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 (δ^{15}N and δ^{18}O) and particulate organic matter (POM) (δ^{15}N and δ^{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 δ^{15}N and δ^{18}O values of nitrate were low (δ^{15}N = 2-10‰, δ^{18}O = 5-8‰) during normoxic conditions but increased gradually (highest δ^{15}N=27‰, δ^{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 δ^{15}N ranging from 1.3 to 2.6‰. Low δ^{15}N (~−5‰) and δ^{13}C (−37‰ to −32‰) of POM co-occuring 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 (δ^{15}N = 2 - 6 ‰). Episodic low δ^{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. δ^{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 δ^{15}N-NO_3^- and δ^{18}O-NO_3^- at all depths. Higher POM (POC and PON) contents and C/N values with lower δ^{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 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 studies freshwater ecosystems in this regard are Lake Lugano at the Swiss-Italian border, Lake Kinneret in Israel and Lake Superior in the USA.

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 of them. 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 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.

2 Methods

2.1 Site Description

The Tillari Reservoir is situated 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 volume of 0.45×10^9 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 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

5   (Narvenkar et al., 2013). Direct human impacts on nutrient inventory of the reservoir are relatively minor, as the basin is located amidst thick forest with low human population density.

2.2 Sampling and field measurements

Sampling was conducted at the deepest part of the reservoir (depth > 50 m). Water samples from pre-fixed depths were collected with Niskin samplers (5 l) attached to nylon ropes and equipped with reversing thermometers to measure

10   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-L acid-cleaned plastic carboys and transported to the laboratory within 3-4 hours.

2.3 Laboratory analyses

15   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

20   to 2015 samples were collected on a seasonal basis. The facility for nitrate isotope analysis was created in 2014 and samples from 2014 and 2015 were analysed 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 SPOM and DIN (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

5   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

10   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

15   (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

20   buffer (A-AA buffer) solution. In 20 ml crimp vials, samples and standards were diluted with LNSW for a final concentration of 20 nmoles and a final volume of 15 ml. Two international nitrite standards (N23 and N20) were added in this step to check the efficiency of N_2O production by the buffer. After addition of the A-AA buffer, the vials were allowed to stand for 1 hour and then the reaction was stopped by adding 0.5ml of 10M NaOH.

The "chemical" method yielded a very low blank (~ 0.5 μM) and worked well for the low concentration samples. The

25   international standards were run before and after each batch of samples, while the internal nitrate standards were run after

every 5 samples. Analytical precision (one standard deviation) was better than 0.3‰ for $\delta^{15}N$ and better than 0.7‰ for $\delta^{18}O$. Results are expressed in δ notation ($\delta^{15}N$ and $\delta^{18}O$), as per mil (‰) deviation from atmospheric nitrogen and Vienna Standard Mean Ocean Water (VSMOW), respectively.

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

5    $\delta^{15}N$ of NH_4^+ was measured by the "ammonia diffusion" method (Holmes et al., 1998). Briefly, 500 ml of sample was collected in duplicates to which 1.5g of MgO was added to elevate the pH. The diffused NH_4^+ was trapped onto acidified glass-fiber filter sealed between two porous Teflon membranes. The sample bottles were kept in an incubator-shaker (20˚C, 80 rpm) for two weeks for complete diffusion of NH_4^+. After two weeks, the GF filters were removed from each sample, dried in a NH_4^+-free environment, packed into tin cups and immediately analysed using CF-EA-IRMS. Results were

10   corrected for blank, percent recovery and fractionation. Analytical precision was better than 0.6‰.

2.4.3 Analyses of $\delta^{13}C$ and $\delta^{15}N$ of SPOM and surface sediment

The frozen filters were acid-fumed with 36% HCl to eliminate carbonates and air 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 SPOM along with particulate C and N contents were analyzed in the same

15   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 $\delta^{15}N = 7.5‰$.

