

Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake

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Abstract

We determined the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of water-column particulate organic matter (POM), dissolved inorganic carbon, and nitrate, together with water chemistry and phytoplankton biomass and species composition every month in eutrophic Lake Lugano. As primary productivity increased during spring, the $\delta^{13}\text{C}$ of photic-zone POM increased from -34‰ to -24‰ . This ^{13}C enrichment reflects decreasing C-isotope fractionation between organic and inorganic carbon pools in response to decreasing surface water $[\text{CO}_2(\text{aq})]$. Variations in the $\delta^{15}\text{N}$ of surface-water POM ($+2\text{‰}$ to $+8\text{‰}$) collected during the productive period were attributed to isotope effects associated with nitrate uptake, nitrogen fixation, and mixing of different organic matter sources. The apparent N-isotope enrichment (ϵ) associated with nitrate assimilation varied with $\epsilon = -1.0\text{‰} \pm 0.9$ for diatoms and $\epsilon = -3.4\text{‰} \pm 0.4$ for green algae. The mechanisms controlling the N-isotopic composition of surface-water nitrate include the combined processes of nitrate assimilation, nitrification, mixing of water masses, and external nitrate loading. There was no consistent relation between the $\delta^{15}\text{N}$ of POM, the $\delta^{15}\text{N}$ of nitrate, and the nitrate concentration in surface waters. Low $\delta^{13}\text{C}$ (-60‰) of POM from the benthic nepheloid layer (BNL) indicated that as much as 80% of the POM in the BNL consisted of methanotrophic bacteria. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of near-bottom POM allowed tracing a “bacteriocline”, which rose with the expanding volume of bottom-water suboxia. Associated with the development of anaerobic conditions and coupled to decreasing $[\text{NO}_3^-]$, the $\delta^{15}\text{N}$ of bottom-water nitrate progressively increased from $+8\text{‰}$ to $+27\text{‰}$, indicating active denitrification in the hypolimnion. This study demonstrates the potential of natural-abundance level measurements of stable carbon and nitrogen isotopes to trace biogeochemical processes in lakes. However, the $\delta^{15}\text{N}$ of POM in eutrophic lakes is of limited use as an indicator of the degree of nitrate utilization and trophic state.

Stable carbon and nitrogen isotope measurements of autochthonous material from aquatic environments have proven to be a powerful tool to better understand biologically driven carbon and nitrogen cycles. Such studies helped to assess the sources and cycling of organic matter (e.g., Cifuentes et al. 1988; Bernasconi et al. 1997; Huon et al. 2002) and to identify microbial processes (e.g., Ostrom et al. 1997; Brandes et al. 1998). Through the carbon and nitrogen stable isotope analysis of sediments, insights may be gained into the trophic evolution of lakes, provided that the processes controlling isotope fractionation during organic matter synthesis and degradation are well understood. For example, the C-isotopic composition of lacustrine organic matter has been used as a proxy indicator for primary productivity, $\text{pCO}_2(\text{aq})$ and CO_2 versus HCO_3^- uptake (Hollander and McKenzie 1991; Ostrom et al. 1997; Hodell and Schelske 1998).

Variations in the isotopic composition of organic and inorganic nitrogen species in aquatic environments can be re-

lated to biologically mediated reactions, including N uptake (e.g., Velinsky et al. 1991; Teranes and Bernasconi 2000), nitrification (Mariotti et al. 1981), denitrification (e.g., Brandes et al. 1998), and organic matter degradation (e.g., Lehmann et al. 2002 and references therein). Numerous marine studies have clearly demonstrated that a relationship exists between the $\delta^{15}\text{N}$ values of autochthonous organic matter and the degree of surface-water nitrate utilization (François et al. 1992; Altabet and François 1994; Sigman et al. 1999). François et al. (1996) and Teranes and Bernasconi (2000) provided evidence that the $\delta^{15}\text{N}$ of lacustrine organic matter record variations in the isotopic composition of surface-water nitrate in response to its utilization by phytoplankton. In general, however, the use of organic matter $\delta^{15}\text{N}$ as indicator of the degree of lacustrine NO_3^- depletion has proven to be less promising (Owen et al. 1999). This is partly due to the fact that phosphorous, rather than nitrogen, commonly limits phytoplankton growth in lakes. Moreover, external nitrogen loading rather than the isotope effect associated with nitrate assimilation may be the predominant factor controlling the isotopic composition of lacustrine nitrogen species (Ostrom et al. 1997; Teranes and Bernasconi 2000).

A profound knowledge of the causes of isotopic variation in the water column of a lake is a prerequisite for the interpretation of isotope data from lacustrine sedimentary archives. In several lake studies, the seasonal and depth-related variability of the isotopic composition of sinking or suspended particulate organic matter (POM) has been discussed

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Acknowledgments

We thank M. Mengis for providing silver nitrate standards for isotopic measurements. Two anonymous reviewers helped to improve the manuscript.

This study was supported by Swiss National Science Foundation grants 21-52332.97 and 20-61626.00.

(Bernasconi et al. 1997; Ostrom et al. 1998; Teranes and Bernasconi 2000). Nevertheless, the factors controlling N-isotope cycling in lakes remain poorly understood. The interpretation of $^{15}\text{N}/^{14}\text{N}$ ratios in lacustrine organic matter is particularly hampered by the paucity of $\delta^{15}\text{N}$ data for ambient inorganic nitrogen species.

This is the first of two companion manuscripts (see Lehmann et al. in press) addressing the biogeochemical cycling of carbon and nitrogen stable isotopes in eutrophic Lake Lugano (Switzerland). We present the first comprehensive data set, covering one seasonal cycle, which combines hydrochemical data, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of suspended particulate organic matter (SPOM), dissolved inorganic carbon, and nitrate, respectively, and data on phytoplankton biomass and species. Our aim was to characterize the most important controls on the anticipated variations in the isotopic composition of POM and nitrate with depth in the water column over an annual cycle and to test the potential of stable carbon and nitrogen isotopes to trace biogeochemical processes and assess the origin of organic matter in eutrophic lakes. In particular, we evaluated the use of organic matter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as recorders of primary productivity and nitrate utilization in a highly productive lake ecosystem. Well-documented marked seasonal changes in the degree of primary production, water column suboxia, and microbial activity (Laboratorio Studi Ambientali 1980–2000; Lazzaretti-Ulmer and Hanselmann 1999) make Lake Lugano an ideal environment in which to study the biogeochemical cycling of C and N isotopes.

