**Biogeosciences
Discussions**

Carbon and nitrogen isotope variations in the water column of Lake Bled (NW Slovenia)

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Abstract

The variability in the stable isotope signature of carbon and nitrogen in particulate organic matter and dissolved species in the water column of the mesotrophic subalpine Lake Bled in NW Slovenia has been determined. After the algae bloom from August to December in 2008, samples were taken from the deepest part of the lake which develops an anoxic hypolimnion for most of the year. C/N ratios and $\delta^{13}C_{POC}$ and $\delta^{15}N_{PN}$ values suggest an autochthonous source for particulate organic matter (POM). According to the isotope model, autochthonous carbon accounted for a major part of the particulate organic carbon (POC), ranging from 0.86 to 0.96 in September and October, while in December the proportion of allochthonous carbon was more pronounced, ranging from 0.57 to 0.59. Low $\delta^{13}C_{POC}$ and $\delta^{15}N_{PN}$ values (from −36 to −33‰ and from 0.8 to 1.8‰), observed below 24 m in August and September, indicate the bacterial origin of POM, mainly from methanotrophic bacteria. $\delta^{15}N_{NO_3}$ and $\delta^{15}N_{PN}$ values decreased with depth. The relations between $\delta^{15}N_{PN}$ and NO_3^- and NH_4^+ concentrations suggest that NH_4^+ is the main assimilation species for nitrogen in POM. Nitrification was active between 12 and 18 m deep in September and October, indicated by increased NO_3^- concentrations and decreased $\delta^{15}N_{NO_3}$ values. The correlation between nitrate concentrations and $\delta^{15}N_{NO_3}$ values suggests active water column denitrification in October 2008. The decrease in $\delta^{15}N_{NO_3}$ values observed in December could be explained by degradation of organic matter, followed by nitrification of the degradation products. During our sampling period, there was no evident influence of sewage, agriculture, or atmospheric deposition on the nitrogen balance in the lake.

1 Introduction

The biogeochemical cycles of carbon (C) and nitrogen (N), two of the most important biogenic elements, are coupled by primary production. During decomposition of the

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particulate organic matter, they are released back to the environment through a more or less complicated network of microbially mediated biochemical reactions. The main characteristic of shallow aquatic basins, such as lakes, is the transfer of a significant part of primary production into the sediment. The coupling of pelagic and benthic biological activity is thus enhanced. Consequently, a large part of the remineralisation of nutrients occurs in the upper layer of sediments, which then acts as an important source of dissolved nitrogen, carbon, etc. for the water column.

Stable C and N isotopes have been proven to be an effective tool for tracing matter and energy flows through biological systems and for evaluating the rates of many ecological processes. Complete understanding of these processes can be achieved only through knowledge of robust and clearly defined source end-points and of the direction and magnitude of isotope alteration during microbial or phytoplankton mediated processes. Variations in ^{13}C in phytoplankton are mostly the result of species composition, its growth rate (Laws et al., 1995; Baird et al., 2001), cell size and geometry (Korb et al., 1996; Popp et al., 1998), membrane permeability, together with several physical and chemical characteristics of the environment, such as temperature, light intensity, pH and carbonate equilibrium (pCO$_2$) (Thompson and Calvert, 1995; Riebesell et al., 2000; Rost et al., 2003). Phytoplankton ^{15}N composition primarily reflects isotope discrimination during nitrate (NO$_3^-$) uptake (Altabet et al., 1991; Holmes et al., 1999; Teranes and Bernasconi, 2000), N$_2$ fixation and N recycling, including zooplankton release (Pantoja et al., 2002). In some studies, it has been shown that assimilation of ammonium (NH$_4^+$) is an import mechanism controlling the isotope composition of phytoplankton (Ostrom et al., 1997; Feber et al., 2004; Lehmann et al., 2004). Fewer studies have attempted to identify sources of NO$_3^-$ in lakes using stable isotope ratios. This is usually difficult, since the isotope composition of NO$_3^-$ and NH$_4^+$ is also affected by microbial processes including assimilation, fixation, mineralization, nitrification and denitrification. Moreover, external natural and/or anthropogenic nitrogen loading can also control the isotope composition of lacustrine nitrogen species. The isotope fingerprints of N derived from atmospheric, fertilizer, soil, and manure-derived sources are

often sufficiently distinct to permit separation. For example, δ^{15}N in synthetic fertilizers varies between -3 and +3‰, while nitrate derived from animal manure has δ^{15}N values ranging from +10 to +25‰ (Kendall et al., 2007). The δ^{15}N values of atmospheric NO_3^- and NH_4^+ are usually in the range of −15 to +15‰ (Kendall et al., 2007). Atmospheric deposition is the dominant source of N compounds in most high mountain and oligotrophic lakes (Ostrom et al., 1997; Wookey et al., 2009; Vreča et al., 2010). In addition, in high mountain lakes, another N source can be snow-pack melting influenced by soil conditions (Bartrons et al., 2010). More anthropogenic nitrate loading derived from fertilizers, manure and/or sewage can be observed in lakes located in urban areas (Townsend-Small et al., 2009).

The principal scope of this study was to characterize the most important processes controlling variations with depth of the isotope composition of particulate organic matter (POM) and nitrate in the water column of the dimictic Lake Bled (NW Slovenia) after the algal bloom. In particular we have assessed the use of organic matter δ^{13}C and δ^{15}N values as recorders of primary productivity and nitrate utilization in a highly productive lake ecosystem, which develops an anoxic hypolimnion for most of the year.

2 Materials and methods

2.1 Sampling site and sampling

Lake Bled is located in the north-west part of Slovenia (Fig. 1). It is a dimictic, sub-alpine water body formed in the Wurm glacial period. According to OECD criteria it is classified as a mesotrophic lake (OECD, 1982). It covers an area of 1.44 km^2 with average and maximum depths of 17.9 and 30.5 m, respectively. It has two natural inflows (Mišca and Solznik) and one outflow proceeding through the Jezernica into the river Sava. The lake is stratified with anoxic hypolimnium most of the year, except during the early spring. In the shallower parts, oxidizing conditions prevail in the whole water column. Thermocline starts at 5 m depth in spring and later moves down to

10 m. Lake Bled is located in an industrially, touristically and agriculturally developed area. In the past, the nutrient status of the lake was high and two amelioration projects were undertaken to improve the situation: (a) a fresh water inflow was diverted from the river Radovna and (b) anoxic water was pumped symphonically into the Jezernica.

