is much lower than those obtained from laboratory cultures (Olleros, 1983; Table 1~~2~~) as well as open-ocean OMZs (Brandes et al., 1998, Voss et al., 2001; Table 1~~2~~) although it is close to the ε^{15} reported from the eutrophic Lake Lugano. Factors controlling denitrification rates in aquatic

systems include temperature, availability of nitrate and organic carbon, oxygen concentration and type of bacterium involved (Seitzinger et al., 1988, Bottcher et al., 1990, and references therein). Sedimentary denitrification is known to incur isotope effect (ε^{15}) of ~0‰ due to almost complete exhaustion of nitrate. The dissolved nitrate concentrations in the Tillari Reservoir are quite low with the highest values being in the range of 10-12 µM (see Results). The hypolimnetic nitrate concentrations were even lower (< 5 µM) during periods of anoxia. Low nitrate availability and sedimentary N-loss may exert major controls on the low ε^{15} observed in the Tillari Reservoir.

Denitrification strongly discriminates among the two N isotopes, leaving behind ^{15}N-enriched in the residual NO_3^-. POM produced by assimilation of this nitrate will also be enriched in ^{15}N. However, lower δ^{15}N-PON at these depths implies that NH_4^+ was the preferred DIN source. For instance, observations in April 2012 showed that denitrification was active below 30m and associated with ammonium build-up, there was nearly a 4‰ depletion in δ^{15}N-PON from 2.5‰ (at 30m) to −2.3‰ (at 40m).

4.2.3 Ammonification:

The isotopic composition of ammonium should reflect that of the sedimentary organic matter being degraded. In Lake Kinneret (Israel), $\delta^{15}N$–NH_4^+ values in the hypolimnion during stratified conditions ranged from 12 to 17 ‰ reflecting the high δ^{15}N of the sedimentary OM ($\delta^{15}N$ = 10‰) (Hadas et al., 2009). In Lake Bled (NW Slovenia), mean $\delta^{15}N$– NH_4^+ value of 3.8‰ was similar to that of sedimentary OM ($\delta^{15}N$ = 4.5‰) (Bratkic et al., 2012). Likewise, the sedimentary OM in the Tillari Reservoir had a δ^{15}N of 2.96‰ similar to the $\delta^{15}N$–NH_4^+ (1.3-2.6‰) thus establishing remineralization of sedimentary OM as the principal NH_4^+ source.

A negative linear relationship between δ^{15}N-PON and ln[NH_4^+] was observed (Fig. 54b) which further indicated uptake of NH_4^+. The fractionation factor (ε) calculated from the slope was −2.4‰. The fractionation factor for ammonium assimilation has been estimated in several field studies (Cifuentes et al., 1988; Bratkic et al 2012) as well as in lab cultures with different organisms (green algae, marine bacteria, etc) (Wada & Hattori, 1978, Wada 1980, Hoch et al 1992). However, such studies in freshwater lakes and reservoirs are scarce. Bratkic et al. (2012) computed fractionation factors of −0.8‰ and −1.4‰ for mean ammonium concentrations of 4.7 µM and 3.3 µM respectively in Lake Bled. Hoch et al. (1992) reported fractionation factor for assimilation by *Vibrio harveyi*, a marine bacterium, to be between −4‰ and −27‰ for ammonium concentrations ranging from 23 to 180 µM. The fractionation factor is expected to approach 0‰ for decreased concentrations of ammonium. For the low to moderate ammonium concentrations recorded (maximum ~12 µM in Figure 5) the fractionation factor computed by us compares well with previously reported values.

4.2.4 Sulphate reduction and evidence for chemosynthesis:

As the summer intensified and oxidized nitrogen was fully utilized, facultative bacteria apparently began to utilize sulphate as an electron acceptor as indicated by the accumulation of H_2S.Mass dependent fractionation during microbial degradation of organic matter with sulphate as an electron acceptor would the residual organic matter enriched in ^{13}C and ^{15}N. However, ~~as H_2S started building up in the water column of the Tillari Reservoir, both δ^{13}C-POC and δ^{15}N-PON became more depleted~~ following the appearance of H_2S, both δ^{13}C-POC and δ^{15}N-PON became more depleted. The δ^{15}N values varied between −8‰ and −5‰ and δ^{13}C values ranged from −37‰ to −32‰ between 30 and 40m depths. The accumulation of H_2S was also accompanied by significant build-up of CH_4 (20-150 µM) and NH_4^+ (1-20 µM) (Naik et al., manuscript in prep.). Increases in POC and PON contents were also observed:

717 from 28 µM to 60 µM for POC and from 4.7 to 8 µM for PON. Bacterial assimilation of
718 ammonium can explain the isotopically light nitrogen, but utilization of biogenic methane is
719 known to lead to extremely low $\delta^{13}C$ values (between −65‰ and −50‰; Whiticar et al.,
720 1986). In our study, the most depleted $\delta^{13}C$-POC value of −37.8‰ was associated with the
721 highest methane concentration of 156 µM. Interestingly, in a study carried out in the waters
722 of Lake Baikal in Siberia, very negative $\delta^{13}C$–DIC values (−28.9 to −35.6‰) were inferred to
723 be derived from methane oxidation while the $\delta^{13}C$-POC values (−31.7 to −33.5‰) were
724 typical of lacustrine organic matter (Prokopenko and Williams 2005). The authors explained
725 this lack of correlation between the two C pools by a possible time lag between the peak
726 methane oxidation and peak productivity. Low $\delta^{13}C$-POC (~−37‰) in Lake Kinneret was
727 attributed to chemosynthetic C fixation using depleted $\delta^{13}C$-DIC derived from methane
728 oxidation (Hadas et al. 2009). It is important to understand the fate of methane in freshwater
729 systems as they are believed to be significant contributors to atmospheric methane emissions
730 (Bastviken et al., 2004). The POM isotopic data of the Tillari Reservoir provides evidence
731 for intense microbial chemosynthesis using sulphide, ammonia and methane as energy
732 donors.