Study site—Lake Lugano (Fig. 1) is located on the southern slope of the Alps at the Swiss–Italian border (271 m above sea level). It has a surface area of 48.9 km² and a catchment area of 615 km², a large portion of which is urbanized and cultivated. Lake Lugano can be divided into three sub-basins, the northern and southern basins, and a smaller basin located in front of the outlet, the Tresa River. This study focuses on the southern basin. It has a maximum depth of 95 m, an area of 20.3 km², and a volume of 1.14 km³. The southern basin has a monoholomictic mixing regime. The winter overturn is followed by the development of thermal stratification of the water column and hypolimnetic suboxia between June and January/February. The mean hydraulic residence time of the southern basin is 1.4 yr. Its most important tributaries are the northern basin and the Veduggio River.

After severe eutrophication between 1960 and 1986, measures to improve water quality of Lake Lugano led to the reduction of external phosphorous loads (Barbieri and Mosello 1992; Barbieri and Simona 2001; Lehmann et al. in press). The southern basin, however, remained eutrophic with an average annual primary productivity of $\sim 340 \text{ g m}^{-2} \text{ yr}^{-1} \text{ C}$ (1990–2000), which was measured at the Figino sampling station (Fig. 1). The external nutrient load to the lake originates mainly from sewage/household sources (85%) and, to a lesser extent, from industrial (10%) and agricultural (5%) sources (Barbieri and Simona 2001). P_{tot} and N_{tot} contents in the southern basin measured in February 1999 are $\sim 0.04 \text{ mg L}^{-1} \text{ P}$ and $1.2 \text{ mg L}^{-1} \text{ N}$, respectively. The autochthonous biomass in Lake Lugano is dominated by cya-

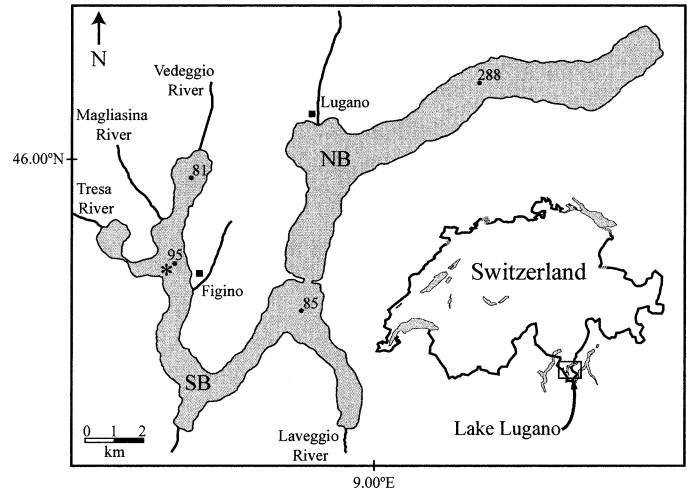


Fig. 1. Map of the Lake Lugano study area. The asterisk indicates the site of water column profiling in the southern basin of the lake (SB), near Figino. Numbers represent water depths.

nobacteria and diatoms, accounting for generally more than 75% of the total phytoplankton; however, the microzooplankton contributes significantly to the overall seston (Barbieri and Simona 2001).

Methods

As part of a long-term lake monitoring and research program, conducted by the Laboratorio Studi Ambientali, Lugano (L.S.A. 1980–2000), monthly water column samples were collected from 11 to 13 different water depths at the Figino sampling locality between February 1999 and February 2000. Temperature, pH, and conductivity were measured with a CTD profiler (Ocean Seven 501, IDRONAUT). Water samples for hydrochemical and isotope analyses were obtained using 5-liter Niskin bottles. In addition, water samples from the most important inputs to the southern basin of Lake Lugano (precipitation, rivers, northern basin) were collected on a regular basis.

Following a routine sampling procedure, samples were always collected and processed at the same hour of the day. In this way we minimized the potentially biasing effect of diurnal variability of photosynthetic activity in surface waters on measured parameters. Suspended particulate matter (SPM) samples were obtained by filtering 2-liter aliquots through precombusted (450°C, 3 h) and preweighed Whatman (GF/F) glass-fiber filters. Filters were dried at 50°C for 12 h and weighed after zooplankton had been removed. To remove particulate carbonate, filters were exposed to fuming HCl (37%) for 12 h in an exicator and subsequently oven-dried (50°C). For measurements of $[\text{NO}_3^-]$, $[\text{NH}_4^+]$, and $[\text{PO}_4^{3-}]$, aliquots of prefiltered water samples were microfiltered through 0.45- μm membrane filters and analyzed colorimetrically with an automatic analyzer (TRAACS 2000, Bran & Luebbe). O_2 concentrations were measured by Winkler titration (American Public Health Association 1989). Alkalinity was determined by potentiometric titration (Titr-

processor 636, Metrohm). The sum of dissolved CO_2 and H_2CO_3 concentrations, denoted as $[\text{CO}_2(\text{aq})]$, was calculated from temperature, pH, and alkalinity. For the determination of CH_4 concentrations, filtrate aliquots were fixed with concentrated NaOH and analyzed by gas chromatography with a flame ionization detector (Perkin-Elmer, model 3920). Primary productivity was measured by ^{14}C assimilation using the Steemann-Nielsen (1952) method, modified by Gächter and Marès (1979). Daily primary productivity was calculated according to Gächter (1972) by interpolation from short-interval, depth-integrating (0–20 m) measurements, taking into account diurnal solar radiation variability. Algal biomass was sampled and quantified as described in Polli and Simona (1992).

For dissolved inorganic carbon (DIC) isotope analyses the pH of a 50-ml aliquot was raised above 10 with NaOH and, subsequently, DIC was precipitated as BaCO_3 with BaCl_2 . Nitrate for $\delta^{15}\text{N}$ analysis was concentrated from water samples by anion exchange according to Silva et al. (2000). Two liters of sample solution were passed through prefiltered anion exchange resin columns (Biorad AG1-X8 in chloride form) and sample nitrate was desorbed with 1 M HCl. The eluate was then neutralized with Ag_2O , filtered to remove the AgCl precipitate, and freeze-dried to obtain AgNO_3 for N-isotope ratio determination.