5 The lake became mesotrophic, however algal and cyanobacterial blooms still occur, although their frequency and duration has diminished (Remec-Rekar and Bat, 2009).

Our study site was the deepest part of the lake, Station D, where the depth of the water column is 30.5 m (Fig. 1). Water samples were collected with a Van Dorn sampler once a month in August, September, October and December 2008. Samples in August

10 were collected only from depths 8, 24 and 28 m. In other months, 5 L samples were taken every two metres from 6 m downwards. Water samples were transferred into pre-cleaned plastic bottles and stored at 4 °C during transport to the laboratory within the next 3 h. At the same time, samples were taken for determination of the isotope composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$). 6 ml of water sample was introduced

15 at the sampling site directly into a gas-tight vial (VACUTAINER Septum Tubes, Labco Limited, UK) with 100–200 µl of H_3PO_4. Vials were previously flushed in the laboratory with pure He to remove any air contamination.

2.2 Chemical and stable isotope analyses

Dissolved oxygen concentration, conductivity, redox potential and pH of the water col-

20 umn were determined in August and September using a Hydrolab H_2O probe. Temperature, pH and alkalinity were determined in all sampling months. Temperature and pH were measured directly in the sample in the field. The field pH was determined on the NBS scale using two buffer calibrations with a reproducibility of ±0.02 pH unit. Temperature was measured with a portable thermometer (Hanna Instruments, HI 91531K). To-

25 tal alkalinity was determined within 24 h of sample collection by Gran titration (Gieskes, 1974) with a precision of ± 1 %. It was used, together with pH, to calculate dissolved inorganic carbon (DIC) and free CO_2 concentrations.

In the laboratory, samples were immediately filtered through pre-combusted (480 °C for 4 h) Whatman GF/C glass-fibre filters and dried at 40 °C. 200 ml of filtered water was further filtered through 0.2 µM filters (Sartorius AG, Germany) and frozen before analysis. These samples were used to determine the concentrations of dissolved nitrogen and phosphorus species (NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}) using standard colorimetric methods (Grasshoff et al., 1983). Total dissolved nitrogen (TDN) and phosphorus (TDP) were determined after oxidation of samples with $K_2S_2O_8$ (Grasshoff et al., 1983). The rest of the water was used for the determination of the isotope composition of nitrate, $\delta^{15}N_{NO_3}$. Samples were passed through a cation exchange resin (BIO-RAD AG 50W-X8, Hydrogen Form, USA) in order to remove cations (especially NH_4^+) and dissolved organic material. In the second step, samples were transferred to an anion exchange resin (BIO-RAD AG2-X8, Chloride Form, USA) according to the method of Silva et al. (2000) as modified by Fukada et al. (2003). The sample was then eluted with 30 ml of 3M HCl. The acid eluent was neutralized with Ag_2O, filtered and dried to obtain solid $AgNO_3$. Dried samples were put into silver capsules and measured on an Isotope Ratio Mass Spectrometer (IRMS) Europa 20–20 with solid-liquid preparation module ANCA-SL. Deeper in the water column (26 and 28 m), where NH_4^+ is the most abundant nitrogen species, samples were only concentrated by evaporation at 60 °C and filtered before measuring the isotope composition of nitrogen.

The concentration and isotope composition of particulate organic carbon (POC) and particulate nitrogen (PN) were determined on filters. Filters used for determination of POC concentration and isotope composition were acidified with 1M HCl to remove carbonate minerals and dried. Concentrations of POC and PN were measured with a Carlo Erba EA1108 elemental analyzer with a combustion temperature of 1020 °C. The precision of measurements was ±3 %. The isotope compositions of POC ($\delta^{13}C_{POC}$) and PN ($\delta^{15}N_{PN}$) were determined on IRMS Europa 20-20 with solid-liquid preparation module ANCA-SL.

$\delta^{13}C_{DIC}$ values were determined after extraction as CO_2 in glass septum tubes. The isotope ratio of extracted CO_2 was determined directly from the headspace by IRMS

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Europa Scientific 20-20 with a ANCA-TG preparation module for trace gas samples, equipped with a Gilson autosampler. In order to determine the optimal extraction procedure for water samples a standard Na_2CO_3 solution was prepared with a known $\delta^{13}C$ value of -10.8 ± 0.2 ‰.

All stable isotope results are reported using conventional delta (δ) notation in per mil (‰) relative to the VPDB standard ($\delta^{13}C$) or AIR ($\delta^{15}N$). Analyses were calibrated against reference materials: NBS 22 (oil) and IAEA-CH7 were used for carbon and IAEA–N1, IAEA-N2 and IAEA-NO-3 for nitrogen. The precision of measurements was usually ± 0.2 ‰ for $\delta^{13}C_{DIC}$ and $\delta^{13}C_{POC}$ and ± 0.3 ‰ for $\delta^{15}N_{NO_3}$ and $\delta^{15}N_{PN}$.

3 Results

The lake was stratified in August, with the thermocline spanning from 6 m to 12 m in October (Fig. 2). In the hypolimnion the average temperature was 7.5 °C, which is very close to values obtained in October (8.5 °C) and December (6.9 °C). The temperatures from 14 m downward did not vary significantly in the sampling months. The uniform profile of temperature observed in December was a strong indicator of vertical mixing at station D. The concentration of dissolved oxygen was measured only in August. It was fairly constant above the thermocline (10.3 mg L^{-1}) and reached a maximum value at a depth of 8 m (20.5 mg L^{-1}). The O_2 concentration further dropped steadily, until it reached a concentration of 0.22 mg L^{-1} at 26 m where anaerobic processes occur. At this depth anoxic conditions are present throughout the year (Remec-Rekar and Bat, 2009).

DIC concentrations did not change much in the autumn months, exhibiting an increase from 3.56 mM to 4.54 mM from surface to bottom of the water column (Fig. 2). $\delta^{13}C_{DIC}$ values increased with depth in September, from -5.9 to -10.3 ‰, and in October from -3.8 to -8.0 ‰. In December, DIC and $\delta^{13}C_{DIC}$ values remained constant throughout the water column with an average DIC concentration of 3.81 ± 0.10 mM and $\delta^{13}C_{DIC}$ of -6.6 ± 0.2 ‰.