733 ***4.3 Monsoon mixing in Tillari Reservoir:***

734 The reservoir gets vertically mixed during the months of July, August and September due to a
735 combination of lower atmospheric temperature, strong winds and inflow of relatively cold
736 water during the southwest monsoon. Nitrate concentrations are moderately high throughout
737 the water column, although variable from one year to another. The mean water-column
738 nitrate concentration were 7.26±2.8 µM (n = 10) in 2011, 9.29±0.8 µM (n = 10) in 2014, and
739 8.13±4.7 µM (n = 9) in 2015. The isotopic composition of nitrate also showed inter-annual
740 variability. While the water column was uniformly nitrate-replete in 2014, the epilimnetic (0-

5 m) nitrate concentrations in 2011 and 2015 were markedly lower than those at deeper depths (Fig.76), ~~indicating nitrate uptake in spite of light-limited conditions. This was also evident in the nitrate isotopic data.~~ except at two deepest samples in 2015. This may indicate nitrate uptake by phytoplankton. However, considering its high concentration in rainwater, ammonium is expected to compete with nitrate for phytoplankton uptake. Moreover, the $\delta^{15}N$ of nitrate in the epilimnion was lower in 2011 and 2015 than in 2014. In fact, elevated values of $\delta^{15}N\text{-}NO_3^-$ (>8‰) occurred throughout the water column in 2014 when the nitrate concentration was also generally higher as compared to the other two years. To investigate the cause of this variability, water samples from six upstream stations along the Tillari River along with a rainwater sample at the main station were collected in 2015. The nitrate concentrations ranged from 1.8 µM at the most upstream station to 9.4 µM close to our main sampling site. The ranges of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- at these stations were 0.4-6.8‰ and 11-27‰, respectively. The ~~precipitation~~ rainwater sample had a nitrate content of 13.89 µM (ammonium = 24.4 µM) and yielded $\delta^{15}N$ and $\delta^{18}O$ values of −2.9‰ and 88.7‰, respectively. Nitrate in wet deposition is usually characterised by high $\delta^{18}O$ (> 60‰) (Kendall et al., 2007; Thibodeau et al., 2013) and low $\delta^{15}N$ (−10 to +5 ‰) (Heaton et al., 2004) values. ~~The nitrate concentrations in the main station were measured to be as high as 13.5 µM (at 15m).~~ Unfortunately, the concentration and isotopic composition of these end members (river runoff and atmospheric deposition) do not explain the data from the Tillari especially from the 2015. Based on the high concentration of nitrate in rainwater, it is tempting to suggest that it could be an important source, but the isotopic data show a mismatch.

The $\delta^{13}C$-POC values in the epilimnion decreased to nearly −30‰ presumably due to a combination of lower primary productivity and inputs of organic matter through runoff. Even though the latter was not measured POC derived from land vegetation is expected to be

isotopically light.The POM data show the ingress of a nearly 30m thick parcel of water from the Tillari River into the reservoir. This ingress is apparent below 5m depth by distinct $\delta^{13}C$ and $\delta^{15}N$ of POM. The $\delta^{13}C$-POC increases from −30.9‰ (±0.1‰) in the upper 5m to −25.4‰ (±1‰) between 5m and 40m. Below 40m, the mean $\delta^{13}C$-POC was −26.5‰ (±1.7‰). The mean $\delta^{15}N$ of the intermediate water parcel was 5.97±2‰, as compared to 5.49±3‰ in the bottom waters and 3.96±2‰ in the upper 5m. The isotopic data correspond well with the ancillary chemical parameters, in that the water parcel had a distinct thermal signature (cooler by nearly 2˚C). It also possessed higher levels of nitrate and lower levels of DO and chlorophyll-*a*.

Thus, looking solely at the high nitrate concentrations in the water column, atmospheric wet deposition ~~seems to be a dominant~~ may be a major nitrate source to the water column during the monsoon season. However, this inference is based on a single measurement where the isotopic composition is also different. Moreover, the river water is also rain-fed and it is not clear why its isotopic composition is much lower at the most upstream station. ~~At the same time, the POM depicts the isotopic signature of the mixing with the upstream waters.~~ At the same time, the isotopic composition of POM indicates influence of the upstream waters. Variable inputs from the atmosphere and by river runoff to the DIN pool probably account for the interannual variability, but more studies are needed to identify and quantify these contributions in detail.