For elemental and isotope analysis, up to 10 mg of bulk particulate material was scraped off the glass fiber filters and loaded into tin capsules. AgNO_3 extract was placed in silver capsules. The atomic C/N ratio and the nitrogen and carbon isotope composition of POM was determined simultaneously using a Carlo-Erba elemental analyzer (EA) (NCS 2500) with autosampler coupled to a Fisons Optima ion ratio mass spectrometer (IRMS). Sample material was combusted in the presence of O_2 in an oxidation column at 1030°C , combustion gases were passed through a reduction column (650°C), and produced N_2 and CO_2 gases were purified and transferred to the IRMS via an open split for on-line isotope measurements. The same EA-IRMS system was used to analyze the N-isotope composition of nitrate. The carbon isotope ratio of DIC was determined on CO_2 generated by the dissolution of BaCO_3 precipitate in a common acid bath of orthophosphoric acid (90°C) and subsequent on-line measurement with a VG (PRISM) mass spectrometer. Stable nitrogen and carbon isotope ratios are reported in the conventional δ -notation with respect to atmospheric N_2 (AIR) and V-PDB (Vienna Pee Dee Belemnite) carbonate standard, respectively. The methods were calibrated with IAEA-N1, IAEA-N2 standards for $\delta^{15}\text{N}$ values, and NBS19 and NBS22 for $\delta^{13}\text{C}$ values. For nitrate isotope measurements, IAEA-N3 and a AgNO_3 working standard (EIL62, $\delta^{15}\text{N} = 18.5\text{‰}$) was used for calibration. Analytical reproducibility is $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in organic matter, $\pm 0.05\text{‰}$ for $\delta^{13}\text{C}$ in carbonate, and $\pm 0.4\text{‰}$ for $\delta^{15}\text{N}$ in nitrate extract.

Results

Primary productivity and water-column hydrochemistry—The seasonal changes of the thermal structure and its strong influence on water column mixing and oxygenation condi-

tions, as well as the seasonal variations in primary productivity, were clearly observed in the evolution of temperature, water density, $[\text{CO}_2(\text{aq})]$, $[\text{O}_2]$, pH, and SPM concentration profiles in the southern basin of Lake Lugano (Fig. 2). Holomixis in winter was followed by the development of thermal stratification between March and August. Elevated surface-water temperatures, together with increasing light intensity and high nutrient availability, promoted phytoplankton blooms during spring and summer. Maximum primary productivity was measured in July with mean daily rates of $1.96 \text{ g m}^{-2} \text{ C}$ (Fig. 3D). Unfortunately, no primary productivity data are available for March and April 1999. However, given the high average primary productivity values (1.5 and $2.0 \text{ g m}^{-2} \text{ d}^{-1} \text{ C}$, respectively) determined for March and April of the previous 10 yr (Lehmann et al. in press), we can assume that rates of primary production reached similar peak values in early spring 1999 (Fig. 3). Rapidly increasing particulate matter concentrations and algal biomass observed in the photic zone are additional evidence for the occurrence of plankton blooms and for high productivity during this period (Figs. 2E and 4). The spring bloom was followed by an interval of reduced primary production, which is also indicated by considerably lower epilimnetic SPM concentrations in May and June. From September onward, primary productivity continuously decreased (Fig. 3D). Elevated photosynthetic activity and inorganic carbon utilization, in spring and summer, resulted in high oxygen concentrations and increased pH values in the epilimnion (Fig. 2C,D). Augmented organic matter fluxes and microbial respiration led to the formation of an oxygen minimum at approximately 15 m depth (Fig. 2D). Moreover, organic matter decomposition resulted in anaerobic conditions in the near-bottom waters from June onward, with the redoxcline steadily rising from the sediment–water interface into the hypolimnion by more than 10 m (Fig. 2D). Simultaneously, a continuously expanding zone of elevated suspended particle concentrations developed above the lake floor, indicating the presence of a benthic nepheloid layer (BNL) (Fig. 2E).

In response to the increased activity in the photosynthesis/respiration cycle, nutrient gradients developed in the water column (Fig. 2). Primary production in the photic zone led to continuous reduction of surface water $[\text{CO}_2(\text{aq})]$, $[\text{NO}_3^-]$, and $[\text{PO}_4^{3-}]$ (Fig. 3). PO_4^{3-} concentrations reached undetectable levels between May and August, whereas NO_3^- concentrations were always higher than $0.4 \text{ mg L}^{-1} \text{ N}$ and, thus, never became biolimiting. Surface-water $[\text{CO}_2(\text{aq})]$ dropped below 0.2 mg L^{-1} ($4.5 \text{ } \mu\text{mol L}^{-1}$) in June and continued to decrease until August (Figs. 2F, 3A). Epilimnetic $[\text{NH}_4^+]$ was mostly lower than $0.03 \text{ mg L}^{-1} \text{ N}$. The increase in both nitrate and phosphate concentrations in the epilimnion between August and February indicates that the replenishment of nutrients from external sources exceeded the consumption by primary producers. CO_2 , oxygen, and nutrient concentrations in the intermediate waters (30 to 70 m) exhibited trends typically resulting from aerobic remineralization of organic particles sinking through the water column, i.e., continuous O_2 consumption and CO_2 /nutrient accumulation (Fig. 2). Intense microbial mineralization of organic matter in anoxic sediments and stagnant near-bottom waters led to the accumu-