20   Surface sediment collected from the reservoir 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

5   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

10   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

15   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. 2a) 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

20   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

25   high ($\delta^{15}N$ = 8-25‰, $\delta^{18}O$ = 24-29‰) (Fig 2b) during the summer stratification presumably due to autotrophic assimilation

whereas relatively lower values (δ^{15}N = 5-8‰, δ^{18}O = 12-15‰) were observed during the monsoon mixing events. Increasing δ^{15}N and δ^{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 δ^{15}N values observed were 27.7‰ (in 2014) and 22.4‰ (in 2012) while the corresponding highest δ^{18}O values were 29.5‰ and 28.8‰, respectively.

5   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 (δ^{15}N = 2-8‰ and δ^{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

10   nearly 12 µM at 40m with a corresponding decrease in δ^{15}N–NH_4^+ from 2.6‰ at 20m to 1.3‰ at 40m (Fig. 4a).

Elevated nitrate concentrations occur throughout the water column during the SW monsoon. The δ^{15}N and δ^{18}O of NO_3^- showed little vertical variations at this time. However, interannual variability was seen in the δ^{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 δ^{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).

15   **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. Primary productivity in the epilimnion led to higher δ^{15}N (2‰ to 6‰) and δ^{13}C (−28‰ to −26‰) in SPOM 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 δ^{15}N ($\sim$−1.4‰) in the epilimnion was observed during

20   the early stratification period (February and March). As the stratification intensified, the δ^{15}N and δ^{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 δ^{15}N and δ^{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, δ^{13}C values of SPOM were lower during monsoon mixing and became more enriched as the stratification intensified. The δ^{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 $\delta^{18}O$. 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.

The isotopic composition of the DIN source exerts the key control on the $\delta^{15}N$ of POM (Altabet, 2006). The epilimnetic POM in the Tillari Reservoir is expected to have $\delta^{15}N$ less than or equal to the $\delta^{15}N\text{-}NO_3^-$. Indeed, the $\delta^{15}N\text{-}POM$ was always lower than the $\delta^{15}N$ of the source nitrate (Fig. 2b). The range of $\delta^{13}C$ values of surface-water POM was typical of lacustrine autochthonous organic matter. As the summer progressed, productivity increased resulting in increased CO_2 uptake and elevated $\delta^{13}C\text{-}POM$. In March, when nitrate was close to detection limit, surface $\delta^{15}N\text{-}POM$ was −1.4‰. The POM resulting from nitrogen fixation by cyanobacteria usually has a $\delta^{15}N$ of 0 to −2‰ (Carpenter et al., 1997). Zeaxanthin, marker pigment of cyanobacteria, was present in significant concentrations (305.1±21 ng/l) within the epilimnion, whereas Chl-*a* concentration was ~1.7 μg l^{-1} (S. Kurian, unpublished data). However, measurements of nitrogen fixation rates in the Tillari Reservoir have yielded very low values during summer (unpublished data). Alternatively, the lower $\delta^{15}N$ values may also result from isotopically light nitrate that is produced in the hypolimnion and diffuses upward into surface waters. Further work is required to investigate the episodic occurrence of low $\delta^{15}N\text{-}POM$.

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

5 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 (Casciotti et al., 2003). The $\delta^{15}N\text{-}NO_3^-$ values ranged from 2-10‰ and the $\delta^{18}O\text{-}NO_3^-$ ranged from 5-8‰ during this period. Nitrate accumulation due to atmospheric

10 deposition and microbial nitrification will have distinct $\delta^{18}O\text{-}NO_3^-$ 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 (Finlay et al., 2007). This is well reflected in the $^{15}N\text{-}^{18}O$ scatter plot where the $\delta^{18}O\text{-}NO_3^-$ data-points from the epilimnion and hypolimnion form completely distinct clusters in February (Fig 3). As the ammonium pool gets used up, the nitrification rate decreases accompanied by a decrease in the extent of fractionation

15 (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

20 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 δ^{15}N. It may be noted that this decoupling of δ^{15}N and δ^{18}O was only observed during the peak stratification period at the thermocline.