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The depth distributions of total phosphorous (TP), NO_3^-, NH_4^+ and dissolved organic nitrogen (DON) concentrations in all sampling periods are presented in Fig. 3. It is seen that TP concentrations were very low in autumn months, ranging from 0.10 µM to 0.31 µM in September and from 0.10 µM and 0.19 µM in October, but more abundant in December. A higher TP concentration of 0.15 µM was observed in September at a depth of 16 m. However, TP concentrations were highest in December, with an average concentration of 0.31 ± 0.03 µM. In September and October dissolved organic phosphorous (DOP) prevailed, over PO_4^{3-} concentrations reaching 91 % at the bottom of the water column, while in December PO_4^{3-} concentrations were more abundant. The vertical profiles for NO_3^- concentration were very similar in September and October, reaching maximum concentrations of 21.1 µM and 28.4 µM, respectively at a depth of 22 m. Average concentrations of 13.4 ± 5.3 µM and 12.6 ± 1.1 µM were observed above the thermocline and 17.3 ± 4.3 and 21.9 ± 7.2 µM below it in September and October, respectively. The lowest NO_3^- concentrations were observed in parallel with the highest NH_4^+ concentrations, which were 37 µM in September and 33 µM in October at the bottom of the water column. In December, NO_3^- concentrations exhibited little variation, with an average value of 18.7 ± 1.3 µM throughout the water column. The NH_4^+ concentrations were low in the upper part of the column, with average concentrations of 4.72 ± 0.81 µM and 3.37 ± 0.58 µM in September and October, but increased below 24 m. The average NH_4^+ concentration in December was 6.48 ± 0.43 µM. DON concentrations in September and October were 1 to 10 times higher than the NH_4^+ concentrations, except at the bottom of the water column. In December, DON concentrations remained relatively constant over the water column, with an average value of 11.79 ± 1.74 µM.

$\delta^{15}N_{NO_3}$ values in the upper layers ranged from +5‰ to +10‰, as reported by Carpenter et al. (1997). $\delta^{15}N_{NO_3}$ values decreased with increasing depth. This decrease occurs at the lower boundary of the thermocline. Until the depth of 24 m $\delta^{15}N_{NO_3}$ values displayed similar patterns in October and December, with −5.6‰ and −6.2‰ at

22 m. NO_3^- was enriched in ^{15}N in October, resulting in an average $\delta^{15}N_{NO_3}$ value of +3.8‰. It became progressively depleted in ^{15}N, reaching −10.3‰ at the bottom of the water column in December. This observation contrasted with our expectations, since all measured parameters indicated a very well mixed water column in December (Figs. 2 and 3).

The C/N ratio of particulate organic matter was determined only in October and December. The ratios increased slightly towards the bottom. Average values in October and December were 7.4 ± 1.0 and 8.3 ± 1.2, indicating the prevalence of autochthonous particulate organic material. The ratios were higher in December, meaning that more allochthonous material was present in the water column. The isotope composition of POM for both $\delta^{13}C$ and $\delta^{15}N$ increased, on average, as months became progressively colder but they decreased with depth in all sampling periods. The lowest values of $\delta^{13}C_{POC}$ and $\delta^{15}N_{PN}$ (−35.7‰ and +0.8‰) were observed at 28 m in August. In other months $\delta^{13}C_{POC}$ values were highest in the upper layers (an average $\delta^{13}C$ value of -29.4 ± 0.6‰) and decreased to the bottom layers (an average $\delta^{13}C$ value of -33.0 ± 1.7‰) (Fig. 2). Values in September and October are more similar than those than in December, which were found to be on the average -29.5 ± 0.7‰, and differed from other periods by an average +3.7‰. $\delta^{15}N_{PN}$ values reached their highest values (Fig. 3) in October, values in September and December being lower. In all cases, values in the anoxic part of the water column at 26 m and 28 m were practically the same, with an average $\delta^{15}N_{PN}$ value of $+3.5 \pm 0.3$‰. In September, $\delta^{15}N_{PN}$ values in the upper part of the water column stayed within the range of +3.3‰ to +5.4‰, except at 24 m, where it dropped to +1.8‰. In October, higher $\delta^{15}N_{PN}$ values of +4.4‰ and +12.2‰ were observed at 6 m and 16 m. $\delta^{15}N_{PN}$ values ranged between +6.5‰ and +5.0‰ in December, with the highest value of +8.0‰ observed at a depth of 6 m.

4 Discussion

The peak in dissolved oxygen (DO) concentration at 8 m depth in August 2008 can
be attributed to the presence and activity of photosynthetic organisms. Their numbers
and activity diminished with depth, along with higher heterotrophic activity, indicated
by decreasing DO. Oxycline and nitricline were correlated with thermocline, indicat-
ing that biological activity decreased with temperature. The progressive cooling of the
epilimnion in winter months, with disappearance of the thermocline, is typical for dim-
ictic lakes in the Alpine region, as it has been also shown in other studies (Hodell and
Schelske, 1998; Teranes and Bernasconi, 2000; Lehmann et al., 2004; Hadas et al.,
2009).

Our results suggest that, during our sampling period, the water column in Lake Bled
can be analyzed as two separate compartments during the time of thermocline, namely
epilimnion and hypolimnion. The epilimnion is much more susceptible to external influ-
ences such as input of allochthonous material by leaching, precipitation and/or nitrogen
fixation. The hypolimnion acts like a closed system, which exchanges relatively small
amounts of different materials with the epilimnion, but much of it is recycled. Resus-
pension from the bottom or diffusion from sediment plays an important role in organic
matter cycling and, in addition, sedimentation from the epilimnion constitutes the main
input of "new material" into the hypolimnium.