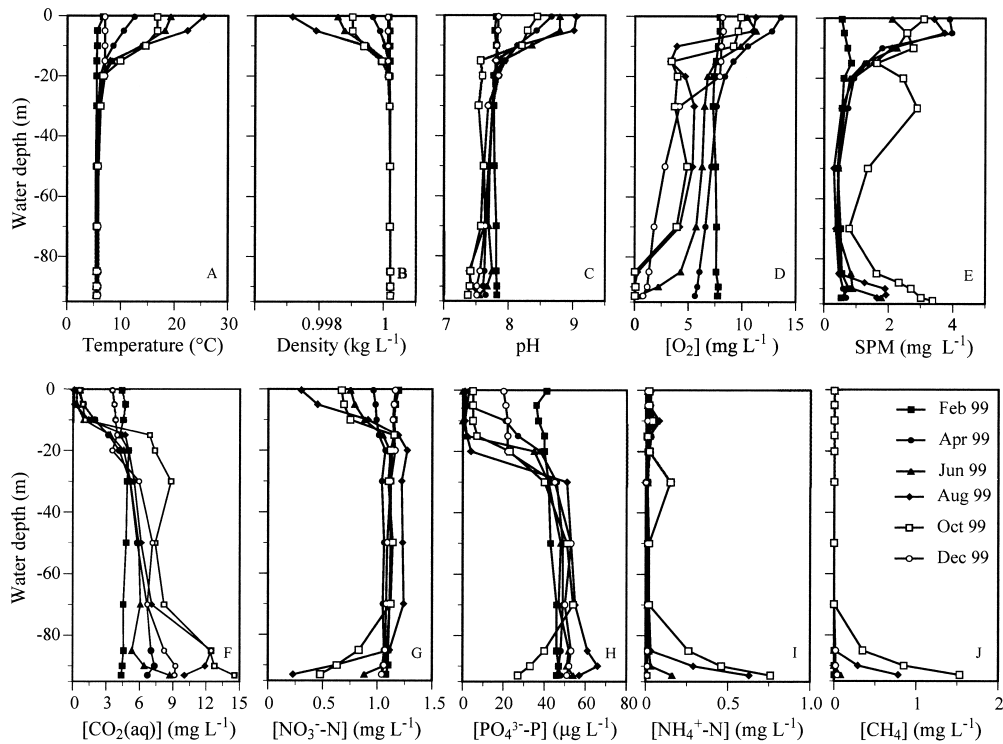


Fig. 2. Depth distribution of hydrochemical (pH, $[O_2]$, $[CO_2(aq)]$, $[NO_3^-]$, $[PO_4^{3-}-P]$, $[NH_4^+]$, $[CH_4]$) and physical parameters (water temperature and density), and concentration of suspended particulate matter at the Figino sampling locality (only bimonthly data are shown).

lation of CO_2 and ammonium in the deep hypolimnion (Fig. 2). With prolonged anaerobic conditions, increasing amounts of methane accumulated in the deepest waters (Fig. 2J), indicating methanogenesis in the sediments and the near-sediment water column. Maximum CH_4 concentrations measured 2 m above the sediment surface were $>1.5 \text{ mg L}^{-1}$ ($\sim 0.1 \text{ mmol L}^{-1}$); however, methane concentrations right at the sediment–water interface can be expected to be one order of magnitude higher (Lazzaretti-Ulmer and Hanselmann 1999). Nitrate concentrations, on the other hand, decreased from ~ 1.1 to $0.2 \text{ mg L}^{-1} \text{ N}$ in the deepest sample (93 m). $[NO_3^-]$ gradients developed with ongoing bottom-water suboxia, suggesting the presence of denitrifying bacteria below the redoxcline (Fig. 2G). With the water-column overturn in February, bottom waters were re-oxygenated and all near-bottom and surface-water gradients collapsed.

Carbon isotope biogeochemistry— The C-isotopic composition of particulate organic carbon (POC) showed a distinct seasonal pattern with low winter and high summer values (Fig. 5A–C). Marked photic zone $\delta^{13}C_{POC}$ gradients developed in July and August. The continuous $\delta^{13}C_{POC}$ increase from $\sim -38\text{‰}$ to -24.0‰ observed in surface waters (0.4–10 m) was associated with increasing primary productivity and phytoplankton biomass. Highest $\delta^{13}C_{POC}$ values in July and August coincided with the lowest CO_2 concentrations (Fig. 3 and Fig 5B). From late summer to fall, a decrease in POC $\delta^{13}C$ by more than 11‰ was associated with decreasing primary productivity. At the same time, surface

water $[CO_2(aq)]$ increased (Fig. 3), indicating the mixing of intermediate waters with elevated $pCO_2(aq)$ into surface waters (Hollander and McKenzie 1991). Variations in the isotopic composition of DIC are closely coupled to the biologic cycle in Lake Lugano. Associated with spring and summer productivity blooms and concomitant with reduced CO_2 concentrations, a $\delta^{13}C_{DIC}$ gradient developed in the epilimnion (Fig. 5D–F). The shift in surface water $\delta^{13}C_{DIC}$ from -10.7‰ to -4.8‰ coincides with the increase in $\delta^{13}C_{POC}$. From November on, the $\delta^{13}C$ of photic zone DIC declined again, reaching values below -10‰ in December and January.

With ongoing high organic matter fluxes during spring and summer (Fig. 2E), the hypolimnetic DIC pool became progressively depleted in ^{13}C (Fig. D–F) because of the release of isotopically light inorganic carbon during the decomposition of organic matter. This well-known feature (e.g., McKenzie 1985) was best observed in near-bottom waters, where the negative shift in the isotopic composition of DIC (-2.3‰) was most pronounced.

Extremely low POC- $\delta^{13}C$ values ($< -60\text{‰}$) were found in the BNL that developed once density stratification ensued (Fig. 5). The rise of the $\delta^{13}C$ minimum from the sediment–water interface into the deep hypolimnion appears to be closely linked to the expansion of the suboxic zone. The marked depletions in ^{13}C relative to photic zone seston and sedimentary organic matter, as well as the concomitant negative $\delta^{15}N_{PON}$ values (Fig. 6), were indicative of a unique origin for the near-bottom SPM. A unique source was further

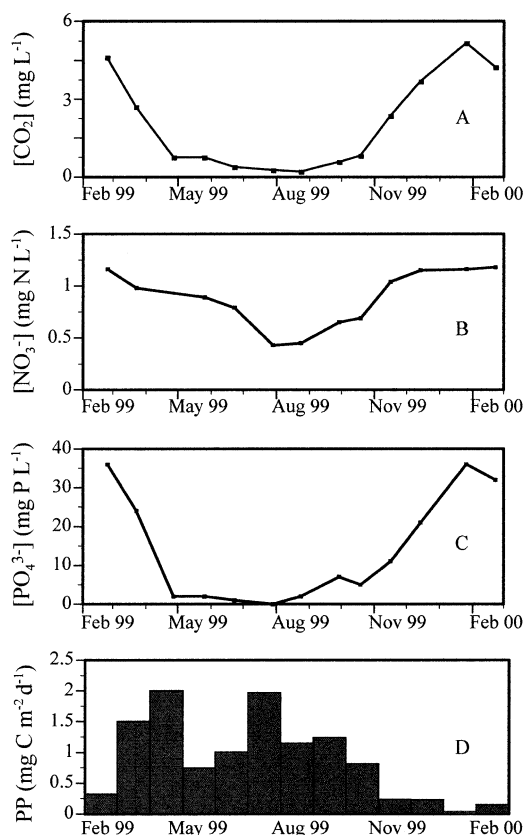


Fig. 3. Annual variations in (A) $[\text{CO}_2(\text{aq})]$, (B) $[\text{NO}_3^-]$, (C) $[\text{PO}_4^{3-}]$, and (D) primary productivity (PP) determined in the epilimnion of Lake Lugano. PP data represent average daily rates. Because of technical problems, no data are available for March and April 1999. March and April values presented here are average values for the period 1990–2000.

indicated by comparatively low C/N ratios (5 to 6.5; data not shown).