5. Summary and Conclusions:

Using stable isotopes of nitrate, ammonium and particulate organic matter, we have been able to identify distinct water column conditions and transformation processes of reactive nitrogen in the Tillari Reservoir. The reservoir gets vertically mixed during the southwest monsoon season as well as in winter; the water column remained stratified during other parts of the

790 year. The most intense stratification occurs during summer just before the monsoon onset.
791 Relative importance of microbial processes such as nitrification, denitrification,
792 ammonification and sulphate reduction in the water column varied depending on intensity of
793 stratification and associated DO levels in the hypolimnion. These processes produced unique
794 isotopic signatures in the dissolved and particulate matter. Our results suggest the occurrence
795 of microbial chemosynthesis using methane and ammonium as primary C- and N- sources,
796 producing organic matter in the anoxic bottom waters that is highly depleted in ^{13}C and ^{15}N
797 content. The thermocline in the Tillari Reservoir has been known to harbour photoautotrophic
798 sulphur bacteria during peak stratification periods (Kurian et al., 2012). We also found strong
799 signatures of nitrification within this zone during summer stratification. Autochthonous
800 production was the principal source of organic matter in the epilimnion which was well-
801 oxygenated at all times, although productivity was significantly lower during the monsoon
802 period due to light-limited conditions. Nitrate was the preferred DIN source in the
803 epilimnion. When nitrate loss occurred in the hypolimnion, the preferred DIN species
804 switched from nitrate to ammonium. Isotopic measurement of precipitation and upstream
805 river samples during one seasonal sampling provided some insight into sources of nitrogen,
806 but the observed inter-annual variability could not be explained. Overall, solar intensity,
807 water depth and redox conditions appear to be the major factors controlling biogeochemical
808 cycling in this pristine reservoir.

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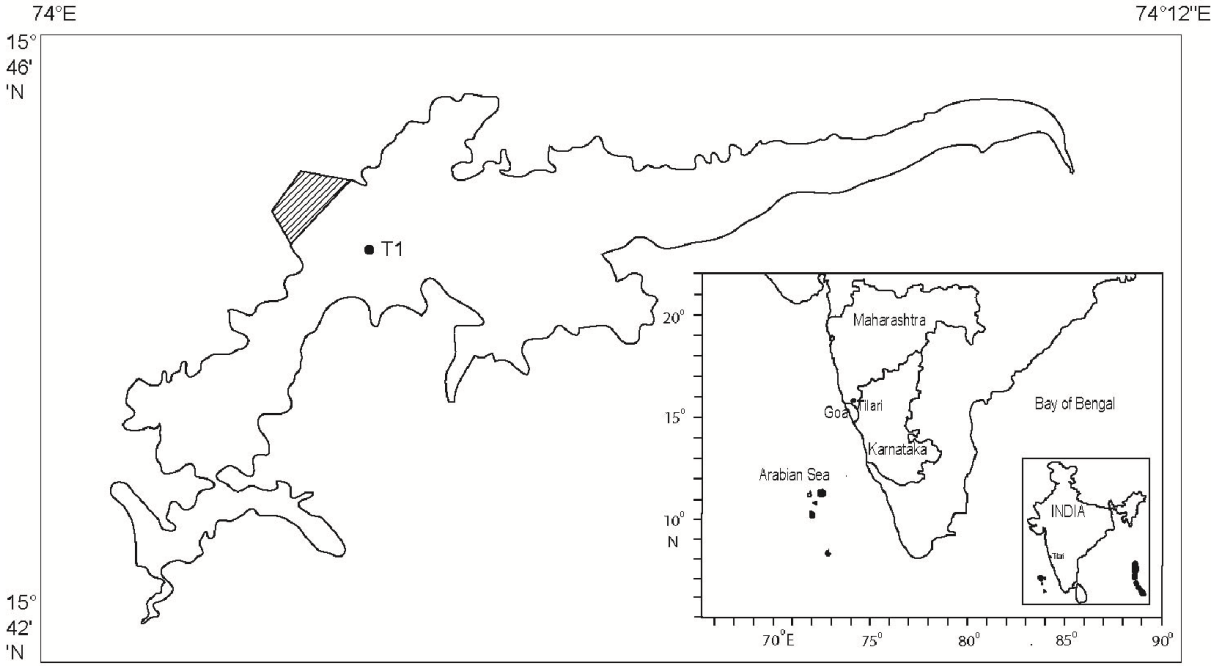
Table 1: Watershed characteristics of Tillari Reservoir (Source : http://www.cwc.nic.in/main/downloads/National%20Register%20of%20Large%20dams%202009.pdf).

Year of completion	Type	Ht. above lowest foundation	Length of dam	Gross storage capacity	Reservoir area	Effective storage capacity
2006	Earthfill /Gravity	73 m	943 m	9274 x $10^3 m^3$	16250 x $10^3 m^2$	447290 x $10^3 m^3$

Table 12: The values of nitrogen (ε^{15}) and oxygen (ε^{18}) isotope effects for denitrification as reported from some natural systems as well as laboratory cultures.

Study Area	ε^{15} (‰)	ε^{18}(‰)	Reference
Cariaco Basin, Venezuela	-1.5		*Thunell et al.*, 2004
Beijiang River, China	-14.8	-8.5	*Chen et al.*, 2009
Boknis Eck, Baltic Sea	-18.9	-15.8	*Dahnke and Thamdrup*, 2013
Lake Lugano, Switzerland	-11.2	-6.6	*Lehmann et al.*, 2003
Groundwater	-27.6	-18.3	*Mengis et al.*, 1999
Denitrifier culture	-30	-15	*Olleros*, 1983
Denitrifier culture	$-10\ to\ -15$		*Kritee et al.*, 2012
Open-ocean OMZs	$-20\ to\ -30$		*Brandes et al.*, 1998; *Voss et al.*, 2001
Shallow groundwater aquifer	-15.9	-8	*Bottcher et al.*, 1990
Tillari reservoir, India	-8.73	-10.74	**This study**

Figure 1: Map of the sampling location (Tillari Reservoir). T1 shows the main sampling location at the deepest point of the reservoir.