The δ^{15}N and δ^{13}C values for the POM were generally low during the nitrification period as also observed in Lake Kinneret (Hadas et al., 2009). The δ^{15}N varied from −4‰ to 3‰ while δ^{13}C varied from −31‰ to −29‰. Assimilation of newly

5 nitrified NO_3^- may be a possible contributor to POM as indicated by the low δ^{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 δ^{15}N-NO_3^- and δ^{18}O-NO_3^-. Linear regression of δ^{18}O versus δ^{15}N yielded

10 slope values of 0.95 and 0.85 in 2014 and 2012, respectively. 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 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

15 8.7‰ and 10.7‰, respectively. The ϵ^{15} is much lower than those obtained from laboratory cultures (Olleros, 1983; Table 2) as well as open-ocean OMZs (Brandes et al., 1998, Voss et al., 2001; Table 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

20 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

25 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

5   The isotopic composition of ammonium should reflect that of the sedimentary organic matter being degraded. In Lake Kinneret (Israel), δ^{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 (δ^{15}N = 10‰) (Hadas et al., 2009). In Lake Bled (NW Slovenia), mean δ^{15}N– NH_4^+ value of 3.8‰ was similar to that of sedimentary OM (δ^{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 δ^{15}N–NH_4^+ (1.3-2.6‰) thus establishing remineralization of sedimentary

10 OM as the principal NH_4^+ source.

A negative linear relationship between δ^{15}N-PON and ln[NH_4^+] was observed (Fig. 4b) 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,

15 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.

20 ### 4.2.4 Sulphate reduction and evidence for chemosynthesis

Mass dependent fractionation during microbial degradation of organic matter by sulphate makes 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. 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: from 28 µM to 60 µM for POC and from 4.7 to 8 µM for PON. Bacterial assimilation of ammonium can explain the isotopically light nitrogen, but utilization of biogenic methane is known to lead to extremely low δ^{13}C values (between −65‰ and −50‰; Whiticar et al., 1986). In our study, the most depleted δ^{13}C-POC value of −37.8‰ was associated with the highest methane

5 concentration of 156 µM. Interestingly, in a study carried out in the waters of Lake Baikal in Siberia, very negative δ^{13}C–DIC values (−28.9 to −35.6‰) were inferred to be derived from methane oxidation while the δ^{13}C-POC values (−31.7 to −33.5‰) were typical of lacustrine organic matter (Prokopenko and Williams 2005). The authors explained this lack of correlation between the two C pools by a possible time lag between the peak methane oxidation and peak productivity. Low δ^{13}C-POC (∼−37‰) in Lake Kinneret was attributed to chemosynthetic C fixation using depleted δ^{13}C-DIC derived from

10 methane oxidation (Hadas et al. 2009). It is important to understand the fate of methane in freshwater systems as they are believed to be significant contributors to atmospheric methane emissions (Bastviken et al., 2004). The POM isotopic data of the Tillari Reservoir provides evidence for intense microbial chemosynthesis using sulphide, ammonia and methane as energy donors.

4.3 Monsoon mixing in the Tillari Reservoir

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

20 column was uniformly nitrate-replete in 2014, the epilimnetic (0-5m) nitrate concentrations in 2011 and 2015 were markedly lower than the deeper depths (Fig.6) indicating nitrate uptake in spite of light-limited conditions. This was also evident in the nitrate isotopic data. To understand this inter-annual variability, water samples from six upstream stations along the Tillari River along with a precipitation 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 location. The ranges of δ^{15}N and δ^{18}O of NO$_3^-$ at

25 these stations were 0.4-6.8‰ and 11-27‰, respectively. The precipitation 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). The $\delta^{13}C$-POC values in the epilimnion decreased to nearly −30‰ presumably due to a combination of lower primary

5   productivity and inputs of organic matter through runoff. 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