Nitrogen isotope biogeochemistry— The $\delta^{15}\text{N}$ of particulate organic nitrogen (PON) exhibited large variations with time and depth (Fig. 6A–C). Before the initial spring phytoplankton blooms and after winter overturn, when POM concentrations are generally low, $\delta^{15}\text{N}$ values in excess of +15‰ (>+20‰ in February 1999) were observed throughout the water column. These high N-isotope values measured in winter must represent refractory or allochthonous organic particles being uniformly distributed in the water column. They did not greatly contribute to the weight-averaged annual isotope signal ($\sim +7.5$). Comparatively heavy winter nitrogen isotope compositions have also been observed in other lakes (Hodell and Schelske 1998; Teranes and Bernasconi 2000) and attributed to either detrital or heterotrophic sources (Teranes and Bernasconi 2000). High $\delta^{15}\text{N}$ values for POM in the mid-water column persisted throughout the year (Fig. 6A–C). Yet, they became less positive during spring and summer, indicating an increased contribution of autochthonous organic matter with lower $\delta^{15}\text{N}$. With increased primary productivity in spring, epilimnetic $\delta^{15}\text{N}_{\text{PON}}$

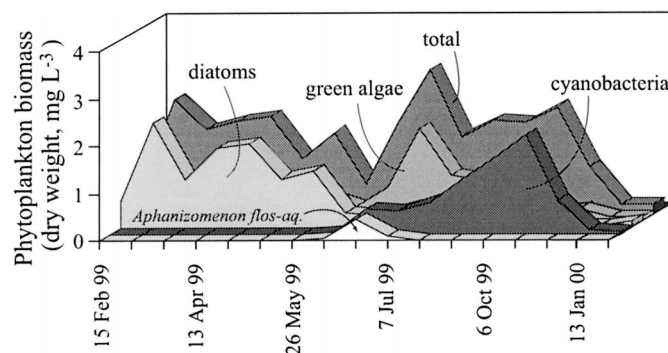


Fig. 4. Phytoplankton biomass in surface waters of the southern basin of Lake Lugano (Figino locality). Data represent mean values for the upper 20 m of the water column. Note the bloom of potentially N_2 -fixing cyanobacteria (*Aphanizomenon flos-aquae*) in June 1999.

values dropped by more than 10‰ to values ranging between +2 and +8‰, with the lowest $\delta^{15}\text{N}$ observed in June (Figs. 6B and 7B).

In the BNL, the strong increase of particle concentration corresponded with a dramatic decrease in the $\delta^{15}\text{N}$ of PON, which reached the minimum values $< -5\%$ in October (Fig. 6B,C). The zone of low- ^{15}N POM expanded into the hypolimnion, suggesting the coupling of processes involved in organic matter synthesis and the rise of the redoxcline between June and October.

After winter holomixis, the water column was characterized by a relatively constant $\delta^{15}\text{N}_{\text{NO}_3^-}$ of $+7.5 \pm 0.6\%$, which prevailed in the mid-waters throughout the year (Fig. 6D–F), whereas between May and December, the N-isotopic composition of nitrate in the photic zone and in near-bottom waters exhibited substantial variations. The most salient feature was a pronounced increase in nitrate $\delta^{15}\text{N}$ above the sediment from +8‰ to +27‰ between June and August, coupled to decreasing $[\text{NO}_3^-]$. A broad subsurface minimum in $\delta^{15}\text{N}_{\text{NO}_3^-}$ was prevalent at 10 to 15 m water depth from July until August. This minimum is associated with the subsurface oxygen minimum (Fig. 2D) and steadily increasing nitrate concentrations, indicating the release of isotopically light nitrate from organic matter. Nitrate $\delta^{15}\text{N}$ values for surface water samples (0–5 m) ranged between +4.5‰ and +8.5‰ (Fig. 7B). During the productive season between March and August, in general, nitrate was more enriched in ^{15}N than the PON. The difference between the N-isotope composition of nitrate and PON, expressed as $\Delta\delta^{15}\text{N}$, was highly variable with time (Fig. 7B). Initial pulses of high productivity during March and April barely affected the $\delta^{15}\text{N}$ of surface-water nitrate. From May until July, surface-water $\delta^{15}\text{N}_{\text{NO}_3^-}$ increased slightly, then continuously decreased to values $< +5\%$, before returning to the initial $\delta^{15}\text{N}$ value ($\sim +7.5\%$) of the well-mixed water column.

Discussion

Carbon assimilation and $\delta^{13}\text{C}$ dynamics in the epilimnion—Fluctuation in the carbon isotope composition of POM