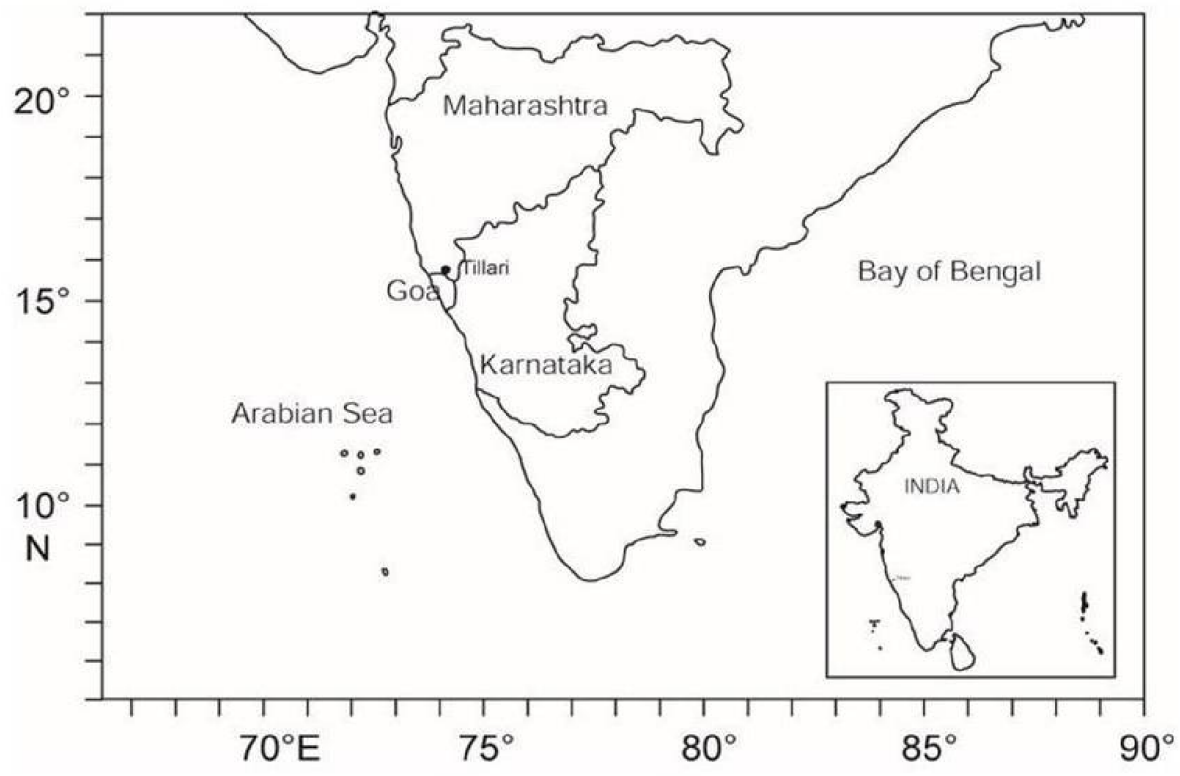


Figure 2:Mean annual variations of δ^{15}N-POM and δ^{13}C-POM at the main sampling location.