4.1 Carbon isotope biogeochemistry in the water column

The isotope data, as well as concentration ranges, imply an autochthonous origin of
organic matter in Lake Bled during the sampling period. The C/N ratio varied between
6.1 and 10.9, indicating that the primary source of organic matter was phytoplankton
detritus. Higher values were observed in December and showed a larger component
of terrestrial organic matter in winter, when no autochthonous material was produced.
Different sources of OM can also be identified using the relationship between $\delta^{13}C_{POC}$
and $\delta^{15}N_{PN}$ (Fig. 4). Three points are included in Fig. 4: phytoplankton values of $\delta^{13}C$

$= -27.7\,‰$ (Čermelj et al., 1996) and δ^{15}N range between -15 and $+20\,‰$ (dotted line in Fig. 4), terrestrial organic matter with δ^{13}C $= -26\,‰$ and δ^{15}N $= +2\,‰$, and sur-face sediment values of δ^{13}C $= -32.5\,‰$ and δ^{15}N $= +4\,‰$ (Lojen et al., 1997). The δ^{15}N range between -15 and $+20\,‰$ was taken for freshwater phytoplankton from the

5 literature (Kendall et al., 2001). Gu et al. (1993) found δ^{15}N values range from $+2$ to $+14\,‰$. François et al. (1996) reported values as low as -2.4 and $-0.2\,‰$, while in the study performed by Vuorio et al., (2006) the δ^{15}N values ranged from -2.1 to $+12.8\,‰$. δ^{15}N values <0 are typical for cyanobacteria. Most of our results are well within the δ^{15}N range; however, the contribution of terrestrial and phytoplankton origin cannot be

10 estimated based on δ^{15}N values. It is also difficult to calculate the contribution of these two sources based on δ^{13}C$_{POC}$ data. Only samples from December and samples from the epilimnium in October failed in the range of all three parameters (Fig. 4). In ad-dition, the δ^{13}C value of phytoplankton was still higher than δ^{13}C$_{POC}$ determined in the epilimnium in all sampling periods. A substantial difference was observed in δ^{13}C

15 values in phytoplankton, depending on phytoplankton taxa, season and lake. The δ^{13}C values ranged between $-34.4\,‰$ and $-5.9\,‰$ in mesotrophic and eutrophic south-west Finnish lakes (Vuorio et al., 2006). The lowest δ^{13}C values were found in chrysophytes ($-34.4\,‰$ to $-31.3\,‰$) and diatoms ($-30.6\,‰$ to $-26.6\,‰$). Cyanobacteria were most variable with δ^{13}C ranging from $-32.4\,‰$ to $-5.9\,‰$. Low δ^{13}C values of $-31.2\,‰$ for

20 diatoms have also been reported from Loch Ness in autumn (Jones et al., 1998) and from lake Kinneret in winter ($-32.0\,‰$, Zohary et al., 1994). In Lake Bled, *Cyanophita*, with *Alhanizomenon slovenicum*, was the most abundant phytoplankton species (45% of total phytoplankton biomass) in the water column in October followed by *Bacillary-oplyceae* (25%), *Crysophita* (20%), *Dynophyta* (8%) and *Chlorophyta* (2%) (Remec-

25 Rekar and Bat, 2009). Since we did not have any additional phytoplankton samples and since it is difficult to separate the POM pool in lake water, the relative contributions of allochthonous and autochthonous carbon to POC were assessed using a model proposed by Pace et al. (2004):

$$\delta^{13}C_{POC} = (1-w) \cdot (\delta^{13}CO_{2(aq)} - \varepsilon_p) + w \cdot (-26) \tag{1}$$

where $\delta^{13}C_{POC}$ and $\delta^{13}CO_{2(aq)}$ are the isotope composition of POC and ^{13}C content of aqueous CO_2 in the form of $DI^{13}C$ that is taken up most by phytoplankton (Laws et al., 1995). Chemical fractionation of ^{13}C between $CO_{2(aq)}$ and HCO_3^- was calculated from

5   DIC, $\delta^{13}C_{DIC}$, pH and temperature (Mook et al., 1974). The $\delta^{13}C$ value for terrestrial plants was taken to be −26‰, a value that was determined in soil taken from lake surroundings. The unknown parameters in Eq. (1) are ε_p and w, the proportion of POC of terrestrial origin. The biological fractionation of ^{13}C photosynthesis, ε_p, was taken to be 21‰ (Bidigare, 1997), a typical value for biological fractionation during

10   photosynthesis. Better estimates for ε_p could be achieved by fitting the model to the incubation data (Pace et al., 2004) or by using experimental models relating ε to the algal specific growth rate and CO_2 concentrations in the water (Baird et al., 2001; Karlsson et al., 2003; Kritzberg et al., 2004; Vuorio et al., 2006), neither of which was possible in our study. Based on our calculation we found that, in the whole water

15   column in September and October, 86 to 96 % of POC was of autochthonous origin. In contrast, in December the proportion of allochthonous carbon to POC was more pronounced, ranging from 0.57 to 0.59.

     $\delta^{13}C_{DIC}$ values suggest two separate sources of $CO_{2(aq)}$ in the lake water column. In the epilimnium, phytoplankton were photosynthetically using mainly CO_2 derived from

20   air-water exchange, while deeper in the water column the phytoplankton could utilized some CO_2 derived from respiration below the thermocline. Thus, as a consequence, we would expect lower $\delta^{13}C_{POC}$ values. Between depths of 8 m and 16 m a decrease of 4 to 5‰ in $\delta^{13}C_{DIC}$ values was observed and was consistent with the same decrease in POC (Fig. 2). The hypolimnetic DIC pool became depleted in ^{13}C because of the

25   release of isotopically light inorganic carbon during the decomposition of organic matter. Bernasconi et al. (1997) and Lehmann et al. (2004) observed a similar decrease in $\delta^{13}C_{DIC}$ values, ranging from −4 to −6‰. Low $\delta^{13}C_{POC}$ values between −33‰ and

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−36‰, determined at the bottom of water column and at the depth of 24 m in October, could also reflect the contribution of methanotrophs. Biomass originating from methanotrophic bacteria have very low δ^{13}C values, because the CO_2 utilized by bacteria is strongly depleted in ^{13}C following CH_4 oxidation (−70‰ ± 15‰) (Lehmann et al., 2002). The bacterial origin of POM in the hypolimnium is additionally supported by low δ^{15}N$_{PN}$, with a minimum value of 0.8‰ measured in August, and by biomarker analysis. The δ^{13}C value of −58‰ was determined in a bacterial biomarker for methanotrophs (Gams Petrišič and Ogrinc, 2011).