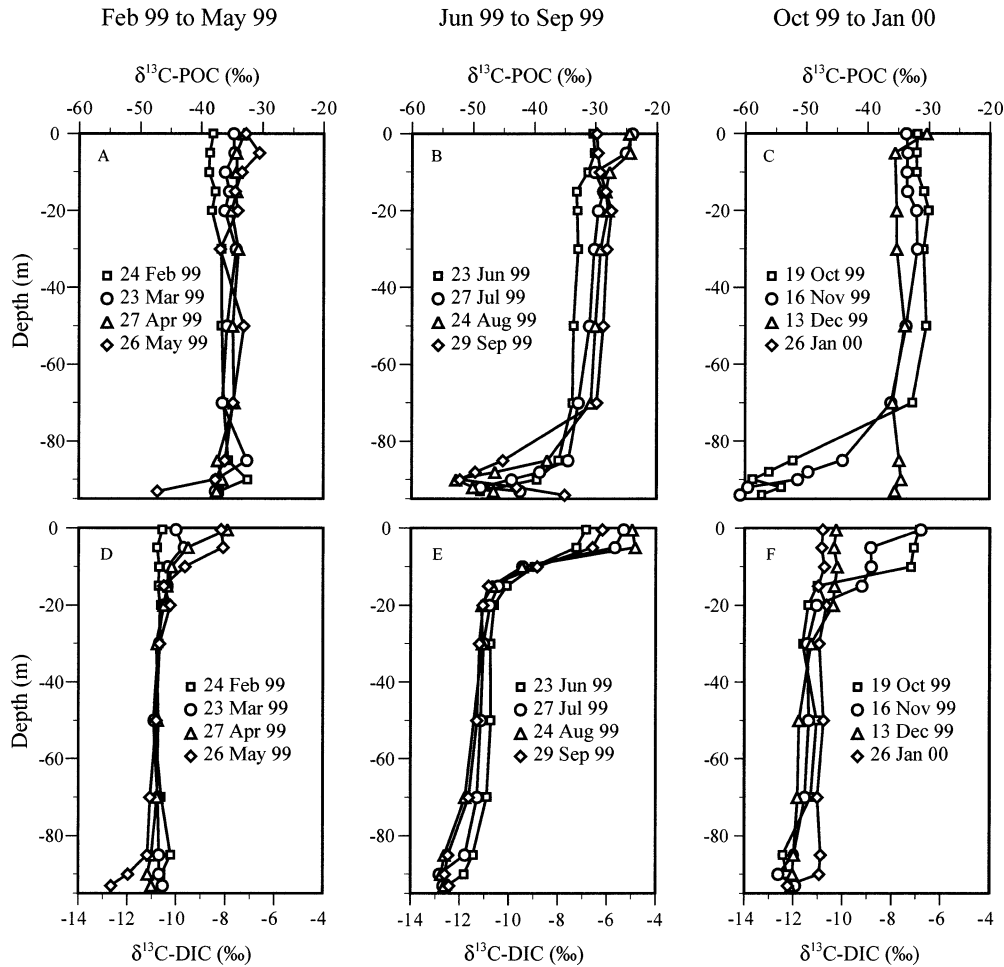


Fig. 5. Annual variation of the $\delta^{13}\text{C}$ (‰) of (A–C) suspended POC and (D–F) DIC with water depth and time.

has been attributed to a variety of controlling factors including primary productivity, aqueous CO_2 concentrations, organic matter sources, and phytoplankton species distribution (e.g., Cifuentes et al. 1988; Rau et al. 1989; Hollander and McKenzie 1991; Bernasconi et al. 1997; Ostrom et al. 1997). The seasonal variations in the carbon isotope composition of epilimnetic POC and DIC in Lake Lugano (Fig. 7A) are consistent with carbon isotope dynamics reported by Cifuentes et al. (1988), Hollander and McKenzie (1991), Bernasconi et al. (1997), and Hodell and Schelske (1998), who all related the annual changes in the $^{13}\text{C}/^{12}\text{C}$ ratios of surface-water POC to the C-isotope fractionation during in situ biosynthesis. Indeed, the close coupling of the C-isotope dynamics with the annual cycle of primary productivity, controlling CO_2 draw-down in surface waters (Fig. 4A,D, Fig. 7A), suggests that particulate material collected from Lake Lugano surface waters mainly originated from autochthonous primary production. Only during winter, when in situ production rates were very low, significant mixing of terrestrial and lacustrine organic matter may have occurred. During photosynthesis, organisms preferentially incorporate ^{12}C into organic matter (O'Leary 1981; Fogel and Cifuentes

1993), leaving the DIC pool progressively enriched in ^{13}C . This is clearly indicated by $\delta^{13}\text{C}$ values of the DIC (Fig. 5D–F, Fig. 7A). The $\delta^{13}\text{C}$ values for both phytoplankton and DIC in surface waters increased with ongoing CO_2 consumption during spring and summer productivity. However, a positive shift of +14‰ in $\delta^{13}\text{C}_{\text{POC}}$ corresponds to a +6‰ shift in the $\delta^{13}\text{C}$ values of DIC. The apparent C-isotope partitioning ($\Delta\delta^{13}\text{C}_{\text{DIC-POC}} \approx \epsilon_{\text{app}} = [(\delta^{13}\text{C}_{\text{DIC}} + 1000)/(\delta^{13}\text{C}_{\text{POC}} + 1000) - 1] \times 10^3$; Farquhar et al. 1989) between phytoplankton and the inorganic surface-water carbon pool ($\geq 95\%$ of it is in the form of HCO_3^-) decreased during spring and summer from -28‰ to -19‰ (Fig. 7A). Thereafter, it increased again to -27.5‰ . Temperature effects (Mook et al. 1974) can account for only a 2.6‰ change; therefore, additional processes, such as changes in the carbon assimilation rate in association with fluctuations in the availability of dissolved CO_2 , must be active. In waters where CO_2 concentrations are low because the rate of CO_2 fixation surpasses the rate at which CO_2 can be resupplied, CO_2 diffusion across the cell membrane of algae is often the limiting factor (O'Leary 1981). Photosynthesizing organisms adapted to CO_2 limitation ($[\text{CO}_2(\text{aq})] \leq 0.44 \text{ mg L}^{-1}$ or $10 \mu\text{mol L}^{-1}$) can actively