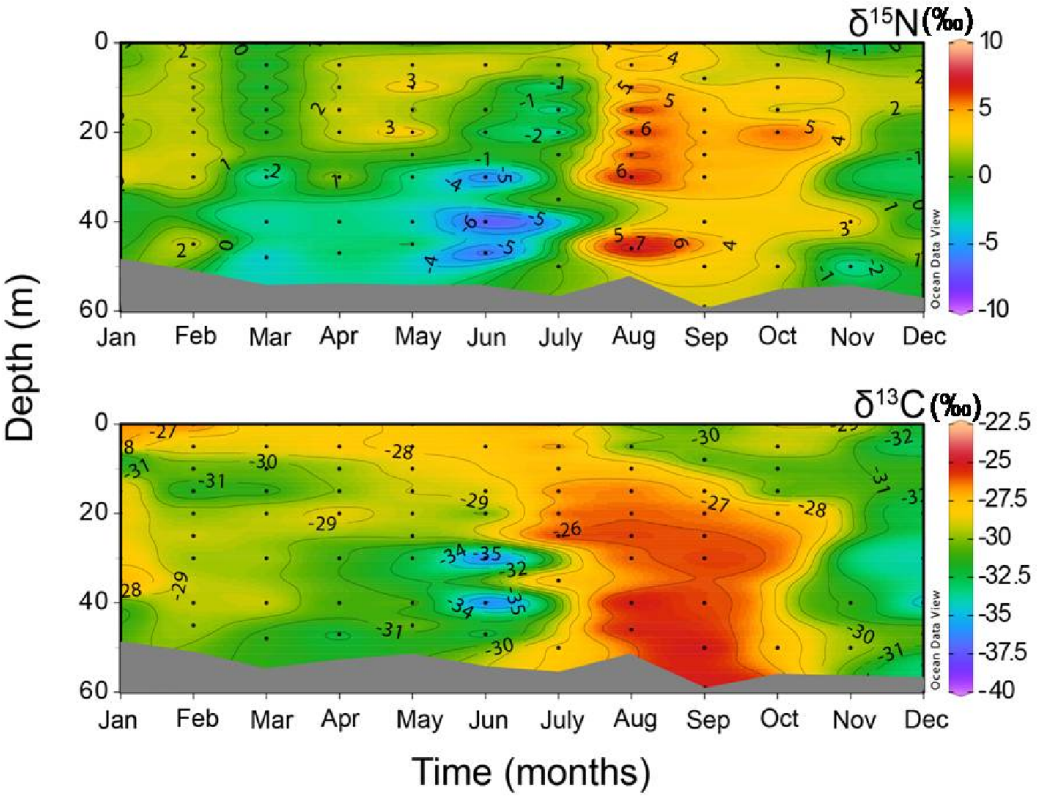
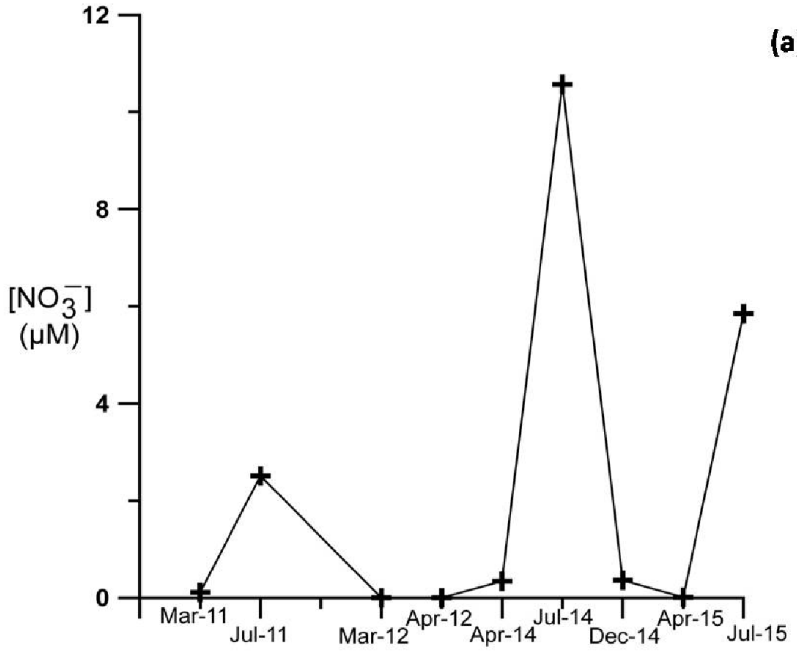


Figure 32: Time-series of nitrate concentrations (a) and δ^{15}N of dissolved nitrate and POM in the epilimnion (0-5 m) (b). The isotopic differences between the dissolved and particulate species have been denoted by arrows. Each data point represents one sample. Each data point represents a single sample.



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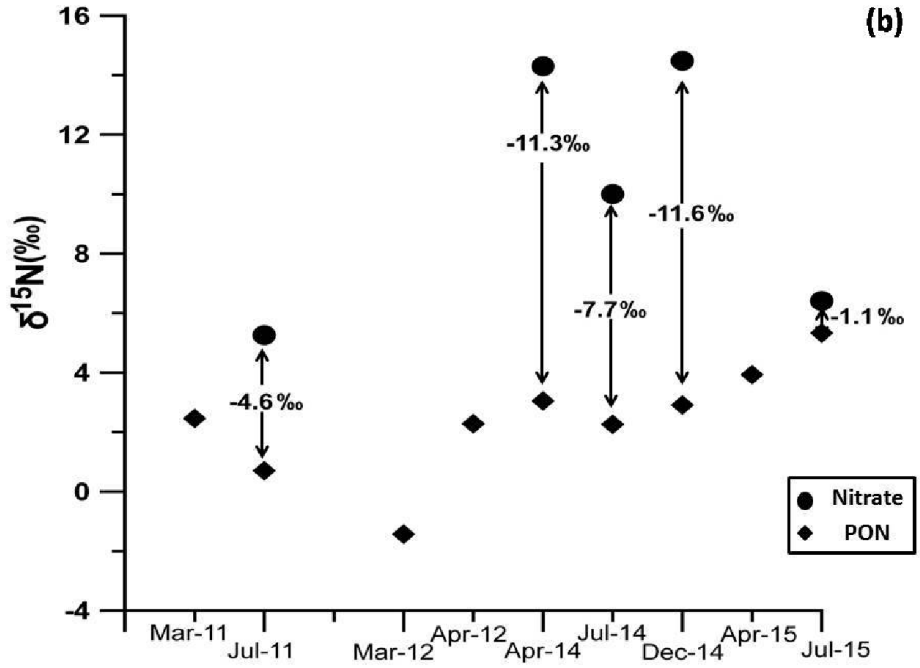