10   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 the dominant nitrate source to the water column during the monsoon season. At the same time, the POM depicts the isotopic signature of the mixing with the upstream waters. Variable inputs from the atmosphere and by river runoff to the DIN pool

15   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

20   mixed during the southwest monsoon season as well as in winter; the water column remained stratified during other parts of the year. The most intense stratification occurs during summer just before the monsoon onset. Relative importance of microbial processes such as nitrification, denitrification, ammonification and sulphate reduction in the water column varied depending on intensity of stratification and associated DO levels in the hypolimnion. These processes produced unique isotopic signatures in the dissolved and particulate matter. Our results suggest the occurrence of microbial chemosynthesis

using methane and ammonium as primary C- and N- sources, producing organic matter in the anoxic bottom waters that is highly depleted in ^{13}C and ^{15}N content. The thermocline in the Tillari Reservoir has been known to harbour photoautotrophic sulphur bacteria during peak stratification periods (Kurian et al., 2012). We also found strong signatures of nitrification within this zone during summer stratification. Autochthonous production was the principal source of organic matter in the

5 epilimnion which was well-oxygenated at all times, although productivity was significantly lower during the monsoon period due to light-limited conditions. Nitrate was the preferred DIN source in the epilimnion. When nitrate loss occurred in the hypolimnion, the preferred DIN species switched from nitrate to ammonium. Isotopic measurement of precipitation and upstream river samples during one seasonal sampling provided insight into sources of nitrogen, but the observed inter-annual variability could not be explained. Overall, solar intensity, water depth and redox conditions appear to be the major factors

10 controlling biogeochemical 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	73m	943 m	9274×10^3 m^3	16250×10^3 m^2	$447290 \times 10^3\,m^3$