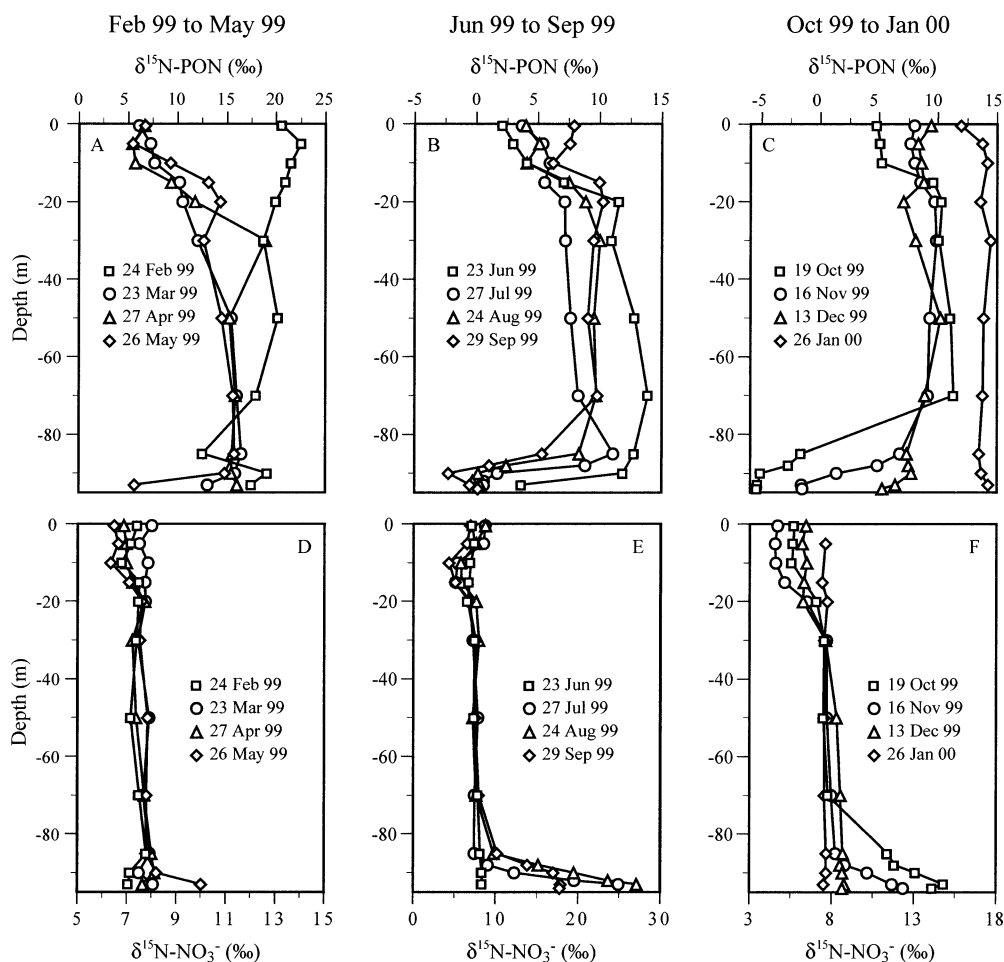


Fig. 6. Annual variation of the $\delta^{15}\text{N}$ (‰) of (A–C) suspended PON and (D–F) nitrate with water depth and time. Note the different scales for $\delta^{15}\text{N}$ values. May–October nitrate $\delta^{15}\text{N}$ data from 50-m water depth and below are from Lehmann et al. (2003).

concentrate DIC in the cell (Fogel and Cifuentes 1993; Huerstas et al. 2000). In an efficient carbon concentrating mechanism (CCM), transported DIC is fixed through carboxylation reactions before it can leak out of the cell. Thus, even though the isotope fractionation due to enzymatic CO_2 fixation is large ($\sim -27\text{‰}$), the overall isotope effect can be quite small ($\sim -5\text{‰}$) (Fogel and Cifuentes 1993). In addition, as the $[\text{CO}_2(\text{aq})]$ progressively decreases and the pH increases during high primary production, HCO_3^- assimilation may be promoted. Laboratory studies have shown that bicarbonate can be an equivalent carbon source, especially under CO_2 -limiting conditions, either through conversion to CO_2 via carbonic anhydrase or direct transport of HCO_3^- across the cell membrane (Korb et al. 1997; Burkhardt et al. 2001). When HCO_3^- is the carbon source, the apparent carbon isotope effect between POC and the total DIC pool is lower, because HCO_3^- is 8‰ to 11‰ heavier than dissolved CO_2 (Mook et al. 1974). Accordingly, the $\delta^{13}\text{C}$ of phytoplankton would increase with the progressive incorporation of ^{13}C -enriched bicarbonate carbon. Data collected during the productive season in the southern basin of Lake Lugano indicate that a relationship exists between aqueous $[\text{CO}_2]$,

the $\delta^{13}\text{C}$ of organic matter, and the carbon isotope fractionation (ϵ_{app}) between the organic and inorganic carbon pools (Fig. 8). A distinct minimum in the C-isotope fractionation between POC and DIC was clearly associated with the lowest $[\text{CO}_2(\text{aq})]$ of 0.11 mg L^{-1} ($2.5 \mu\text{mol L}^{-1}$). Our data, however, do not permit the differentiation between the two possible mechanisms (an efficient CCM or HCO_3^- utilization) causing diminished carbon isotope fractionation at low $[\text{CO}_2(\text{aq})]$.

A marked shift in phytoplankton species composition occurred in June 1999 (Fig. 4). Diatoms were dominating during spring blooms (60–90% of total phytoplankton biomass), whereas green algae and cyanobacteria contributed mostly to summer and fall blooms (70–90%), respectively. A similar sequence of phytoplankton species has been reported from the Swiss Lake Greifen (Hollander and McKenzie 1991). According to Hollander and McKenzie (1991), the variation of algal assemblages may have affected the carbon isotope composition of bulk organic matter inasmuch as different phytoplankton species cope differently with low $\text{CO}_2(\text{aq})$ concentrations. More specifically, during summer, green algae may have flourished when CO_2 became a lim-

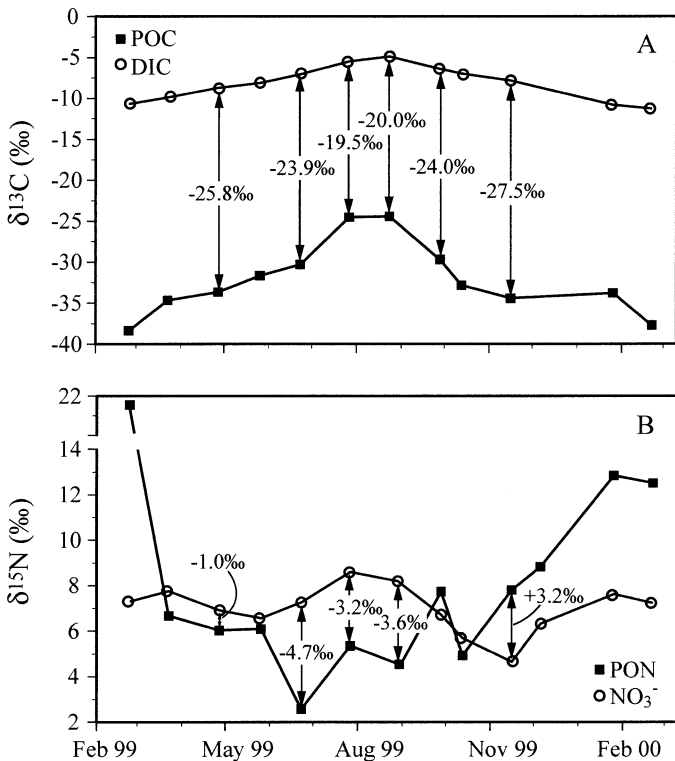


Fig. 7. Time series of (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$ values for surface-water POC, DIC, PON, and nitrate collected between 0 m and 5 m water depth. Arrows indicate the difference in the isotopic composition between particulate and dissolved species, $\Delta\delta^{13}\text{C}_{\text{POC-DIC}}$ and $\Delta\delta^{15}\text{N}_{\text{PON-NO}_3^-}$, respectively. Note the break in the y-axis of panel B.