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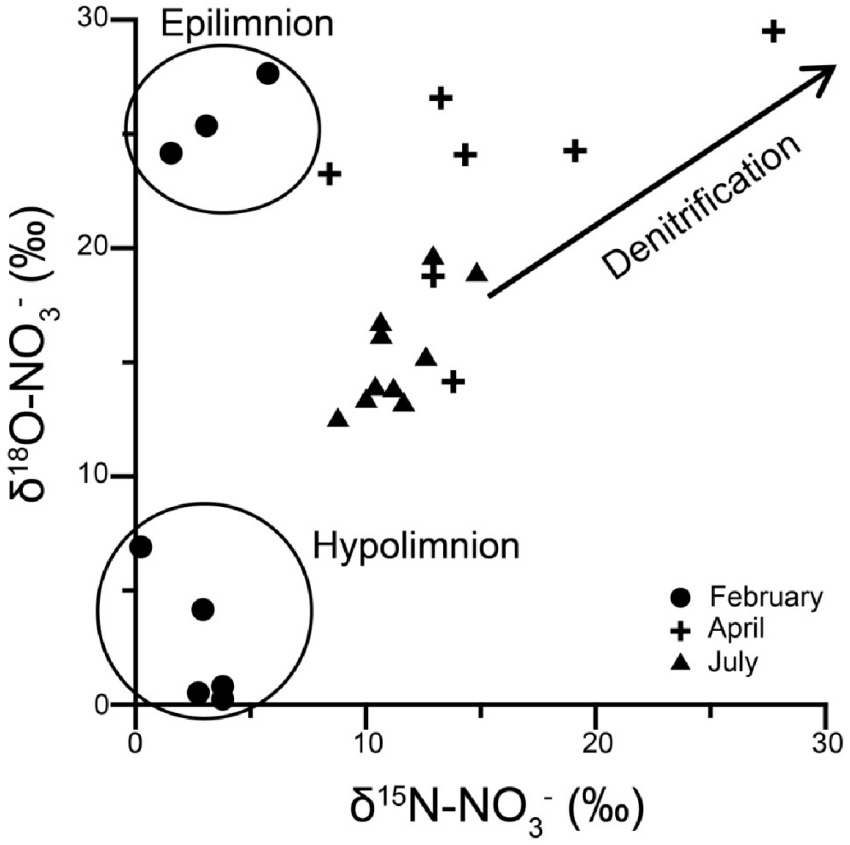
1099 **Figure 43: Nitrogen and oxygen isotopic composition of dissolved nitrate during three**

1100 **different periods in 2014. February represents the early or weak stratification period**

1101 **with two distinct clusters of epilimnetic (0-10 m) and hypolimnetic (15-48 m) samples.**

April is a period of intense water-column stratification and denitrification signal is observed in the bottom waters. July is a period of monsoon holomixis when the water column has uniformly high nitrate values.



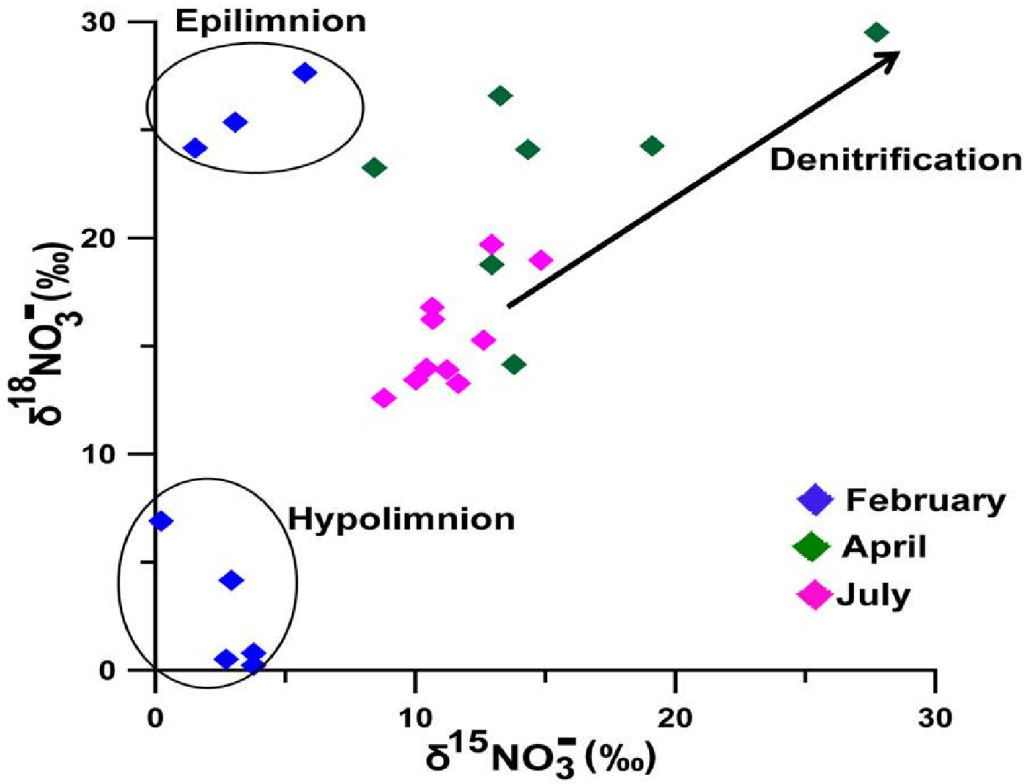
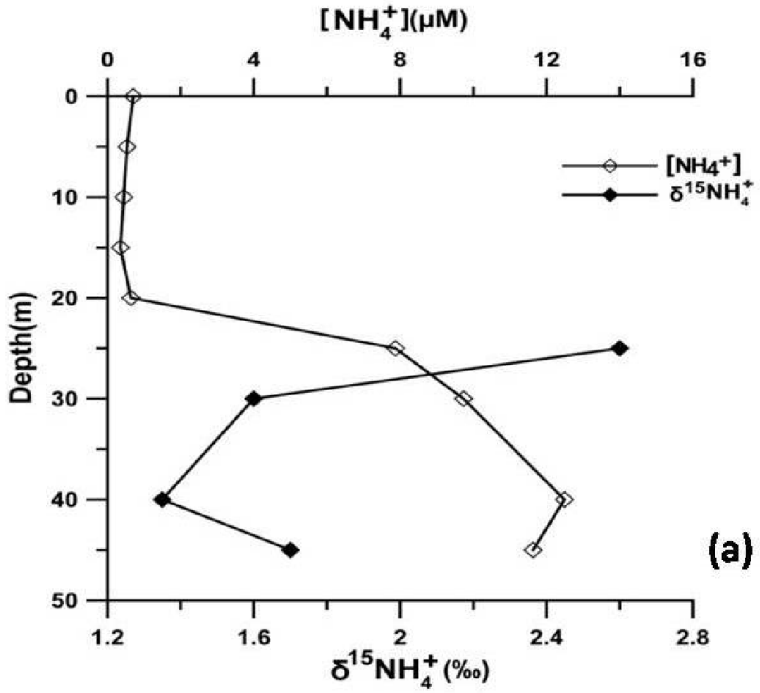


