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**Seasonally varying nitrogen isotope biogeochemistry of particulate organic matter (POM)
in Lake Kinneret, Israel.**

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Abstract

Large temporal variations in the nitrogen isotopic composition ($\delta^{15}\text{N}$) of particulate organic matter (POM) and dissolved inorganic (DIN) species in Lake Kinneret occurred in response to seasonal phasing of dominant nitrogen cycle processes. The lowest $\delta^{15}\text{N}$ POM values (-5.5‰) were observed in early winter, a consequence of isotopic fractionation by chemoautotrophic microbial NH_4^+ assimilation and during the first phase of the nitrification period, by seasonally dominant NH_4^+ oxidizers. Nitrification itself was strongly fractionating, producing ^{15}N depleted NO_3^- and strongly enriching water column NH_4^+ in ^{15}N . Toward the end of nitrification and into the phytoplankton bloom period in later winter and early spring, there was a corresponding 15 to 30‰ jump in POM $\delta^{15}\text{N}$ due to assimilation of high $\delta^{15}\text{N}$ NH_4^+ . Maximal $\delta^{15}\text{N}$ POM values were thus measured during the algal blooms of *Peridinium gatunense* in 2004 and *Debarya* sp. and *Microcystis* sp. in 2005. Toward the end of the blooms in mid- to late spring, POM $\delta^{15}\text{N}$ values decreased to values similar to $\delta^{15}\text{N}$ for NO_3^- (8 to 12‰) indicating a switch to this DIN source. NO_3^- assimilation into POM appeared to occur without isotopic fractionation. Late spring was also the denitrification period with NO_3^- removal in the newly suboxic hypolimnion. Though $\delta^{15}\text{N}\text{NO}_3^-$ increased in the residual NO_3^- , complete consumption by denitrification suggests no net effect as supported by a lack of increase in $\delta^{15}\text{N}$ POM at this time. Oligotrophic conditions are found in the epilimnion in late summer and early autumn and low $\delta^{15}\text{N}$ POM was found (3.3‰) during the bloom of the filamentous N_2 fixing cyanobacteria, *Aphanizomenon ovalisporum* and *Cylindrospermopsis cuspidis*. The $\delta^{15}\text{N}$ -DON isotopic signature at this time was significantly higher, 8 to 12‰, suggesting a phytoplankton source from earlier in the seasonal cycle. Overall, the largest known seasonal variations in N isotope composition have been observed in Lake Kinneret providing a uniquely useful biogeochemical tool for studying N cycling in lakes as well as detecting long-term changes in N source and cycling in response to watershed land use changes and climate change.

Introduction

The nitrogen and carbon isotopic composition of particulate organic matter (POM) in aquatic environments reflects and integrates the influence of biogeochemical processes in the water column and sediments including primary production, redox-sensitive nitrogen transformations, and carbonate chemistry. Both source (allochthonous, autochthonous) and transformation processes encode information in the natural distribution of stable isotopes making these measurements valuable, in situ indicators of N and C elemental cycling in these ecosystems (Bernasconi et al. 1997; Kendall et al. 2001; Altabet 2006). Seasonal variation in N ($\delta^{15}\text{N}$) and C ($\delta^{13}\text{C}$) isotopic composition for example, were used to deduce that riverine plankton as opposed to allochthonous sources was the dominant source of POM in an extensive survey conducted in large river systems in the United States (Kendall et al. 2001).

There have been a number of studies of POM and DIC (dissolved inorganic carbon) $\delta^{13}\text{C}$ in lakes. They generally show greater seasonal variability than those observed for marine ecosystems, due to both shifts in plankton species composition and larger relative changes in carbonate chemistry (Stiller 1977; Takahashi et al. 1990; Zohary et al. 1994). In contrast, there are relative few studies of lake N isotope biogeochemistry despite its potential for elucidation of N cycle dynamics (Lehmann et al. 2004). $\delta^{15}\text{N}$ POM is influenced by the isotopic composition of the dissolved inorganic N (DIN) sources for phytoplankton, isotopic fractionation during partial utilization of these sources, as well as transfer to higher trophic levels (Altabet 2006). The $\delta^{15}\text{N}$ of DIN is in turn controlled by allochthonous sources, sinks from the system, as well as internal N cycling. Allochthonous sources are usually dominated by fluvial inputs, whose $\delta^{15}\text{N}$ can be directly measured and biological N_2 fixation which adds combined N with a relatively low $\delta^{15}\text{N}$ of between 0 and -2‰ (Carpenter et al. 1997; Brandes and Devol 2002; Mahaffey et al. 2005). Internal cycling processes that have been shown to produce large N isotope fractionation effects include nitrification and denitrification. Whereas microbial degradation and mineralization can increase $\delta^{15}\text{N}$ POM values (Altabet and McCarthy 1985; Saino and Hattori 1987; Owens and Law 1989), no significant effect has been detected where the $\delta^{15}\text{N}$ of NH_4^+ produced has been measured. Previously, one of the most thorough studies of lake N isotope biogeochemistry was carried out in Lake Lugano, Italy (Lehman et al. 2004). Being a

subtropical lake in a semi-arid region, Lake Kinneret, Israel is a useful contrast more amenable to geochemical budgeting with only one significant tributary serving as the source for both water and nutrients.

Lake Kinneret, Israel and its watershed have been subject to both anthropogenic degradation and recent restoration efforts (Hambricht et al. 2000) that likely altered C and N isotopic composition. Historically, an intense annual winter-spring phytoplankton bloom was typically dominated by the dinoflagellate *Peridinium gatunense* followed by characteristically 7-10 times lower summer and autumn biomass from July till the end of the annual cycle (Pollinger 1986; Berman et al. 1992). Decomposition and degradation of the *Peridinium* biomass during a short period of 4 to 6 weeks largely fueled heterotrophic activity leading to anoxia in the hypolimnion in late spring. In the last decade, though, there has been an increase in the appearance of cyanobacteria, particularly the filamentous nitrogen fixing *Aphanizomenon ovalisporum*, and *Cylindrospermopsis cuspis* in summer-autumn (Pollinger et al. 1998; Hadas et al. 1999; Zohary 2004) and the absence of *Peridinium gatunense* blooms in some years. The appearance in Lake Kinneret of cyanobacteria capable of N₂ fixation, may add to new production, causing alterations in the biogeochemistry likely reflected in the $\delta^{15}\text{N}$ of POM during summer and autumn.

While the $\delta^{13}\text{C}$ of integrated water samples of POC (particulate organic carbon) and individual taxa within the food web of Lake Kinneret have been studied previously (Stiller 1977; Zohary et al. 1994; Hadas et al. 2001), there has been no corresponding effort to use $\delta^{15}\text{N}$ to study Lake Kinneret's N cycle. In the present study, we followed the temporal and depth-related fluctuations in the $\delta^{15}\text{N}$ of POM (a complex mixture comprised of organic material derived from both microbial and phytoplanktonic biomass) during an annual cycle, in order to distinguish the effects of the different dominant processes involved in the nitrogen cycle in Lake Kinneret, Israel; i.e, nitrification, denitrification, and N₂ fixation. Distinct seasonal phasing of these processes appears to have produced the largest known seasonal variations in $\delta^{15}\text{N}$ POM.

Methods

Study site: Lake Kinneret, Israel, is a freshwater, warm monomictic lake, stratified for 7-8 months of the year. Our study site, Station A, is located in the central and deepest part of the lake

(42 m depth). Water input is dominated by inflow from the upper Galilee and the Golan Heights, via the Jordan River. Loss is mainly by pumping for domestic and agricultural use via the Main Water Carrier (MWC) and evaporation. However in years with very heavy winter rains, high lake level results in drainage to the lower Jordan River. During stratification, an aerobic warm (24-28°C) epilimnion and anoxic relatively cold (14–16°C) hypolimnion are formed. Annual primary production in the epilimnetic euphotic layer averages ca. 1.8 g C m⁻² d⁻¹ (Berman et al. 1995). The nitrogen cycle in Lake Kinneret is largely seasonally and spatially compartmentalized and can be divided into periods and regions dominated by nitrification, denitrification, and N₂ fixation as result of changes in stratification, hypolimnion O₂ concentration, and epilimnion DIN content, respectively (see Results section).