Table 2: 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**
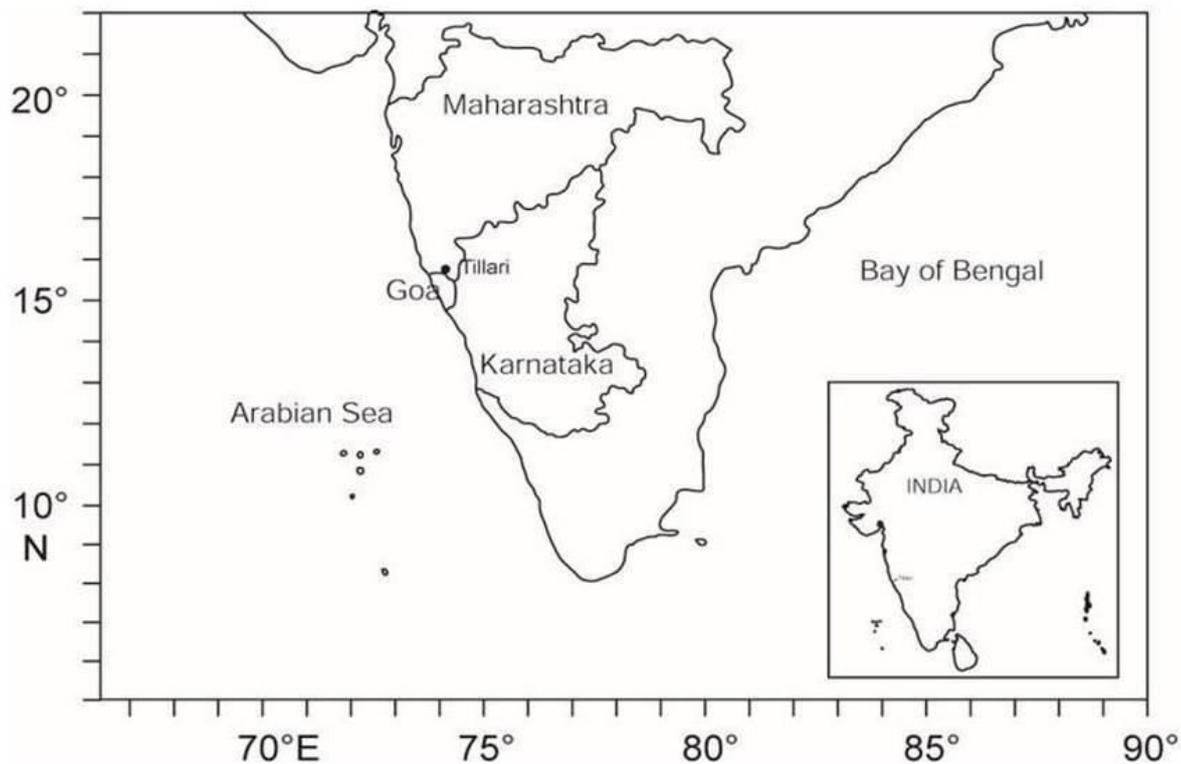
Figure 1: Map of the sampling location (Tillari Reservoir).
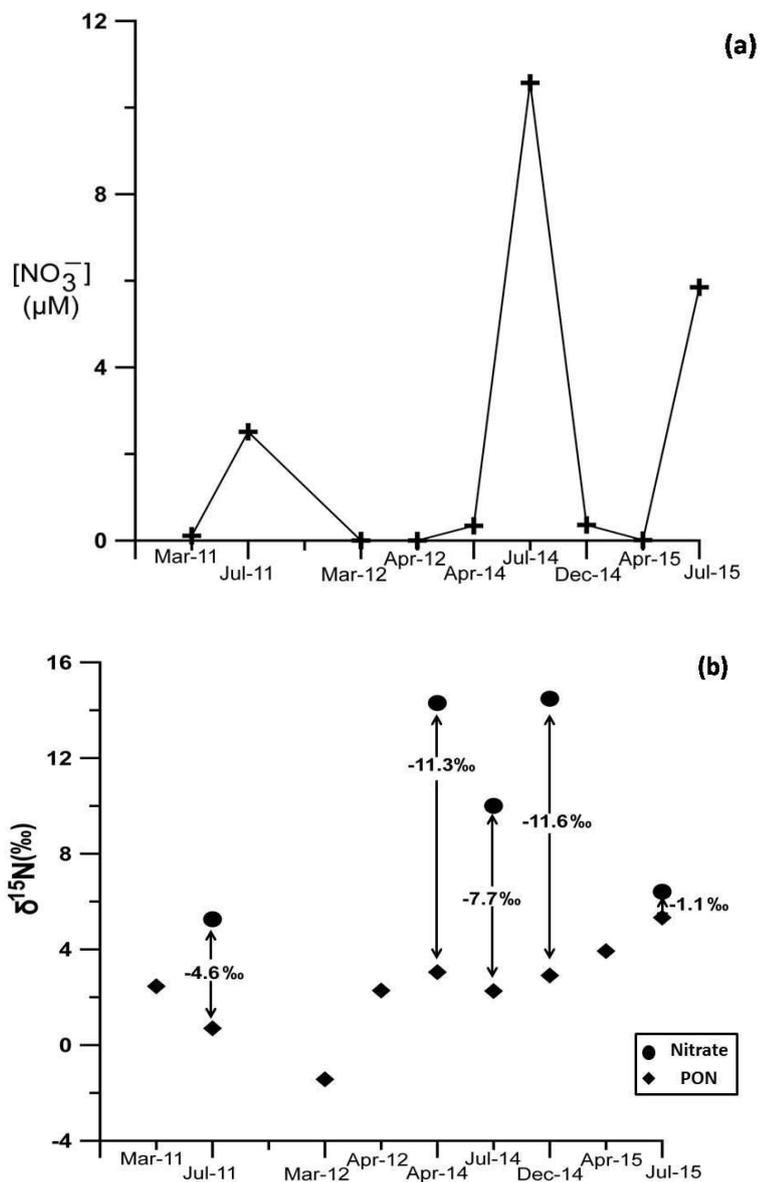
Figure 2: Time-series of nitrate concentrations (a) and δ¹⁵N of dissolved nitrate and POM in the epilimnion (0-5m) (b). The isotopic differences between the dissolved and particulate species have been denoted by arrows.