Figure 54: (a) The depth-wise variations of ammonium concentration and $\delta^{15}N\text{-}NH_4^+$ in May 2012. (b) ~~Correlation between~~ Plot of $\delta^{15}N$-PON versus $\ln(NH_4^+)$. ~~The line indicates the linear regression during ammonium utilization with ε = -2.4‰.~~ The negative linear correlation yields a fractionation factor (ε) of -2.4‰.Each data point represents a single sample.



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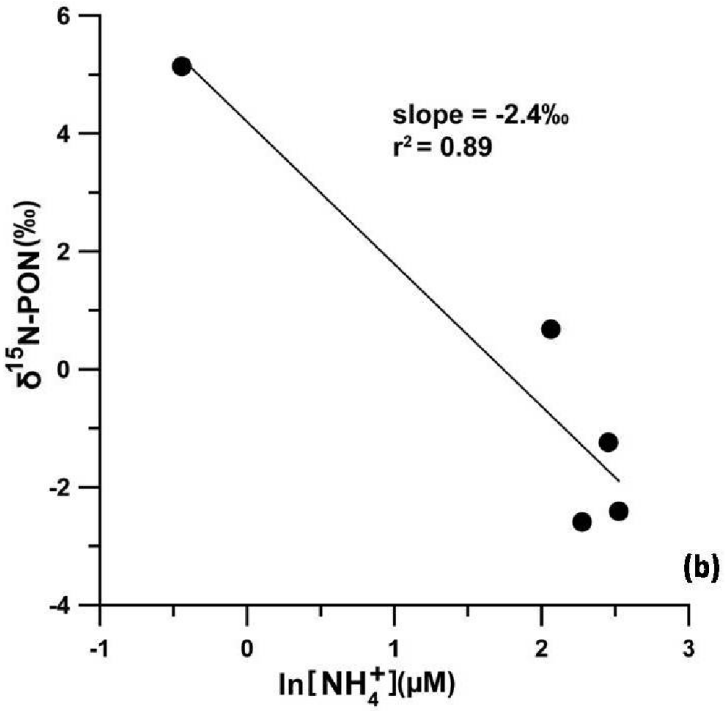

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1121 **Figure 65: Schematic diagram depicting major biogeochemical processes taking place in**

1122 **the Tillari Reservoir over an annual cycle. This information is based on monthly**

1123 **sampling in the reservoir for several years (Shenoy et al., manuscript in preparation)**