NH₄⁺ concentrations ranged from 0 to about 120 μmol L⁻¹ (the highest values in the hypolimnion before overturn). NO₃⁻ concentrations varied widely (0 to 50 μmol L⁻¹) in response to punctuated inputs from the watershed via Jordan River flood events, production by nitrification of NH₄⁺ in winter, and removal by algal uptake or denitrification. Two seasonal NO₂⁻ maxima typically occur, during the nitrification period in January (about 10 to 15 μmol L⁻¹) and during denitrification in May (7 to 10 μmol L⁻¹).

Sampling and field measurements – Sampling took place between Jan 2004 to Dec 2005 covering twice each of the three main N cycle processes in the lake; i.e nitrification, denitrification, and N₂ fixation, respectively. Depth profiles of water samples from station A, in the deepest part in the lake (42 m), were collected using either a 5 L poly vinyl chloride (PVC) sampler or by pump. Samples were placed into dark sterile bottles and then transferred to the laboratory. One liter was used for chlorophyll and chemistry measurements and another 1.5 liters (in duplicate) were used for δ¹⁵N and/or δ¹³C measurements. Each bottle was separately filtered through a 47 mm diameter, pre-combusted GF/F filter which was subsequently kept frozen till analysis. Filtrate was preserved by acidification with HCl to pH 2.5.

POM δ¹⁵N, δ¹³C - Isotopic measurements were conducted using a continuous He carrier flow, elemental analyzer-isotope ratio mass spectrometer system (EA-CF-IRMS; Europa Euroboprep – Finnigan MAT 251 or Eurovector-GV IsoPrime). Filters with POM samples of approximately 5-10 mg POM were first HCl fumed to remove inorganic C, dried, and then

placed within tightly squeezed tin foil capsules. During analysis, samples were automatically dropped into a combustion tube at 1000°C coincident with injection of high purity O₂ and followed by passage over a Cu reduction furnace at 600°C. The CO₂ and N₂ product gasses were separated by a GC column and were introduced via open slit to the mass spectrometer. Masses 28, 29 and 44, 45, 46 were integrated for N₂ and CO₂ isotopic determination, respectively. Injections of tank N₂ and CO₂ accompanied each sample analysis to correct for instrument drift. In-house organic standards of known isotopic composition were used to calibrate $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ results against atmospheric N₂ and PDB references, respectively. All samples were analyzed in duplicate and standards were analyzed after every 6 samples. Reproducibility was nominally $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

NH₄⁺, NO₂⁻, NO₃⁻, dissolved organic nitrogen (DON), and Chl concentration measurements – Lake water samples were filtered through a 0.45 μm filter (Schleicher & Schuell) for NH₄⁺, NO₂⁻, and NO₃⁻ concentration analysis and measured spectrophotometrically according to Standard Methods (APHA 2001) or with a Flow Injection System (QuikChem 8000 Automated ion Analyzer, Lachat Instruments). Chlorophyll *a* was measured by fluorometry after acetone extraction using the method of Holm-Hansen et al. (1965).

$\delta^{15}\text{N}$ of Dissolved NH₄⁺ and NO₃⁻ - $\delta^{15}\text{NO}_3^-$ analysis on 20 mL aliquots followed the method of McIlvin and Altabet (2005). NO₃⁻ was reduced to NO₂⁻ with Cd and the NO₂⁻ was subsequently reduced to N₂O using sodium azide buffered to pH 4 to 5 using acetic acid. When sufficiently abundant, NO₂⁻ may be analyzed by skipping the Cd reduction step (to be reported elsewhere). $\delta^{15}\text{NH}_4^+$ was analyzed by oxidation to NO₂⁻ using hypochlorite or hypobromite followed by the reaction with azide to N₂O (Zhang et al. 2007). For either $\delta^{15}\text{NO}_3^-$ or $\delta^{15}\text{NH}_4^+$ analysis, pre-existing NO₂⁻ was removed by addition of sulfanilic acid. In every instance, $\delta^{15}\text{N}_2\text{O}$ analysis was done as described previously using an automated purge-trap system (PT-CF-IRMS; GV IsoPrime). In every sample batch, unknowns were run together with replicates of 3 working standards with known $\delta^{15}\text{N}$ values. For NH₄⁺ and NO₃⁻ internationally recognized standards were used (NH₄⁺ - IAEA N1 0.49‰, NITS USGS25 -29.44‰, NITS USGS26 52.87‰; NO₃⁻ - USGS34, and USGS35). These standards were chosen to span the expected range in natural $\delta^{15}\text{N}$. Generally these methods have near-0 analytical blank, are applicable to concentrations

down to $< 0.5 \mu\text{mol L}^{-1}$, and have reproducibilities of about $\pm 0.3 \text{ ‰}$. Though not discussed here, these methods were also used for NO_3^- and NO_2^- $\delta^{18}\text{O}$ determination.

$\delta^{15}\text{N}$ of *DON*: Since there is almost no dissolved inorganic nitrogen (DIN) in the epilimnion water column of Lake Kinneret in summer and autumn, the only other dissolved combined nitrogen fraction is DON. The persulfate oxidation method was used to convert DON to NO_3^- (Knapp et al. 2005). 14 mL samples were reacted with 6 mL of reagent (175 mL of 1N NaOH plus 25 g of recrystallized $\text{K}_2\text{S}_2\text{O}_8$ made up to 500 mL with dH_2O) for 3 h at 100°C . Afterwards, 50 mg of MgO was added to neutralize the now acidic samples. The samples were then shaken and centrifuged to separate excess MgO prior to processing for $\delta^{15}\text{NO}_3^-$. Isotopic analysis was done as described above.

$\delta^{15}\text{N}$, $\delta^{13}\text{C}$ of phytoplankton end members isolated from Lake Kinneret- Algal and cyanobacterial species which bloomed or were abundant in the lake during the study period were collected by towing horizontally 23 or 63 μm phytoplankton mesh nets. The species were separated by differential filtration and characteristic buoyancies of each of the species. *Aphanizomenon ovalisporum*, *Microcystis* spp. are buoyant and can be easily isolated from other components of the plankton. *Microcystis* floating near-shore was collected directly using a pump. *Peridinium gatunense* sinks quickly and was thus easily separated. The collected material was microscopically examined for purity of the isolated species and only nearly mono-species samples were frozen and lyophilized (Hato). 0.5-1.0 mg of the lyophilized dry samples was analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as described above.

Nitrate reductase activity - Dissimilatory nitrate reductase (NR) activity is the first step in the denitrification process. NR activity was used to detect active denitrification and was measured on 500 mL of lake water samples as described in Hochman et al. 1986.

Results and Discussion

Identification of seasonally dominant processes

Nitrification – During the long, summer- autumn, period of strong thermal stratification, the upper layer of the mid-depth chemocline presents a vertically restricted environment where

coexistence of NH_4^+ and O_2 stimulates localized nitrification. However with thermocline deepening and eventual overturn in winter, the entire water column becomes conducive to nitrification. In late 2004, the anoxic hypolimnion was characterized by high NH_4^+ concentrations (~ 98 to $115 \mu\text{mol L}^{-1}$) and no detectable NO_2^- or NO_3^- (Figs. 1, 2, 3A). The water column became isothermal and oxygenated by 03 January 2005 with subsequent increase in NO_3^- . This seasonal pattern is typical for Lake Kinneret; similar observations were made in the previous year (2003-2004) with the overturn occurring in the last week of December (Figs. 2, 3A). The year 2005 was unique, though, in that a peak in NO_2^- concentration was maintained for more than two weeks, reaching values of $18 \mu\text{mol L}^{-1}$ (Fig. 2). NO_2^- appearance co-occurred with NH_4^+ disappearance, indicative of the first stage of nitrification; microbial NH_4^+ oxidation. The second stage, NO_2^- oxidation to NO_3^- appeared to be delayed by two weeks accounting for the NO_2^- peak. Usually, NO_3^- accumulated simultaneously with NH_4^+ decrease due to similar rates of NH_4^+ and NO_2^- oxidation (Cavari 1977). Samples taken on 09 January 2005 for bacteria molecular characterization revealed that 90% of the 16S rRNA clone library were related to *Nitrosomonas* (National Center for Biotechnology Information Gene Bank accession numbers: DQ154782 - DQ154799). By the end of January in 2004 and 2005, nitrification had almost reached completion, no NO_2^- and very low NH_4^+ concentrations were observed. NO_3^- concentration reached 33 and $27 \mu\text{mol L}^{-1}$ at 3 m depth in 2004 and 2005, respectively due to nitrification plus storm water NO_3^- input via the Jordan River (Fig. 2).