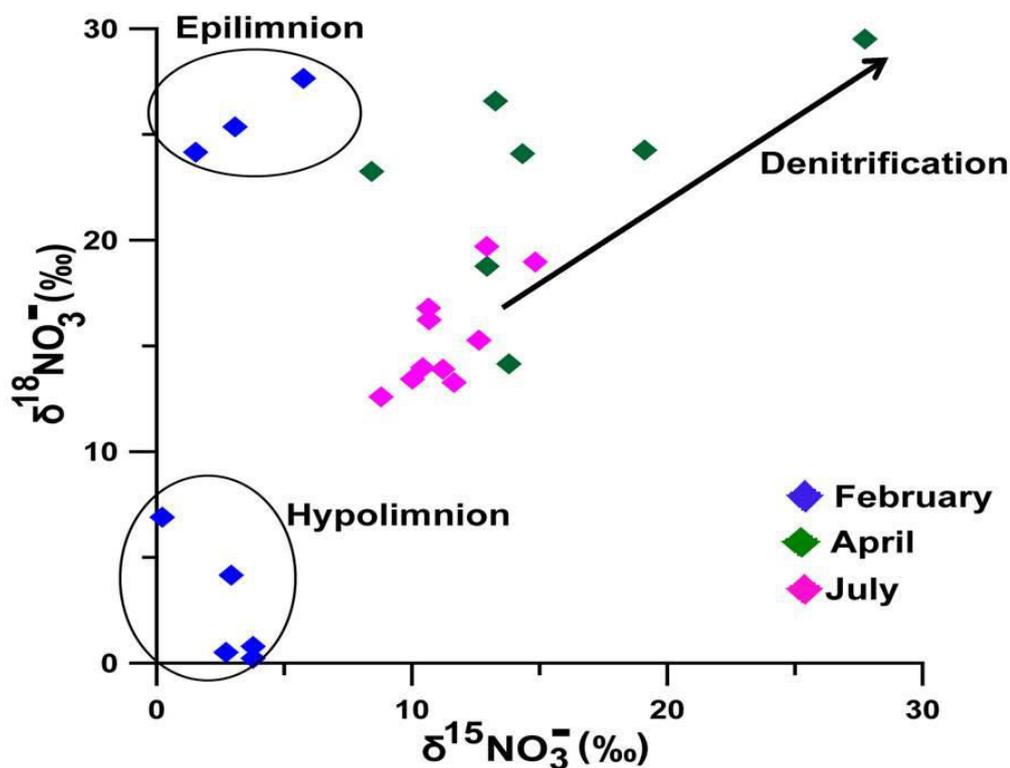
Figure 3: Nitrogen and oxygen isotopic composition of dissolved nitrate during three different periods in 2014. February represents the early or weak stratification period with two distinct clusters of epilimnetic and hypolimnetic samples. April is a period of intense water-column stratification and denitrification