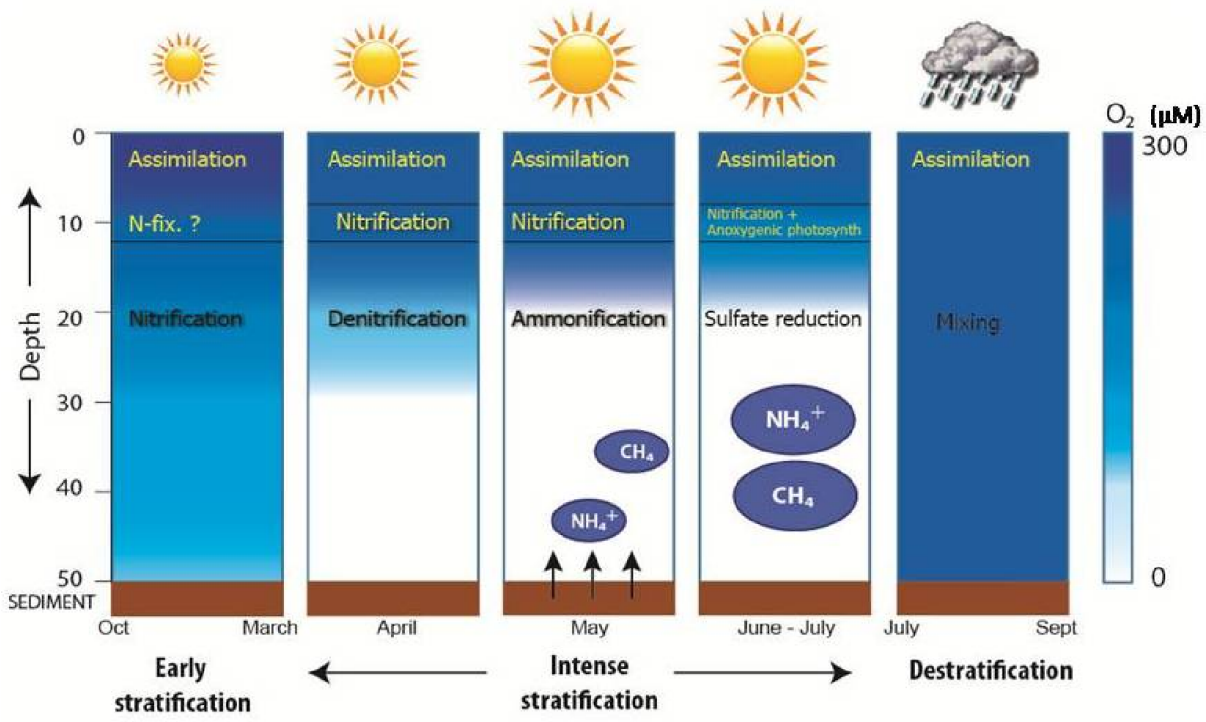
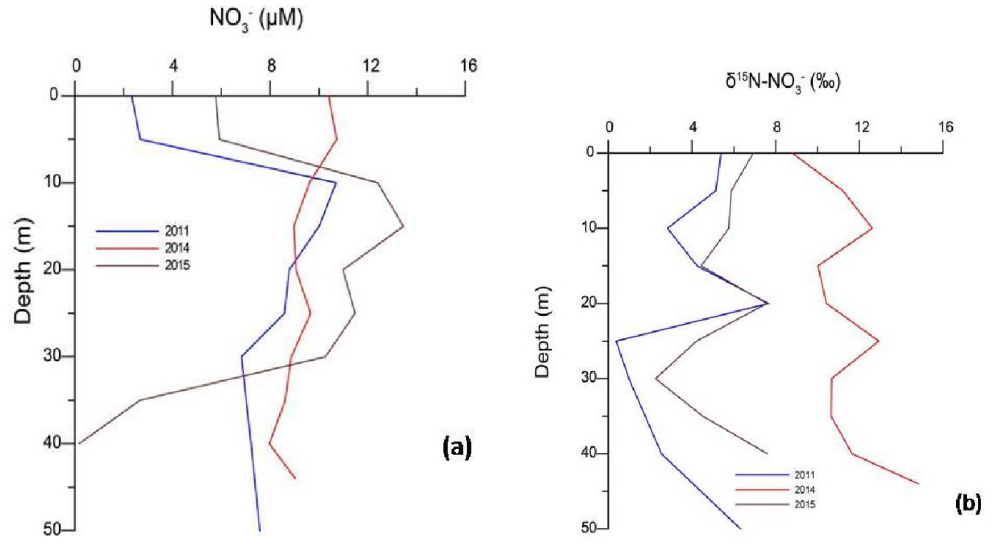


Figure 76: Vertical profiles of NO_3^- (a) and $\delta^{15}N\text{-}NO_3^-$ (b) during monsoon mixing in 2011, 2014 and 2015. Each profile is from one field trip during the peak SWM in a given year with each data point representing one sample.



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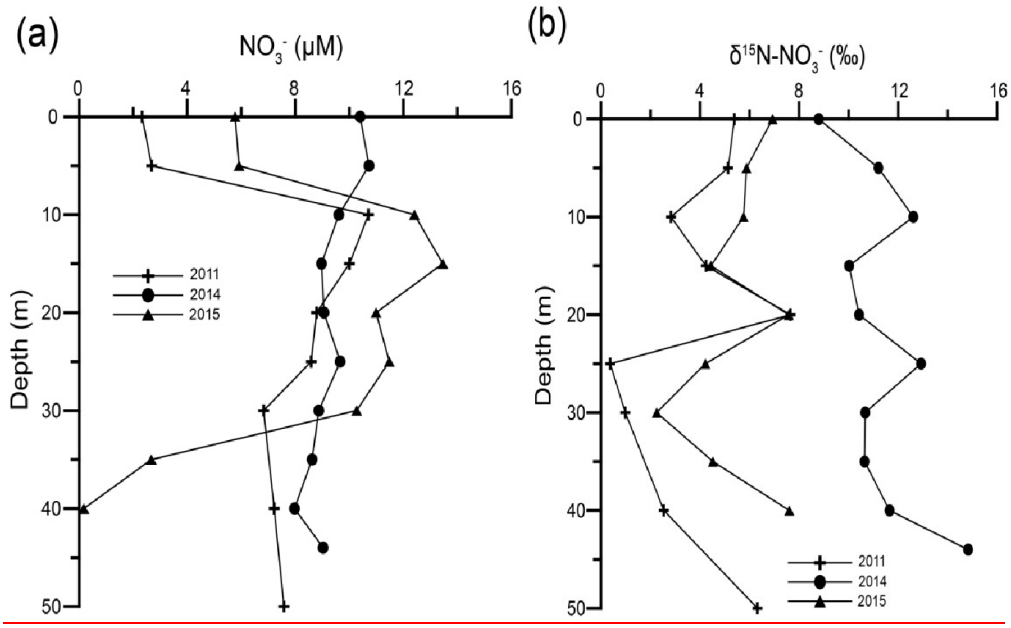

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