Denitrification – Denitrification in the sediments probably occurs throughout the whole year (Cavari and Phelps 1977). However, sedimentary denitrification has minimal effect on the overlying system's N isotope geochemistry due to transport limitation of the overall rate which has a very low isotopic fractionation potential (Brandes and Devol 1997). Water column denitrification, which can strongly affect the system's N isotope balance, began with the first appearance of suboxia at the sediment-water interface in late March. However, much more intense denitrification typically takes place in late April and May after the spring bloom when microbial degradation of abundant organic matter (OM) first consumed available O_2 and then used NO_3^- as an oxidant (electron acceptor). For example between March and June 2004 when low hypolimnic O_2 concentrations were established, NO_3^- concentrations decreased from 37 to $0 \mu\text{mol L}^{-1}$ in the hypolimnion (Fig. 3A). Correspondingly high NR activity was measured at the beginning of May 2004 at 30 to 40 m depth, reaching maximal values of $71 \mu\text{g N l}^{-1} \text{h}^{-1}$ (O.

Hadas unpubl. data). At this time, NH_4^+ began to accumulate in the lower part of the hypolimnion due to supply from the sediments and continuing degradation of OM derived from sinking *Peridinium gatunense* cells (Fig. 3A).

N₂ fixation - In summer and early autumn, very low DIN concentrations measured in the epilimnion (Fig. 4C) had significant potential for stimulating N_2 fixation. Since 1994, there has been a shift in the lake's phytoplankton population for this time toward cyanobacteria with blooms of the filamentous N_2 fixers, *Aphanizomenon ovalisporum* and *Cylindrospermopsis cuspidis*. Although, Lake Kinneret is also phosphate depleted in summer and autumn, phosphate pulses may be responsible for stimulation of N_2 fixation. A drop in $\delta^{15}\text{N}$ POM during late summer and autumn was observed in both years of the study with lowest $\delta^{15}\text{N}$ values in August 2005 (3.3‰, Fig. 4A) consistent with atmospheric N_2 nitrogen fixation (*see below*).

Seasonal and vertical variations in isotopic composition

NH_4^+ and NO_3^- isotopic composition - The isotopic dynamics of NH_4^+ , NO_2^- , and NO_3^- reflected their sources and inter-conversions between these forms. As DIN sources for autotrophs, these species were also a primary control on $\delta^{15}\text{N}$ POM. NO_2^- , as an intermediary in nitrification and denitrification, was present at very low concentrations and thus unlikely to have direct influence on $\delta^{15}\text{N}$ POM. $\delta^{15}\text{NH}_4^+$ varied seasonally but on average had high $\delta^{15}\text{N}$ values (Fig. 3B). During stratified conditions, NH_4^+ in the hypolimnion had $\delta^{15}\text{N}$ values between 12 and 17‰ (Fig. 3B). These high values most likely reflected remineralization of high $\delta^{15}\text{N}$ sediment OM. In support of this inference, organic-rich fluffy layers overlying the sediments at Station A had an average $\delta^{15}\text{N}$ of ~10‰ (Fig. 3B). $\delta^{15}\text{NH}_4^+$ would be expected to further increase as a result of isotopic fractionation during NH_4^+ oxidation to NO_2^- (nitrification) by chemoautotrophic ammonia oxidizers (Delwiche and Steyn 1970; Nadelhoffer and Fry 1994). Such a change was clearly seen during the 2004 and 2005 nitrification period when $\delta^{15}\text{NH}_4^+$ increased by 30‰ over a few week period (Figs. 4B, 5). The magnitude of the isotope fractionation effect for NH_4^+ oxidation varies phylogenetically from 10 to 40‰ (Casciotti et al. 2003). Here we estimated the fractionation factor to be 22‰ assuming closed-system Rayleigh distillation. While N isotopic fractionation for NO_2^- oxidizing bacteria had not been as well studied, the $\delta^{15}\text{N}$ of NO_3^- should reflect efficient

oxidation of low $\delta^{15}\text{N}$ NO_2^- produced by NH_4^+ oxidation. In support, $\delta^{15}\text{NO}_3^-$ during this time was offset to lower values (Fig. 4B). The $\delta^{15}\text{N}$ of the product NO_3^- reached a stable value near the initial one for $\delta^{15}\text{NH}_4^+$ with exhaustion of the NH_4^+ pool. This result is also predicted by Rayleigh fractionation given nitrification as the dominant process consuming NH_4^+ and producing NO_3^- . In winter 2004, the nitrification period was not as well sampled, but stable post-nitrification values for $\delta^{15}\text{NO}_3^-$ were also observed in the epilimnion but were $\sim 5\%$ higher than in 2005. It should be noted that NO_3^- input from winter flooding of the Jordan may also influence $\delta^{15}\text{NO}_3^-$ at this time. In Jan 2005, the Jordan input had a $[\text{NO}_3^-]$ of $80 \mu\text{mol L}^{-1}$ and throughout the year $\delta^{15}\text{NO}_3^-$ was between 5 and 7 ‰ (sample provided by A. Nishri). These values also characterized the $\delta^{15}\text{N}$ of the dominate combined N inputs to the lake.

In late spring (May) well after the nitrification and river input periods, NO_3^- concentrations decreased in the epilimnion due to autotrophic assimilation and in the hypolimnion due to denitrification. Surprisingly, there was no evidence for isotopic fractionation during NO_3^- uptake by phytoplankton since $\delta^{15}\text{NO}_3^-$ in the epilimnion did not increase with decreasing $[\text{NO}_3^-]$ in both years. However, there was a large increase in $\delta^{15}\text{NO}_3^-$ in the hypolimnion as a result of denitrification but the effect appeared isolated to this region of the lake.

$\delta^{15}\text{N}$, $\delta^{13}\text{C}$ of isolated Lake Kinneret phytoplankton – To understand the seasonal variations in POM $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (see below), bloom-dominant phytoplankton species, that had been isolated in prior years as well as during the study, were examined for their isotopic signatures (Table 1). Almost all bloom forming phytoplankton had high $\delta^{15}\text{N}$ values between 11 and 17‰ regardless of species; the dinoflagellates *Peridinium gatunense* and *Peridiniopsis* sp., the cyanobacteria *Microcystis* sp. (Green), and the chlorophyte *Debarya* (Table 1, Fig. 4A). These $\delta^{15}\text{N}$ values are clearly not inherent characteristics of these species but correspond to the isotopic composition of DIN source and the magnitude of isotopic fraction for DIN assimilation during the late stages of these blooms. In contrast, *Aphanizomenon ovalisporum* collected during its massive bloom in October 1994 under N impoverished conditions, had low $\delta^{15}\text{N}$ (0.3‰) consistent with N_2 fixation by this filamentous cyanobacterium and is an isotopic signature inherent to this

mode of N acquisition. $\delta^{13}\text{C}$ for these phytoplankton also varied widely (-30 to -14‰) but likely reflected the CO_2 concentration particular to each collection period.

$\delta^{15}\text{N}$ of Dissolved Organic Nitrogen (DON) – The persulfate oxidation method used is in reality a determination of total nitrogen $\delta^{15}\text{N}$. However, we have restricted its application to epilimnetic waters between June and November of each year when DIN was undetectable making these the first lake DON $\delta^{15}\text{N}$ determinations to be reported. DON concentration appeared to vary widely with season from $32.5 \mu\text{mol L}^{-1}$ in June to $16.7 \mu\text{mol L}^{-1}$ in August 2004, though the range in 2005 was relatively small (Table 2). Varying DON concentration indicated some measure of lability and involvement in N cycling, though maintenance of high concentration during N impoverished conditions indicates a large biologically resistant fraction. DON was relatively ^{15}N enriched with $\delta^{15}\text{N}$ between 7.5 to 12‰ and overlapping with much of the summer and autumn range in POM $\delta^{15}\text{N}$ (Table 2). Algal production within the lake was thus the likeliest source for DON. Lowest values for DON $\delta^{15}\text{N}$ were detected during the N_2 fixation period (7.5‰) and may be the result of dissolved compounds released by the blooming N_2 -fixing cyanobacteria. However, in late autumn just prior to overturn, DON $\delta^{15}\text{N}$ increased while $\delta^{15}\text{N}$ POM decreased suggesting uncoupling at this time.