signal is observed in the bottom waters. July is a period of monsoon holomixis when the water column has uniformly high nitrate values.
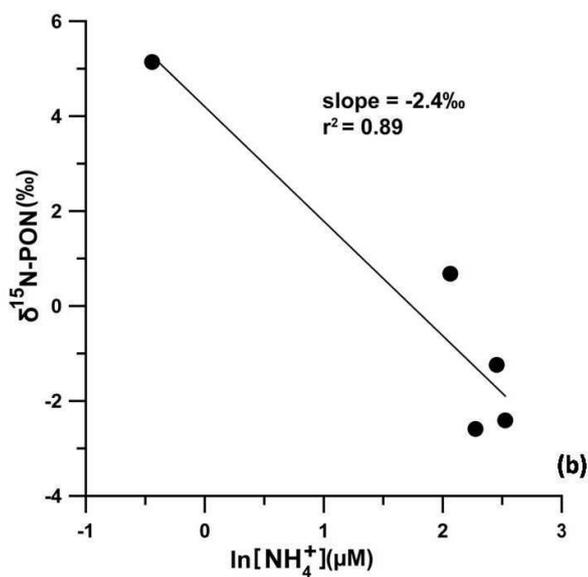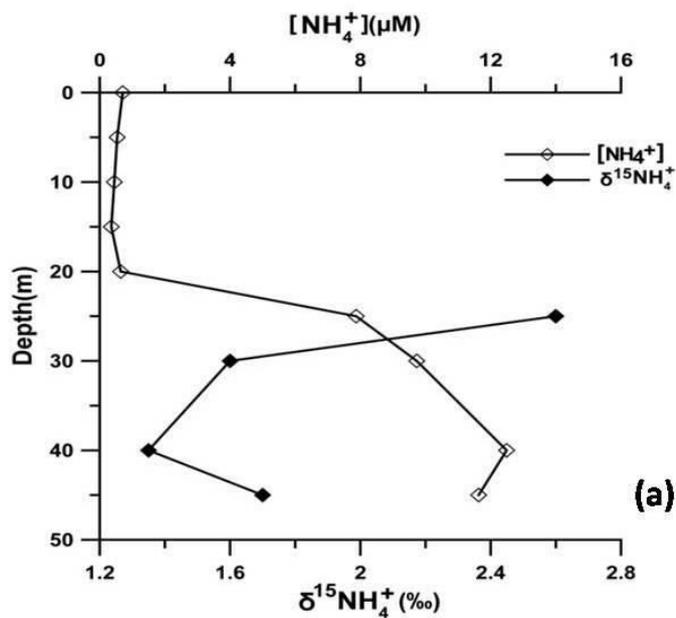
Figure 4: The depth-wise variation of ammonium concentration and δ^{15}N-NH$_4^+$ in May 2012(a) Correlation between δ^{15}N-PON and ln(NH$_4^+$)(b). The line indicates the linear regression during ammonium utilization with ε = -2.4‰.