4.2 Nitrogen isotope biogeochemistry in the water column

The N isotope composition of POM suggests that nitrogen fixation is not an important source of N for phytoplankton or cyanobacteria in the epilimnium. This process results in organic matter, which is depleted in ^{15}N. This is not the case in our study, since the average δ^{15}N$_{PN}$ values were +4.3‰ and +5.4‰ in September and October. Fixation is also a highly energy consuming process and occurs only when other nitrogen sources are depleted. Assimilation is favoured, because it requires less energy input. In our study, nitrate and ammonium concentrations were never less than 5 μM and thus never limiting. Lehmann et al. (2004) obtained similar results. Hadas et al. (2009) attributed low isotope values of δ^{15}N in POM (+3.3‰) to nitrogen fixation during the bloom of N_2-fixing cyanobacteria. It was possible, however, that observed values were partly due to the slight autumn peak of primary production. Information about the autumn phytoplankton composition (Remec-Rekar, personal communication, 2009) indicated that, in the upper layers (down to 7 m), *Aphanizomenon* sp. and *Aphanocapsa* sp. (cyanobacteria) and *Cyclotella*, *Fragillaria* and *Asterionella* (diatoms) were the dominating species. Our average δ^{15}N$_{PN}$ value of +4.4 ± 0.2‰ from 6 m to 8 m is well within the range reported by Vuorio et al. (2006) for diatoms (from −2.1‰ to +12.8‰) but higher than values reported for cyanobacteria (from −2.1‰ to −1.6‰). The average δ^{15}N$_{PN}$ value from hypolimnion (anoxic depths excluded) was +6.1 ± 2.3‰, again

indicating the prevalence of diatoms. It is important to include seasonal variations in taxons when interpreting isotope data, since it was found that, for cyanobacteria for example, this variation ranges between 2 and 4‰ (Vuorio et al., 2006).

Variations in the $\delta^{15}N_{PN}$ values have been understood to reflect primarily the isotope effects during nitrate uptake (Altabet et al., 1991; Holmes et al., 1999). Phytoplankton preferentially incorporates ^{14}N during nitrate assimilation and thus $\delta^{15}N_{PN}$ should be less than or equal to that of nitrate. In marine environments, the $\delta^{15}N$ values for phytoplankton and sedimenting organic matter have shown a negative correlation with nitrate concentration. This relation should follow the theoretical model of Rayleigh kinetic fractionation (Altabet and François, 1994). According to this model, the change in the substrate and product isotope composition is described by the following equation:

$$\delta^{15}N_{NO_3(f)} = \delta^{15}N_{NO_3(f-1)} - \varepsilon \cdot \ln(f) \qquad (2)$$

and the product's isotope value is given by

$$\delta^{15}N_{PN} = \delta^{15}N_{NO_3-} + \varepsilon \qquad (3)$$

where f represents the residual fraction of NO_3^-, ε is the biological enrichment factor for NO_3^- consumption in phytoplankton (in ‰) and $\delta^{15}N_{PN}$ and $\delta^{15}N_{NO_3}$ are nitrogen isotope values for POM and NO_3^- (in ‰). Eqs. (2) and (3) require a linear relation between $\delta^{15}N_{PN}$ and $\ln[NO_3^-]$, the slope of the curve giving ε. We should emphasize that Rayleigh's distillation model only applies for systems with limited reactant availability. In our lake, and also in many other aquatic natural environments, there is a constant input of NO_3^-; the calculated enrichment factor is therefore only an apparent factor. The correlations between $\delta^{15}N_{PN}$ and $\ln[NO_3^-]$ were positive in all sampling months, which means that NO_3^- assimilation was not a major source of N in POM and the slope of the curve was not typical for biological enrichment. There is only one exception in September at the depth of 10 m (Fig. 3) where the sudden decrease in concentration could be explained by intensive assimilation. Similar behaviour was reported for Lake Lugano

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in Switzerland (Bernasconi et al., 1997; Lehmann et al., 2004). On the contrary, in Baldeggersee (Teranes and Bernasconi, 2000), negative correlations between $\delta^{15}N_{PN}$ and $\ln[NO_3^-]$ indicated that NO_3^- assimilation took place. In their study the derived value of $-2.96\,‰$ for ε as within values for fractionation factors found for marine phytoplankton (Altabet and François, 1994) and lacustrine phytoplankton (Kendall et al., 2001). In lakes, phosphorous is usually the limiting factor of primary production and not nitrogen, and evidence for a correlation between nitrate concentration and $\delta^{15}N_{PN}$ is thus scarce. When the ratio of nitrate to phosphate is greater than 16 (Redfield ratio), phosphate depletion occurs. Our ratios were much higher than 16 throughout the water column, except at depths of 26 m and 28 m, where nitrate was consumed by denitrifying bacteria. Thus in Lake Bled phosphorous was really the factor controlling primary production. In addition, Teranes and Bernasconi (2000) concluded that, in lakes with shorter resident times, allochthonous organic matter input with distinctively different isotope value could influence the isotope composition of NO_3^-, obscuring variations in $\delta^{15}N_{PN}$ values related to the degree of nitrate utilization. The residence time in Lake Bled was shortened from 4 years to 1.1 year after the amelioration projects took place (Remec-Rekar and Bat, 2003), suggesting much more extensive recycling of N than in other lakes (Bernasconi et al., 1997; Hodell and Schelske, 1998; Teranes and Bernasconi, 2000; Lehmann at al., 2004; Hadas et al., 2009). This means that hypothetical input to the Lake Bled would have to be large or should have very different isotope values. In the former an increase in nitrate concentrations should be observed, while in the latter case the isotope value of the source should differ significantly from the measured ones.

Lehmann et al. (2004) suggest possible reasons for anomalous isotope values: non-constant fractionation factor during assimilation, other sources of N (NH_4^+, N_2), allochthonous NO_3^- or OM input, intensive nitrification, OM degradation and influence of sedimentary OM after the turnover. Other possible, and very likely, reasons are a shift in cyanobacteria and phytoplankton species composition and change in growth conditions, on which the fractionation factor largely depends (Faganeli et al., 1989; Montoya