POM $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ – Very large seasonal variations in $\delta^{15}\text{N}$ POM were observed at all depths during the two years of the study. The lowest $\delta^{15}\text{N}$ POM values were observed during the first phase of the nitrification period (as low as -5.5‰) corresponding to light $\delta^{13}\text{C}$ POM values previously recorded for this period (Zohary et al. 1994; Hadas et al. 2001). Very low $\delta^{15}\text{N}$ POM was likely the consequence of N isotopic fractionation during chemoautotrophic NH_4^+ assimilation into biomass by sulfide, methane and ammonia oxidizing microbes. Along with nitrification, this process would also contribute to isotopic enrichment of the remaining water column NH_4^+ . Assimilation of the low $\delta^{15}\text{N}$ NO_3^- was not a likely contributor to the POM isotopic signature since NH_4^+ , when abundant, is usually the preferred DIN source. This inference was supported by the large increase in $\delta^{15}\text{N}$ POM that subsequently occurred upon exhaustion of NH_4^+ by nitrification.

$\delta^{15}\text{N}$ POM dramatically increased from -5.5 ‰ to 26 ‰ between the end of December 2004 and the end of February 2005 (Fig. 4A) paralleling the sharp rise in $\delta^{15}\text{NH}_4^+$ (Fig. 5). Peak $\delta^{15}\text{N}$

POM was reached as ^{15}N enriched NH_4^+ was completely consumed with little net isotopic discrimination as required by mass balance (Fig. 4; Fry and Sherr 1984; Altabet and McCarthy 1985). These observations are in contrast to river systems in the USA and in San Francisco Bay where low $\delta^{15}\text{N}$ POM were observed during phytoplankton blooms (Kendall et al. 2001; Cloern et al. 2002) probably due to a lack of DIN isotopic enrichment from nitrification. The developing phytoplankton bloom at this time also probably competed for NH_4^+ with chemoautotrophic NH_4^+ assimilation. Since each likely had distinct fractionation factors for NH_4^+ assimilation, observations must reflect their average effect. Further evidence for NH_4^+ as the source for POM was seen in consistently higher $\delta^{15}\text{N}$ POM as compared to relatively low $\delta^{15}\text{NO}_3^-$ in the epilimnion during this period (Fig. 4B). A less dramatic $\delta^{15}\text{N}$ maximum of 14.3‰ in February 2004 suggested interannual variation in the amplitude of this event. These high $\delta^{15}\text{N}$ POM values following the large increase in $\delta^{15}\text{NH}_4^+$ were also associated with the blooms of the dinoflagellate *Peridinium gatunense* in spring 2004 or *Debarya* sp. and *Microcystis* sp. in 2005 which prefer NH_4^+ (Berman et al. 1984).

Moderately high $\delta^{15}\text{N}$ POM values were subsequently observed in May of both years (Fig. 4A; 8 to 12‰). During this late spring period, NH_4^+ was depleted but NO_3^- was relatively abundant in the epilimnion making this the likely source for new production. Accordingly, $\delta^{15}\text{N}$ POM decreased with time toward $\delta^{15}\text{NO}_3^-$ values of near 10‰. Based on measured water column POC concentrations and primary production rates, POM turnover time in the lake is less than a month (0.5 to 0.8 month⁻¹ for the epilimnion and whole water column respectively). Accordingly, this change over time represents the $\delta^{15}\text{N}$ of newly produced POM with little influence from material produced earlier in the seasonal cycle. Because of the relatively low $\delta^{15}\text{NO}_3^-$ observed in the epilimnion at this time, NO_3^- utilization probably accounted for decreasing $\delta^{15}\text{N}$ POM during the bloom's peak in March - May of both years. In 2005 when *Debarya* sp. dominated, this was particularly clear as $\delta^{15}\text{NO}_3^-$ and $\delta^{15}\text{N}$ POM converged toward the end of the bloom in May. The $\delta^{15}\text{N}$ POM at 3 m depth at this time was 9.9‰ as compared to the isolated *Debarya* $\delta^{15}\text{N}$ of 10.6‰. In 2004 when *Peridinium gatunense* bloomed, a similar switch from NH_4^+ to NO_3^- utilization occurred, particularly since this species is known to be capable of nitrate uptake when ammonia is limiting (Figs. 1, 4C; Berman et al. 1984). *Peridinium gatunense* reached a biomass of 413 g m⁻²

(95% *Peridinium*) at the end of February 2004 with $\delta^{15}\text{N}$ signature ranging between 13 to 17.4‰. The $\delta^{15}\text{N}$ POM decreased by May 2004 to about 11-12‰ (*Peridinium* consisted 99% of the biomass) very close to the $\delta^{15}\text{N}$ found for pure *Peridinium gatunense* collected and isolated from the lake in previous years (11.2‰). Since $\delta^{15}\text{N}$ POM converged with epilimnetic $\delta^{15}\text{NO}_3^-$ and the latter did not increase with NO_3^- depletion, phytoplankton NO_3^- assimilation appeared to occur without isotopic fractionation. It is well known that the magnitude of the fractionation factor varies as function of growth conditions and species such that little fractionation could be readily obtained (Montoya and McCarthy 1995; Waser et al. 1998; Needoba et al. 2003).

May is also the period of denitrification in the hypolimnion when $\delta^{15}\text{NO}_3^-$ increases in this region of the lake. Denitrification is well known to be strongly fractionating, leaving behind ^{15}N enriched NO_3^- that when assimilated transfers this isotopic signature to OM. However, the $\delta^{15}\text{NO}_3^-$ data for the epilimnion during this period did not exhibit any increase during the denitrification period. It appeared that the thermocline was an effective barrier to the upward transport of hypolimnion NO_3^- that has become ^{15}N enriched due to denitrification. The POM $\delta^{15}\text{N}$ values observed appear to simply reflect epilimnion $\delta^{15}\text{NO}_3^-$ which in turn reflected $\delta^{15}\text{NH}_4^+$ prior to the nitrification period. However as a principal N loss term, denitrification must be indirectly responsible for the overall high $\delta^{15}\text{N}$ values for the lake through influence of its overall isotopic balance.

During summer-autumn stratification, the euphotic zone of Lake Kinneret was depleted of DIN, a condition favorable to N_2 fixation. Since 1994, there has been a shift in phytoplankton population at this time toward cyanobacteria blooms of the filamentous nitrogen fixers, *Aphanizomenon ovalisporum* and *Cylindrospermopsis cuspidis* (Pollinger et al. 1998; Hadas et al. 1999; Zohary 2004). Summer-autumn decreases in $\delta^{15}\text{N}$ POM were observed in both years of the study with lowest $\delta^{15}\text{N}$ POM values of 3.3‰ at 1-3 m depth, at the end of August 2005 (Fig. 4A), indicative of atmospheric N_2 fixation. The relative importance of N_2 fixation has been debated, Berman (2001) pointed to DON as the main N source for the cyanobacteria blooms. Although DON could not be excluded as the source for nitrogen for cyanobacteria at this period, our $\delta^{15}\text{N}$ data for DON ranging from 7.5 to 11.7‰ in the epilimnion, could not explain the low $\delta^{15}\text{N}$ POM values during the cyanobacteria blooms. Despite increasing hypolimnion $[\text{NH}_4^+]$ during this period,

$\delta^{15}\text{NH}_4^+$ continued to be $>10\text{‰}$ indicating that vertical NH_4^+ transport could not account for decreasing $\delta^{15}\text{N}$ POM. More likely, upward diffusing NH_4^+ was nitrified in the chemocline and the product NO_3^- could be a DIN source for phytoplankton. In this case, this NO_3^- would be expected to have similarly high $\delta^{15}\text{N}$ since here nitrification went to completion. By contrast, *Aphanizomenon ovalisporum* isolated from the first bloom in autumn 1994 had an average $\delta^{15}\text{N}$ value of 0.3‰ , indicating N_2 fixation, as had been found in the ocean (Minagawa and Wada 1986; Montoya et al. 2004). In addition, C: N ratios dropped during the blooms of *Aphanizomenon* and *Cylindrospermopsis* indicating release from N stress due to N_2 fixation. N_2 fixation was thus the mostly likely cause for decreasing $\delta^{15}\text{N}$ POM in later summer and autumn in Lake Kinneret.