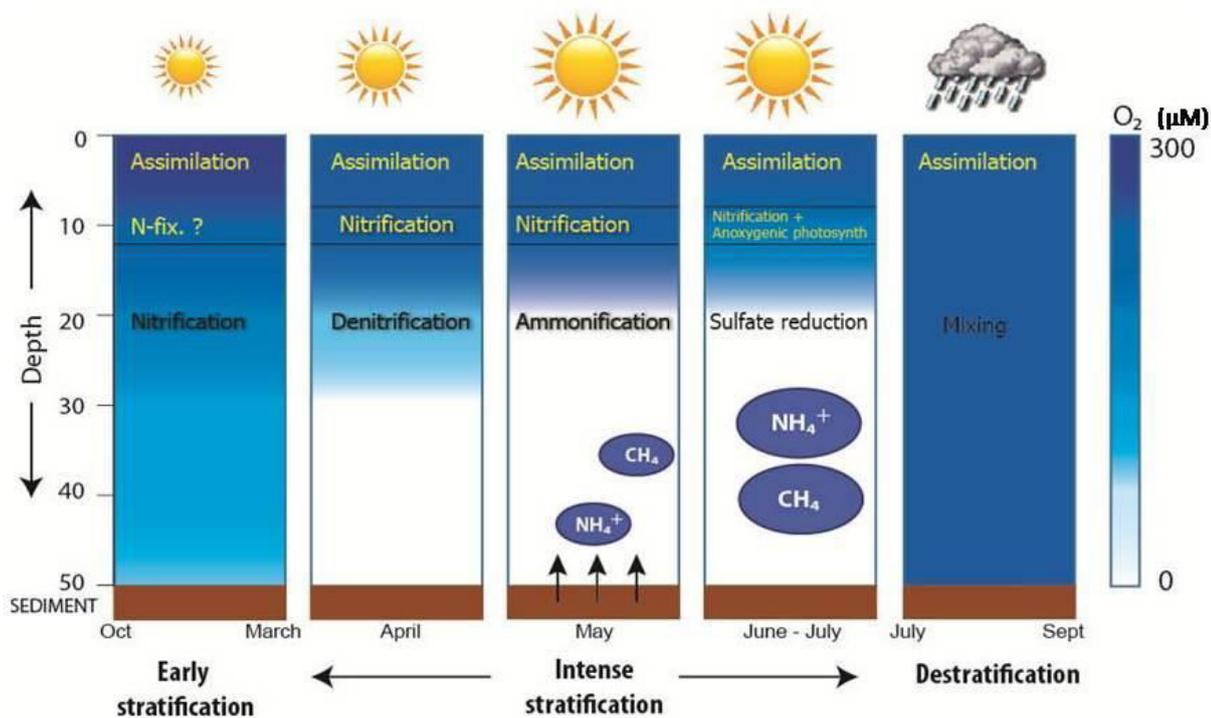
Figure 5: Schematic diagram to depict different biogeochemical processes taking place in the Tillari Reservoir over an annual cycle.
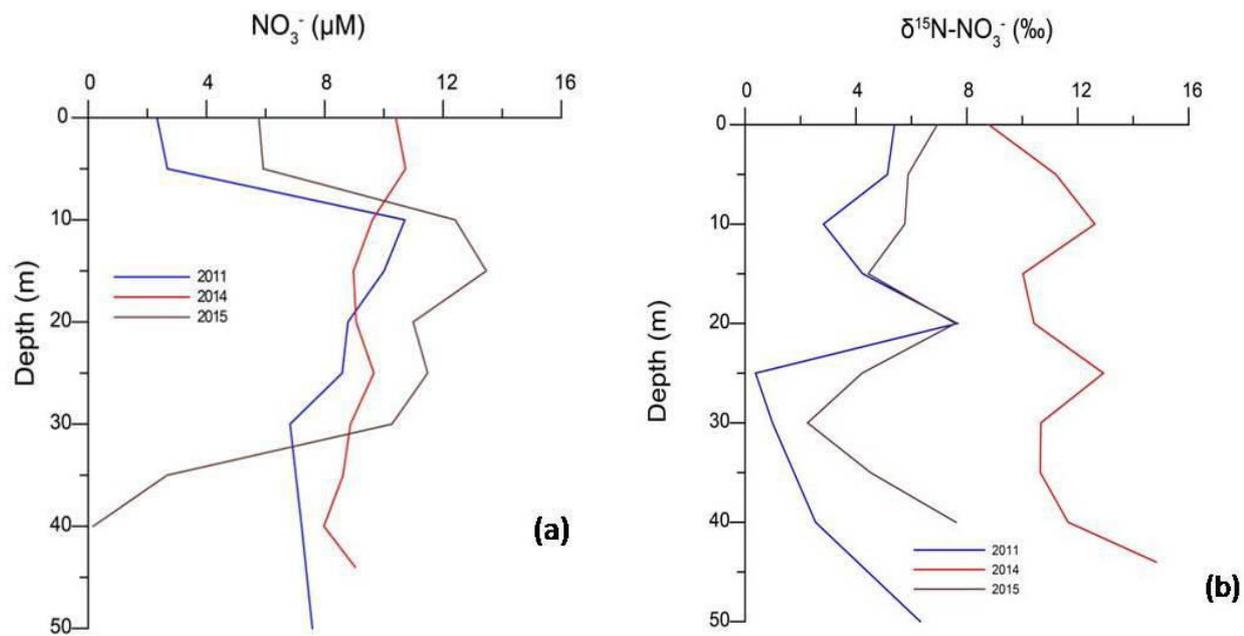
Figure 6: Vertical profiles of NO_3^- (a) and $\delta^{15}N\text{-}NO_3^-$ (b) during monsoon mixing in 2011, 2014 and 2015.