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and McCarthy, 1995; Hadas et al., 2009). Ostrom et al. (1997) reported that, in a cold coastal marine environment, the assimilation of nitrate was not the primary mechanism controlling the isotope composition of phytoplankton. The authors proposed that the assimilation of ammonium may have contributed to the high $\delta^{15}N_{PN}$ values. It was found that ammonium was also the principal source for cyanobacteria in the eutrophic Shelburne Pond (Burlington, USA) (Feber et al., 2004). The difference in concentrations between autumn and winter sampling showed that NH_4^+ consumption was higher in the autumn. If nitrification was the main NH_4^+ sink, the $\delta^{15}N_{PN}$ and $\delta^{15}N_{NO_3}$ values would be lower, followed by even lower $\delta^{15}N_{PN}$ values. It appears, therefore, that NH_4^+, more than NO_3^-, was the source of N for particulate matter in our study too. The negative linear relationship between $\delta^{15}N_{PN}$ and $\ln[NH_4^+]$ was indicative of active NH_4^+ consumption in September and October (Fig. 5). No such correlation was observed in December. Fractionation factors were calculated to be $-0.8\,‰$ in September and $-1.4\,‰$ in October and were lower than the fractionation factor of $-10\,‰$ associated with N incorporation of ammonia in a field experiment (Cifuentes et al., 1988). The fractionation factor for ammonium assimilation is typically greater than the fractionation factor for nitrate assimilation. In addition, the isotope composition of ammonium should have lower $\delta^{15}N$ values than nitrate, and it is thus expected that organisms using ammonia as a nitrogen source will have lower $\delta^{15}N$ values than those using nitrate as a source. The concentrations of NH_4^+ were not sufficient for isotope measurements in the water column, except at the bottom where we found that $\delta^{15}N$ values of NH_4^+ averaged $+3.8 \pm 0.1\,‰$. $\delta^{15}N_{NH_4}$ values in the water column found in high mountain lakes in the Pyrenees ranged from -9.4 to $+7.4\,‰$, depending on snow-pack melting or soil conditions (Bartrons et al., 2010). On the other hand, the average $\delta^{15}N_{NH_4}$ values of $+2.2 \pm 1.6\,‰$ in sediment pore waters were found to be uniform, with no variations corresponding to catchment or lake characteristics, and similar to values found in our study at the bottom of the water column. The isotope composition of ammonium is usually similar to that of organic matter from which it was mineralized. Indeed, in our study

we found that $\delta^{15}N$ of NH_4^+ is similar to that of sedimentary organic matter which has an average $\delta^{15}N$ value of $+4.5 \pm 0.7\,‰$ in the upper 15 cm (Lojen et al., 1997). However, the high content of ^{15}N in POM suggested a source rich in ^{15}N. In October at some depths, $\delta^{15}N_{PN}$ values were remarkably higher reaching $+12.2\,‰$ at a depth of 16 m.

5 This may be attributed to microzooplankton grazing, release of nitrogen compounds during degradation, or regeneration of nitrogen in the euphotic layer.

$\delta^{15}N_{NO_3}$ values determined in Lake Bled ($+3.6$ to $-10.3\,‰$) were clearly distinct from the isotope composition of POM or sediments (Fig. 2), except in September and October at the bottom of water column. This indicates that other processes are contributing

10 to the reservoir of nitrate in Lake Bled. $\delta^{15}N_{NO_3}$ values in the water column found in high mountain lakes in Pyrenees were more negative, ranging from -11.4 to $-3.5\,‰$. Other anthropogenic sources are chemical fertilizers or sewage-derived nitrate and nitrate from atmospheric deposition. An influence of sewage derived nitrate was unlikely since generally $\delta^{15}N_{NO_3}$ values are greater than $8\,‰$ and sometimes even more than

15 $+20\,‰$ (Aravena et al., 1993) and sewage input in Lake Bled has decreased by 80 % in the last 30 years (Remec-Rekar and Bat, 2003). However, Owens (1987) observed $\delta^{15}N$ values between $+2$ and $+4\,‰$ in sewage which are very close to $\delta^{15}N_{PN}$ values measured in particulate matter in the epilimnion in October. Ostrom et al. (1998) attributed the very low average $\delta^{15}N_{NO_3}$ of $-4.1\,‰$ determined in Lake Superior to

20 atmospheric deposition. However, it should be noted that Lake Superior has been classified as ultra-oligotrophic and 95 % of the watershed is forested. Thus the isotope composition of nitrate was consistent with its origin from atmospheric nitrate. Lake Bled and its surrounding area received approximately 250 mm of rainfall per square metre in October, which was in accordance with the long-term average observed in this area

25 (Remec-Rekar and Bat, 2009). No major precipitation events were observed, except in October when 4.8 mm of rain fell on the lake in the night before sampling. The observed amount could not influence the concentrations and isotope composition of nitrate in the Lake.

Plotting $\delta^{15}N_{NO_3}$ values versus nitrate concentrations often reveals that weather denitrification and mixing of nitrate from various sources are the main processes influencing concentrations and isotope composition of nitrate in the study environment (Mayer et al., 2002). The negative linear relationship and relatively high discrimination coefficient ($r^2 = 60$) were both indicative of an active water column denitrification in October 2008 (Fig. 6). Water column denitrification influences $\delta^{15}N_{NO_3}$ more than sediment denitrification (Lehmann et al., 2003; Hadas et al., 2009). In high mountain lakes in the Pyrenees a positive correlation between $\delta^{15}N_{NO_3}$ and NO_3^- concentrations was observed, indicating nitrate loading from the catchment due to atmospheric deposition and the added nitrification influence (Bartros et al., 2010). In December, we could not confirm this relationship, presumably because the water column was well mixed and thus oxygenated. The decreased $\delta^{15}N_{NO_3}$ values in December can be explained first by degradation of isotopically heavier organic matter and later by nitrification of degradation products. This conclusion was further supported by high NH_4^+ concentrations due to ammonification. The obtained results are in a good agreement with those of Hadas et al. (2009), but contrast with those of Lehmann et al. (2004). The nitrifying community is a constant consumer of NH_4^+; on the other hand, the heterotrophic community is its reliable supplier (Gardner et al., 1987). These results together imply that there was no influence of sewage or farming on the nitrogen balance in the lake during the sampling period. In addition, we were able to determine some points in the lake at which nitrification was active. These were at depths between 12 m and 18 m in September and October, where the $\delta^{15}N_{NO_3}$ values decreased between −8.4 and −7.2 ‰ and nitrate concentrations increased.

In conclusion this study demonstrates that the combination of carbon and nitrogen isotope measurements lead to a better understanding into the processes and factors controlling the cycling of both elements in the water column of a mesotrophic alpine lake with high recycling efficiency. The successful use of N isotopes in tracing the origin and cycling of nitrogen has further direct implication for management practice to preserve the water quality of lakes.