During chemocline formation in May 2004 and 2005, lower $\delta^{15}\text{N}$ POM was also observed in this subsurface layer, opposite to the expected effect from POM degradation. In this instance, low $\delta^{15}\text{N}$ POM was the result of intense microbial chemoautotrophic activity (Fig. 4A.). During autumn when the chemocline was sharp and deepened below the euphotic zone, a drop of 6‰ in $\delta^{15}\text{N}$ POM could be observed within the chemocline, probably due to isotopic fractionation during NH_4^+ uptake fueled by chemoautotrophic H_2S oxidation (O. Hadas unpubl. data).

Through most of the time-series, $\delta^{13}\text{C}$ POM was typically between -30 and -25‰ (Fig. 6). Higher values developed in the epilimnion during the bloom period but lagged the maxima in $\delta^{15}\text{N}$ POM by a month. Increased POM $\delta^{13}\text{C}$ (as high as -18‰ but more typically -21‰) was consistent with reduced isotopic fractionation during C fixation when dissolved $[\text{CO}_2]$ decreased due to increased temperature and intense photosynthesis (Berman-Frank et al. 1998). Much lower POM $\delta^{13}\text{C}$ (-32‰) was observed in May within the chemocline and benthic boundary layer (hypolimnion) due to chemosynthetic activity of sulfide oxidizers (Hadas et al. 2000, 2001). Light C isotopic composition is known to occur during assimilation by chemoautotrophic ammonia, sulfide and methane oxidizing bacteria (Cavanaugh et al. 1981; Del Giorgio and France 1996; Kiyashko et al. 2001). The lowest values were observed in Dec 2004 to Jan 2005 (as low as -37‰) coincident with low $\delta^{15}\text{N}$ POM. Low $\delta^{13}\text{C}$ POM was probably caused by intense chemosynthetic C fixation associated with sulfide oxidation and nitrification using low $\delta^{13}\text{C}$ DIC supplied by methane oxidation.

Comparison of $\delta^{15}\text{N}$ values for N inputs and lake N inventories - A remaining question is the cause of the overall high $\delta^{15}\text{N}$ values for Lake Kinneret as compared to N inputs. Denitrification as a strongly fractionation loss term is typically responsible but only if there is a residual of ^{15}N enriched nitrate which can reenter the system's N cycle. The complete exhaustion of hypolimnetic NO_3^- and no obvious effect on $\delta^{15}\text{N}$ POM during the denitrification period suggest that its influence can only be indirectly responsible for the overall high $\delta^{15}\text{N}$ for the lake. In fact, during and subsequent to the denitrification period, the high $\delta^{15}\text{N}$ signature of NH_4^+ accumulating in the hypolimnion (12 to 17‰; Fig. 3A) must reflect ammonification of high $\delta^{15}\text{N}$ OM. This OM could be either recently derived from that year's *Peridinium* bloom or previously stored in sediments. The eventual large size of the NH_4^+ pool in the hypolimnion clearly preconditions the isotopic signals produced in the subsequent destratification in the next year. These $\delta^{15}\text{NH}_4^+$ values were contrasted with dominant combined N source to the lake from storm water NO_3^- runoff during winter via the Jordan River (Cavari 1977; Serruya 1978), with $\delta^{15}\text{N}$ signature ranging from 5 to 7‰ throughout the year. Therefore, a highly fractionating N removal process was required to produce the much higher lake $\delta^{15}\text{N}$. Other significant N losses such as outflows and sediment burial would not produce ^{15}N enrichments in N remaining in the lake. Clearly, some ^{15}N -enriched nitrogen must escape denitrification. This may come about by the relative timing of nitrification and NH_4^+ assimilation phases and the overall partitioning between them. Denitrification has generally been recognized not only as a major process for N elimination but also the source of high $\delta^{15}\text{N}$ signatures in lakes (Seitzinger 1988; Mengis et al. 1997), but in Lake Kinneret and similar systems the mode of coupling with nitrification may also be essential. N and N isotope budgeting will be dealt with in another paper.

The N isotopic composition of Lake Kinneret POM and DIN varied dramatically with season as a function of the annual sequence of dominant N-cycle processes. The winter-time nitrification period was characterized at first by low $\delta^{15}\text{N}$ POM due to the preference for ^{14}N - NH_4^+ by chemoautotrophic NH_4^+ assimilation and phytoplankton assimilation into OM. Nitrification itself was strongly fractionating leaving ^{15}N -enriched NH_4^+ and ^{15}N -depleted NO_3^- in the water column. In late winter early spring, $\delta^{15}\text{N}$ POM rose rapidly indicating that the phytoplankton bloom at this time preferred NH_4^+ as its N source. After NH_4^+ exhaustion, $\delta^{15}\text{N}$ POM decreased to values similar to epilimnion $\delta^{15}\text{NO}_3^-$, the clear DIN source at this time. The similarity in $\delta^{15}\text{N}$ POM and

$\delta^{15}\text{NO}_3^-$ toward the bloom's end as well as no increase in $\delta^{15}\text{NO}_3^-$ with NO_3^- depletion in the epilimnion, demonstrated little isotopic fractionation during NO_3^- assimilation by the dominant bloom-forming species. $\delta^{15}\text{NO}_3^-$ increased in the hypolimnion during its depletion by denitrification but did not appear to directly influence the $\delta^{15}\text{N}$ of POM produced in the hypolimnion. After NO_3^- exhaustion in the epilimnion in early summer, and the presence of filamentous nitrogen fixing cyanobacteria, low $\delta^{15}\text{N}$ POM was measured during the N_2 fixation period. Within the chemocline formed during the stratified period, chemoautotrophic processes strongly influenced $\delta^{15}\text{N}$ POM as well as $\delta^{13}\text{C}$ POM. In both cases, low values were reached due to strong isotopic fractionation by microbes in both C and N acquisition. Measurement of $\delta^{15}\text{N}$ POM and $\delta^{13}\text{C}$ provides a useful tool for better identification of chemocline location and the dominant processes therein.

Overall, time-series N isotopic analysis is an important approach for studying lake N cycle with even larger signal amplitude as compared to prior successful open ocean studies (Altabet 2006). This is particularly so when seasonal stratification and destratification produces large swings in redox state and favored N cycle process as seen in Lake Kinneret. We also anticipate long-term changes in Lake Kinneret $\delta^{15}\text{N}$ due to changes in land use in the watershed of the lake producing changes in allochthonous N sources entering the lake via the Jordan River.

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Table 1: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) of the principal blooming phytoplankton species in Lake Kinneret

Species	Origin	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
<i>Peridinium gatunense</i>	Lake bloom (Apr 1976)	11.2	-19.6
<i>Peridiniopsis</i> sp.	Lake (Aug 2005)	16.5	-13.6
<i>Aphanizomenon ovalisporum</i>	Lake bloom (Oct 1994)	0.3	-21.0
<i>Microcystis</i> sp. brown	Lake bloom (May 1997)	9.2	-23.9
<i>Microcystis</i> sp. green	Lake bloom (Feb 1996)	14.9	-29.9
<i>Microcystis</i> sp. green	Lake bloom (May 1997)	11.3	-28.4
<i>Debarya</i> + 30% dinoflagellates	Lake bloom (May 2005)	10.6	N/A

Table 2: Concentrations ($\mu\text{mol L}^{-1}$) and $\delta^{15}\text{N}$ of dissolved organic nitrogen (DON) in the epilimnion of Lake Kinneret during summer and autumn 2004 and 2005

Date	Depth (m)	N-DON ($\mu\text{mol L}^{-1}$)	$\delta^{15}\text{N}$
23 Jun 04	3	32.5	9.1
14 Jul 04	3	24.0	9.6
11 Aug 04	1	16.7	8.2
12 Sep 04	3	23.6	8.9
25 Oct 04	1	23.4	9.6
29 Jun 05	3	20.3	7.5
31 Jul 05	3	20.1	7.9
23 Aug 05	3	19.3	7.5
06 Oct 05	3	N/A	10.9
09 Nov 05	3	18.3	11.7

Figure Legends:

Fig. 1. Vertical profiles of Lake Kinneret's chemical and physical properties at Sta. A in 2004 (Upper panel) Oxygen mmol L^{-1} (filled circles), Temperature $^{\circ}\text{C}$ (open squares).

(Lower panel) N-NH_4^+ $\mu\text{mol L}^{-1}$ (filled diamonds), N-NO_2^- $\mu\text{mol L}^{-1}$ (open squares), N-NO_3^- $\mu\text{mol L}^{-1}$ (filled triangles).

Fig. 2. Dissolved inorganic nitrogen concentrations at Sta. A during the nitrification period in 2003-2004 as well as 2004 – 2005.

Fig. 3. Hypolimnetic dissolved inorganic nitrogen concentrations and nitrogen isotopic composition at 35-40 m. (A) N-NH_4^+ $\mu\text{mol L}^{-1}$, N-NO_2^- $\mu\text{mol L}^{-1}$, N-NO_3^- $\mu\text{mol L}^{-1}$. (B) $\delta^{15}\text{N-NH}_4$ (filled diamonds), $\delta^{15}\text{N-POM}$ of the sediment fluffy layer (solid arrow), $\delta^{15}\text{N-Jordan}$ River N input (dashed arrow). Shaded area represents time of stratified lake.

Fig. 4: N isotopic time series for Lake Kinneret at Sta. A (A) $\delta^{15}\text{N-POM}$ at 1-3 m depth, 19-21 m depth, 38-40 m depth. (B) $\delta^{15}\text{N-NH}_4$, $\delta^{15}\text{N-DON}$, $\delta^{15}\text{N-NO}_3^-$ in the epilimnion (1-3 m depth). (C) Dissolved inorganic nitrogen concentrations in the epilimnion at 1m depth N-NH_4^+ $\mu\text{mol L}^{-1}$, N-NO_2^- $\mu\text{mol L}^{-1}$, N-NO_3^- $\mu\text{mol L}^{-1}$.

Fig. 5: Expanded view of covariation in the $\delta^{15}\text{N}$ of POM, NH_4^+ , and NO_3^- during the nitrification and subsequent bloom period in Nov 2004 – May 2005. $\delta^{15}\text{N-NH}_4$ (filled diamonds), $\delta^{15}\text{N-NO}_3^-$ (open circles), $\delta^{15}\text{N-POM}$ at 1-3 m depth (open diamonds), 19-21 m (filled squares), 40 m (filled circles).

Fig. 6: (A) $\delta^{13}\text{C-POM}$ at 1-3 m depth, 19-21 m depth, 38-40 m depth and (B) integrated chlorophyll values at Sta. A during the study period.