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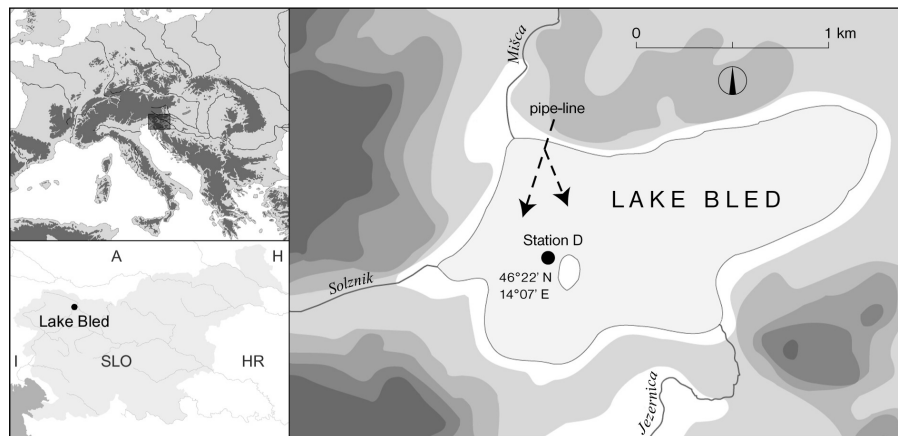**Fig. 1.** Location of Lake Bled with sampling point D, the deepest Western Basin, marked.
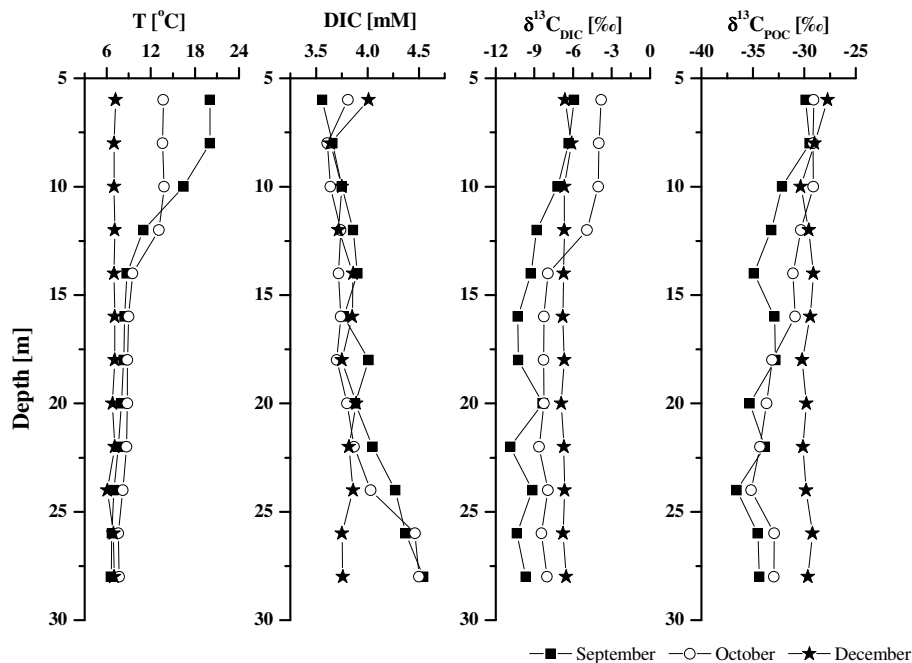
Fig. 2. Depth profiles of temperature (T), concentration of dissolved inorganic carbon (DIC), and isotope composition of dissolved inorganic and particulate carbon ($\delta^{13}C_{DIC}$, $\delta^{13}C_{POC}$) in the water column during September to December 2009 at sampling point D.
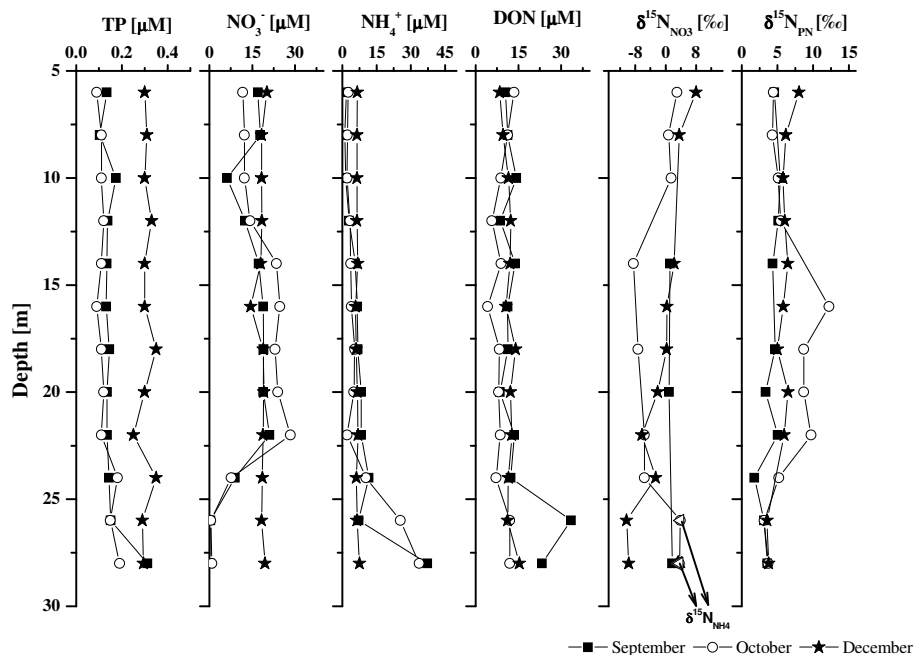
Fig. 3. Depth profiles of total phosphorous (TP), concentrations of nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic nitrogen (DON), together with the isotope composition of nitrate ($\delta^{15}N_{NO_3}$) and particulate nitrogen ($\delta^{15}N_{PN}$) in the water column from September to December 2009 at sampling point D.