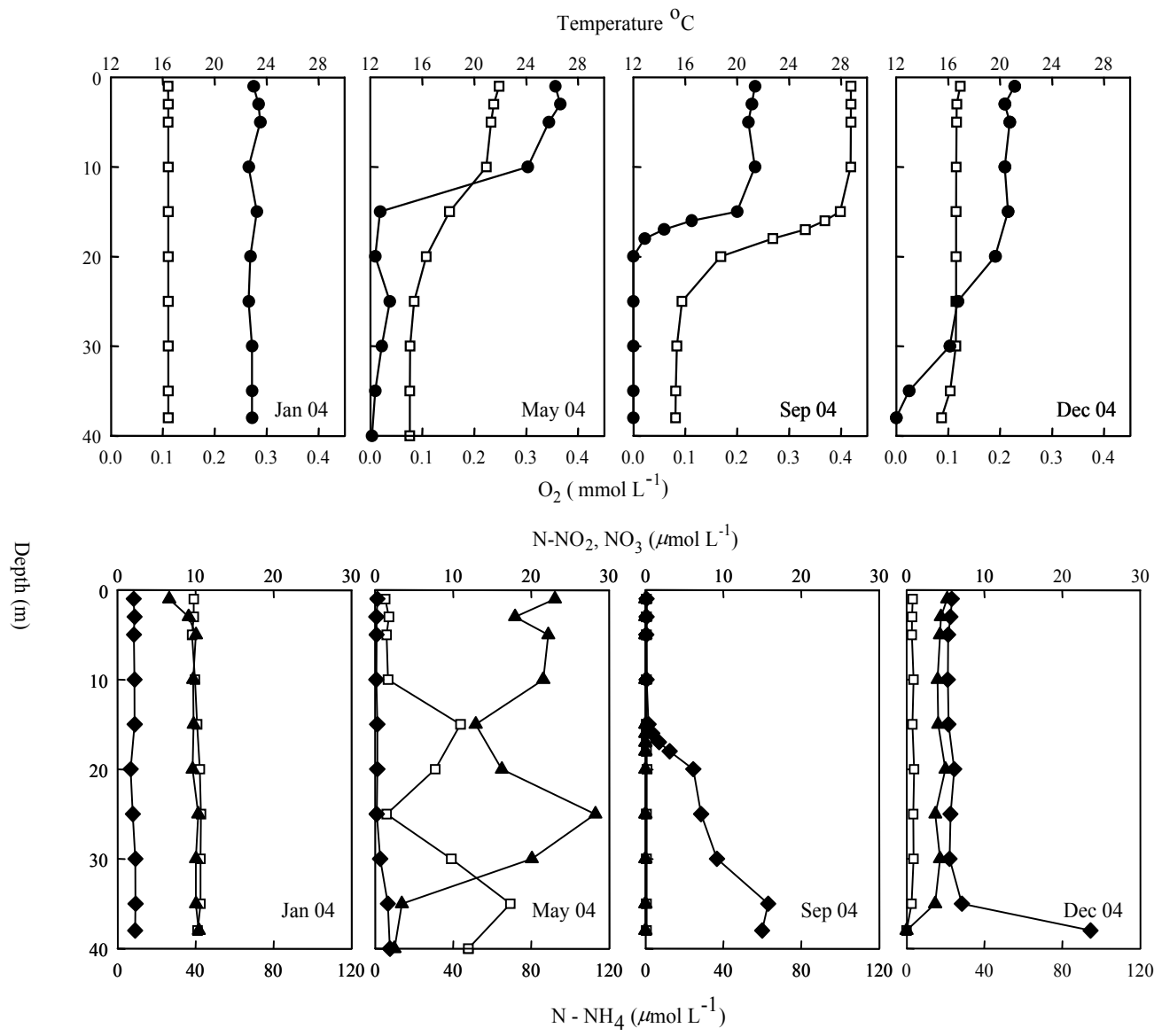


Fig. 1

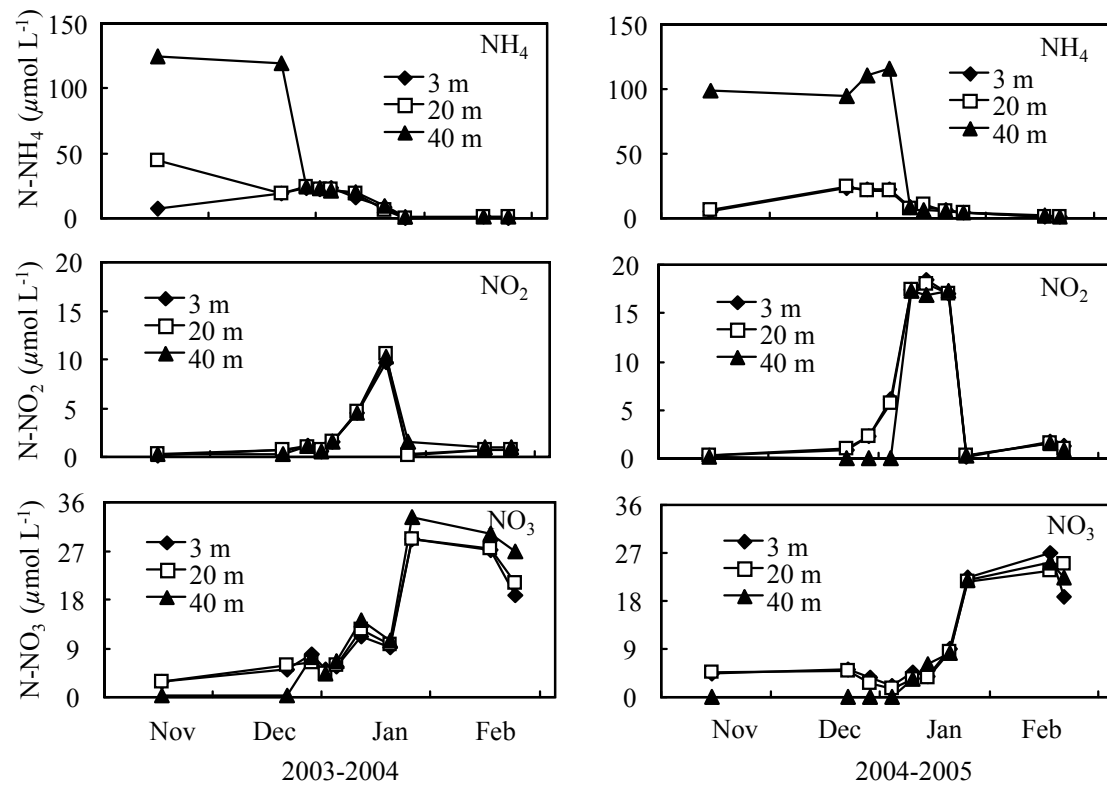


Fig.2

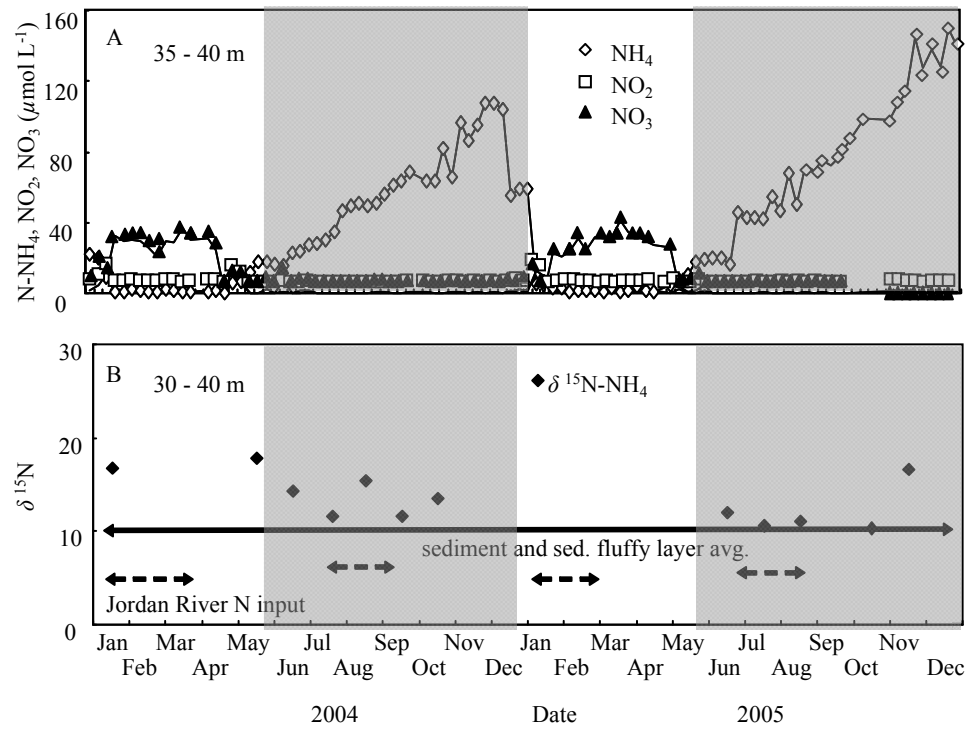


Fig.3

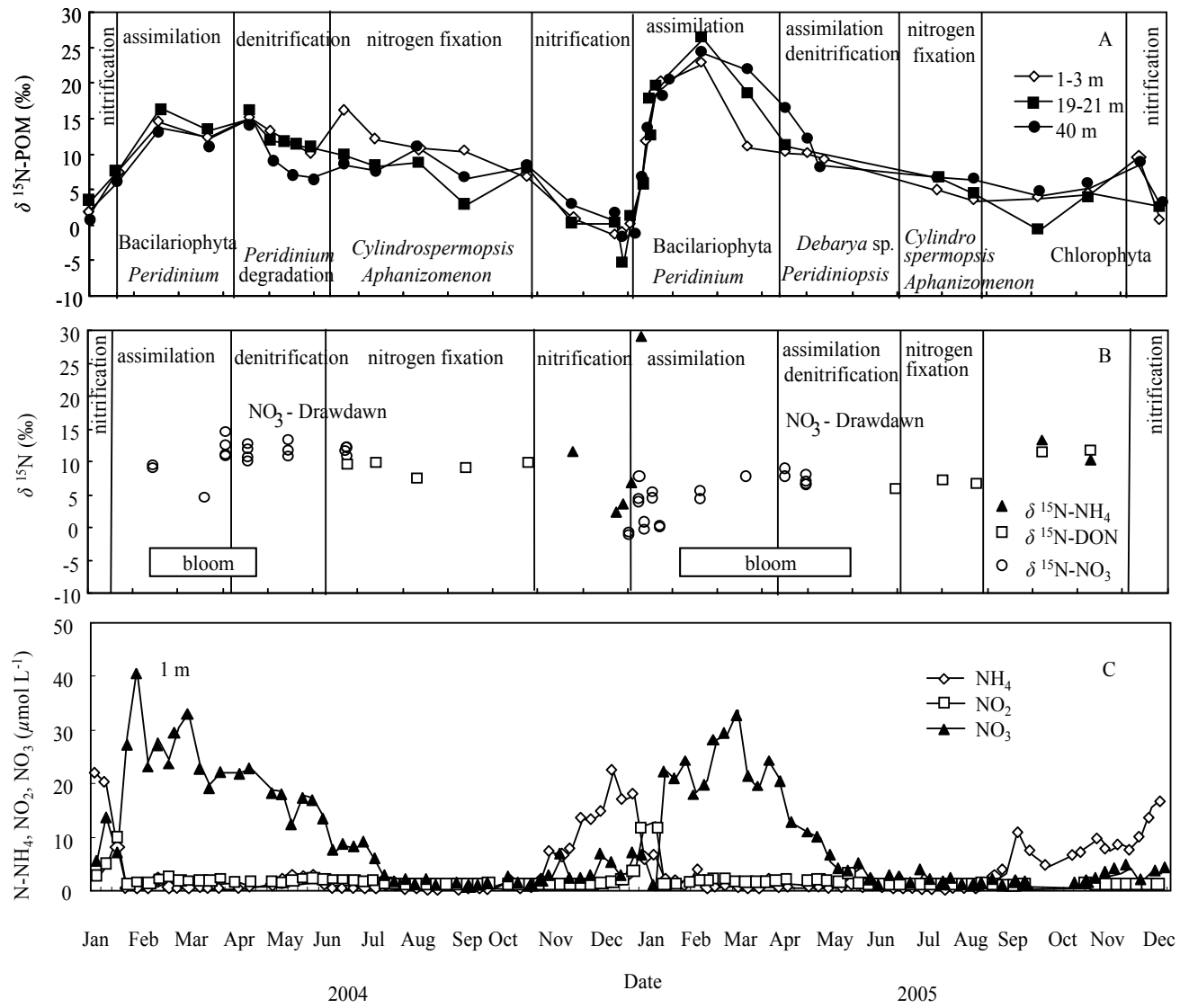


Fig. 4

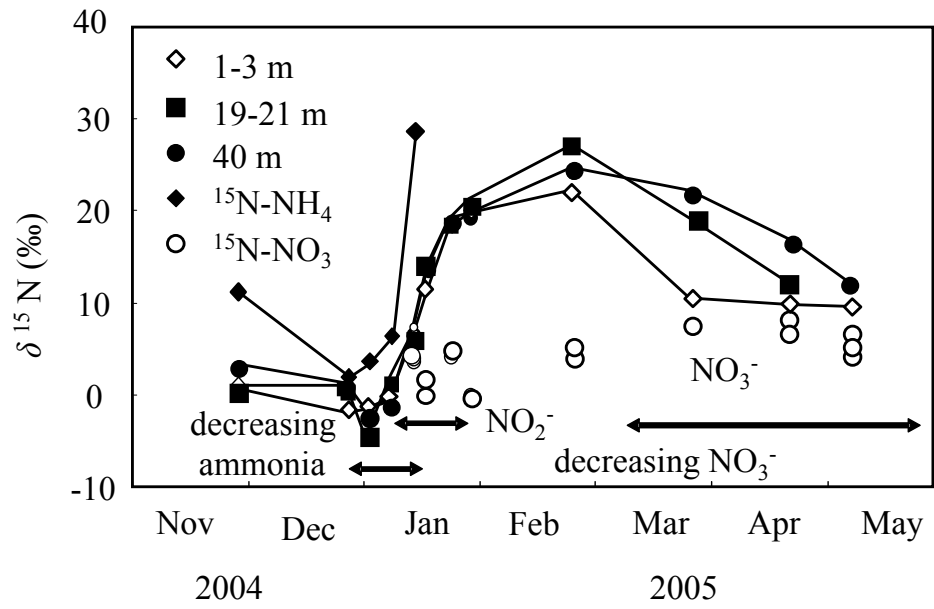


Fig. 5

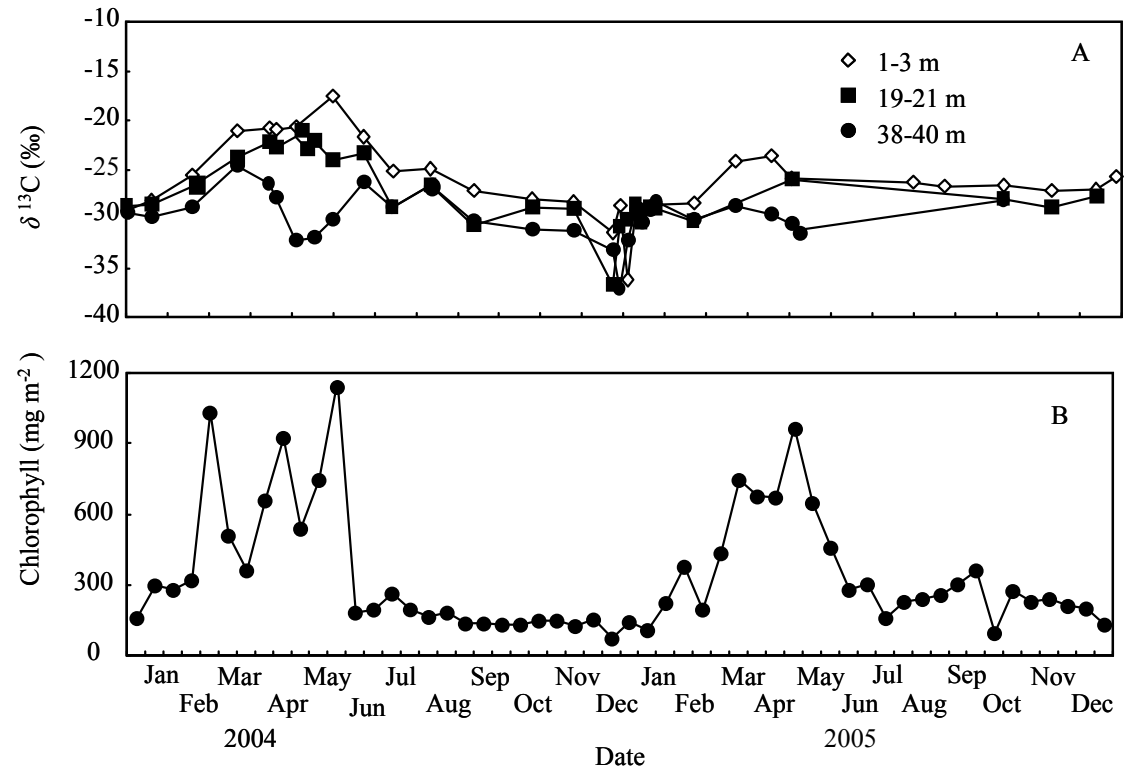


Fig. 6

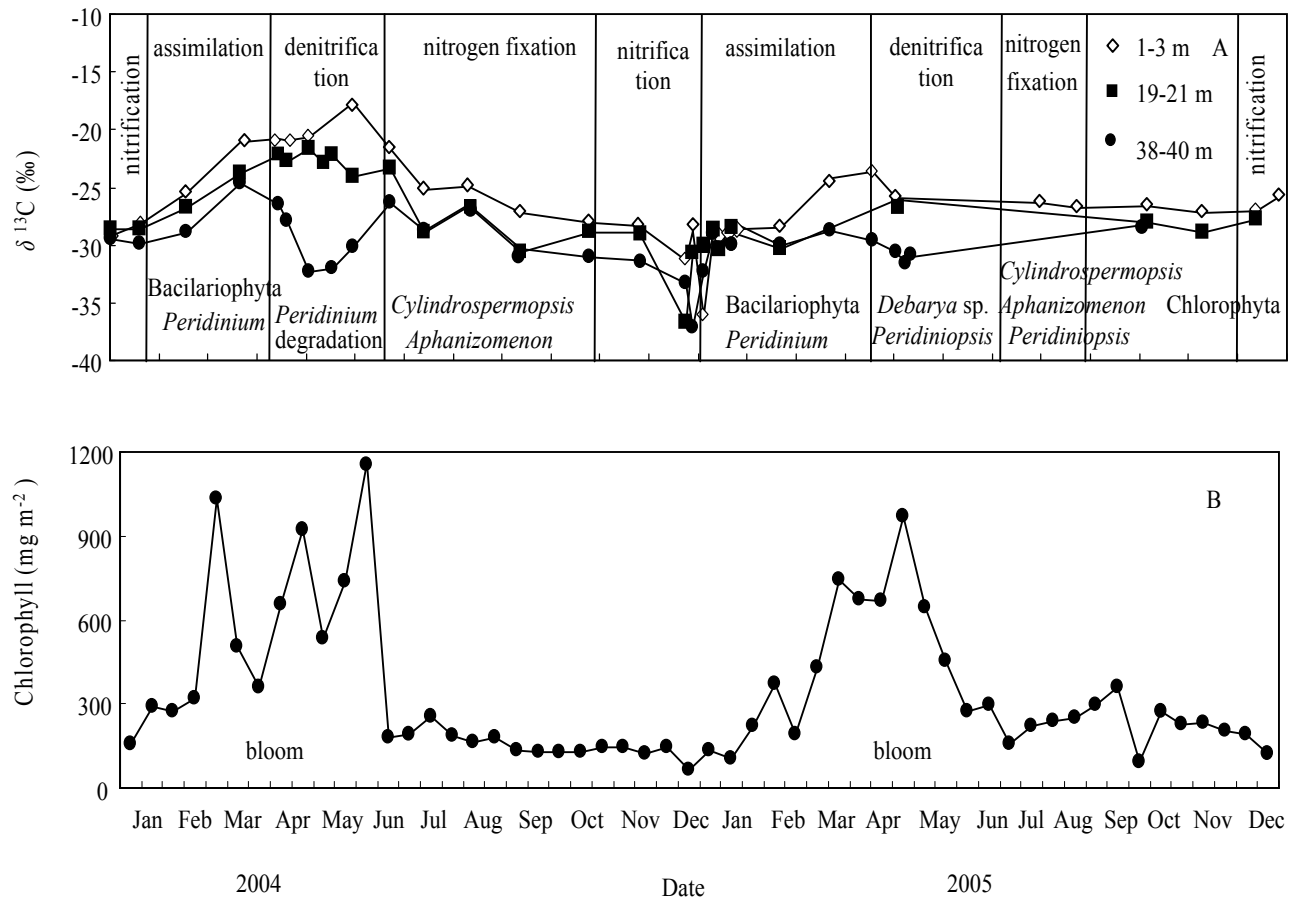


Fig. 6
 Reviewer's suggestion preferred by the associate editor