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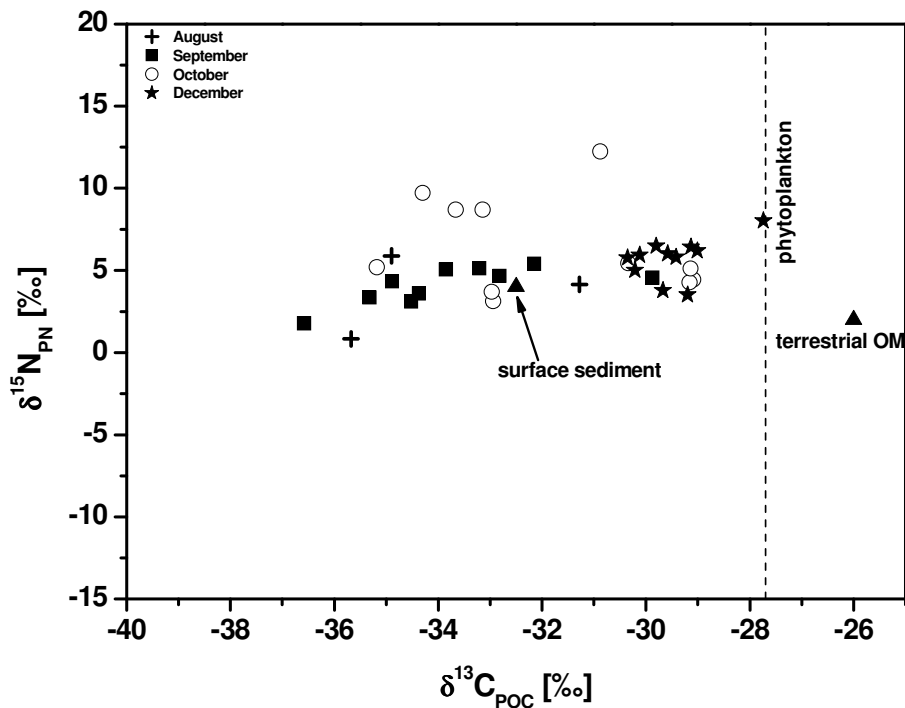
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Fig. 4. Relationship between $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$ in suspended particulate organic matter during all sampling periods, together with the isotope composition of sedimentary and terrestrial organic matter and the isotope range of phytoplankton (Čermelj et al., 1996; Kendall et al., 2001).

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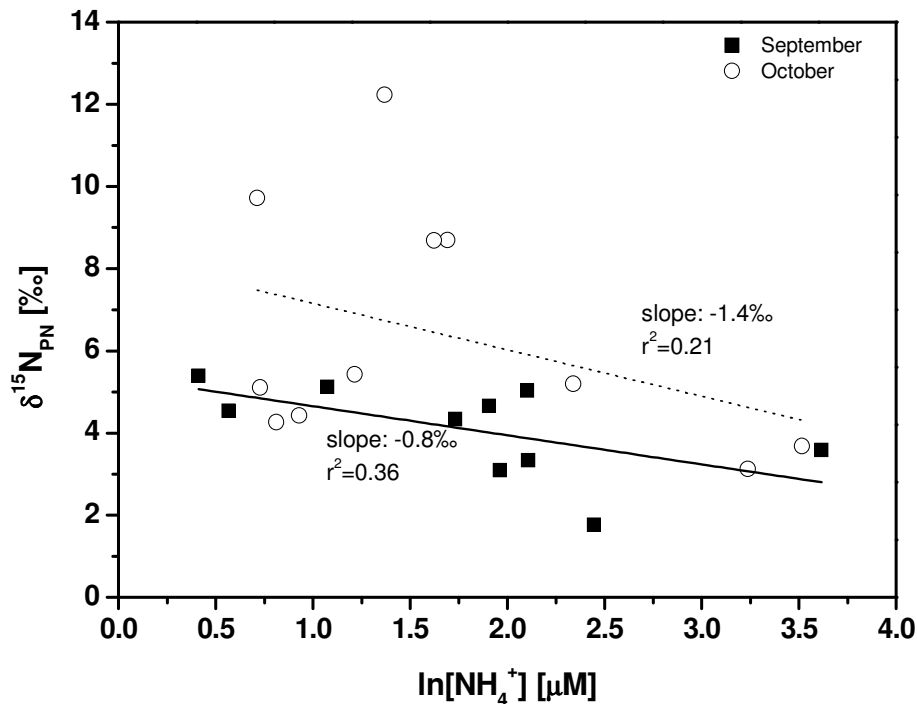
Fig. 5. Dependence of $\delta^{15}N_{PN}$ values on the natural logarithm of ammonium concentration ($\ln[NH_4^+]$). The lines indicate the linear regression during ammonium utilization with isotope effects of $\varepsilon = -0.8$ and -1.4 in September and October 2009.
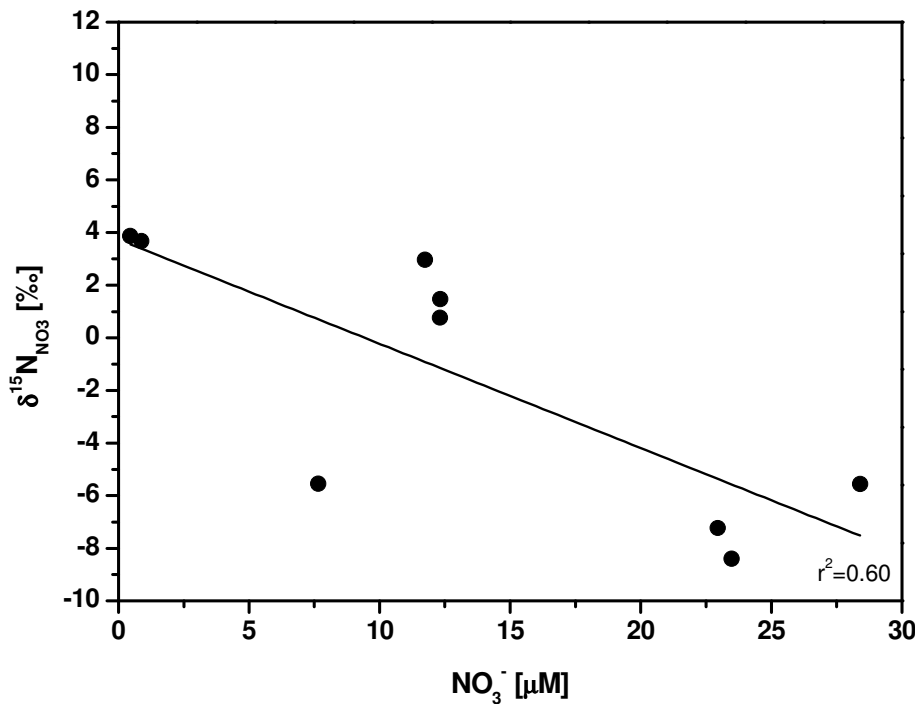
Fig. 6. Relation between $\delta^{15}N_{NO_3}$ values and NO_3^- concentrations, together with a regression line indicating the denitrification that occurred in October 2009.