iting carbon source in the epilimnion and, alternatively, may have utilized bicarbonate that was enriched in ^{13}C by at least 8‰ with respect to dissolved CO_2 . Furthermore, data by Burkhardt et al. (1999) provide evidence that differences in cell size or general taxonomic differences can affect the carbon isotope fractionation. In fact, the most pronounced increase in $\delta^{13}\text{C}_{\text{POC}}$ at the end of June, corresponding to the most pronounced decrease in the apparent C-isotope fractionation, directly coincided with the main shift in the phytoplankton population.

Independent of the mechanisms involved in carbon fixation during photosynthesis, Lake Lugano data provide additional evidence that the $\delta^{13}\text{C}$ values of autochthonous organic matter and estimated ϵ_{app} reflect CO_2 concentrations (Fig. 8). A logarithmic form was favored to describe the relationship between $[\text{CO}_2(\text{aq})]$ and ϵ_{app} (Fig. 8A). In contrast to earlier sediment trap and water column studies in Lake Lugano (Bernasconi et al. 1997) and Lake Greifen (Hollander and McKenzie 1991), in which similar regressions were made using ϵ_{app} values based upon calculated $\delta^{13}\text{C}_{\text{CO}_2}$, we now use ϵ_{app} values calculated from $\delta^{13}\text{C}$ values of the total DIC, thus making allowance for the uncertainty of the carbon species utilized by the autotrophs. Either way, it is not possible to satisfyingly account for the (comparatively small) effects of temperature-dependent isotope equilibrium between CO_2 and HCO_3^- if a change in the inorganic carbon

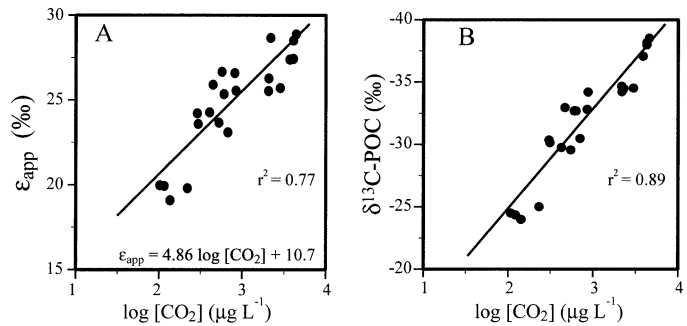


Fig. 8. Correlation between surface water (0–5 m) $[\text{CO}_2(\text{aq})]$, the isotopic composition of POM, and the apparent C-isotope fractionation between the organic and inorganic carbon pools (ϵ_{app}). ϵ_{app} is defined as $[(\delta^{13}\text{C}_{\text{DIC}} + 1000)/(\delta^{13}\text{C}_{\text{POC}} + 1000) - 1] \times 10^3$ (Farquhar et al. 1989). The correspondence between $[\text{CO}_2(\text{aq})]$ and the C-isotope system demonstrates the use of $\delta^{13}\text{C}$ as an indicator of pCO_2 in surface waters.

species assimilation occurred during the productive season. The use of $\delta^{13}\text{C}$ values as indicators of the concentration of CO_2 in surface waters, however, remains a valid approach, as the effect of C-isotope fractionation during photosynthesis clearly predominates.

The lowest $\delta^{13}\text{C}$ values for bulk organic matter were observed after winter overturn, when ^{13}C -depleted respired DIC was mixed from the hypolimnion into the surface waters (Fig. 7A). The low $^{13}\text{C}/^{12}\text{C}$ ratios and the maximum C-isotope fractionation were the result of low primary production without CO_2 limitation. The contribution from strongly ^{13}C -depleted POC originating from methanotrophic activity in the stratified deep hypolimnion (see below) cannot account for the low $\delta^{13}\text{C}$ values. The $\delta^{15}\text{N}$ values for particles from the BNL were much lower than the $\delta^{15}\text{N}$ values observed in the mixed water column during winter.

Particularly during periods of low primary productivity, the input of organic matter from external sources may be an additional factor influencing the C-isotope composition of the POC. However C/N ratios (~ 8 , data not shown) do not indicate a significant input from terrestrial sources, which typically display C/N ratios higher than 20 (compilation in Meyers and Ishiwatari 1993). Yet the interpretation of bulk organic matter $\delta^{13}\text{C}$ in less productive lakes with relatively large terrestrial inputs is likely to be more problematic and the use of the C-isotopic composition of POM as indicator of $[\text{CO}_2(\text{aq})]$ and primary productivity in surface waters may be limited.

$\delta^{15}\text{N}$ in the epilimnion: Reliable indicator of nitrate utilization?—Variations in the $\delta^{15}\text{N}$ of PON have often been interpreted to be the result of isotope effects associated with nitrate uptake (e.g., Altabet et al. 1991; Holmes et al. 1999). Phytoplankton preferentially incorporate ^{14}N during nitrate assimilation (Fogel and Cifuentes 1993). Hence, if nitrate assimilation is the major control on the N-isotopic composition of autochthonous organic matter, its $\delta^{15}\text{N}$ should be less than or equal to that of the nitrate. As the nitrate concentration in the photic zone progressively decreases during the productive period, residual nitrate should become pro-

gressively enriched in ^{15}N , and, in turn, newly produced phytoplankton should display steadily increasing $\delta^{15}\text{N}$. The $\delta^{15}\text{N}$ of organic matter, in this case, will reflect the degree of nitrate utilization in surface waters. Indeed, in marine environments, the $\delta^{15}\text{N}$ values for phytoplankton and sedimenting organic matter have been shown to correlate negatively with the nitrate concentration (Altabet et al. 1991; Altabet and François 1994; Holmes et al. 1999). In lakes, evidence for a correlation between nitrate concentration and the $\delta^{15}\text{N}$ of POM is scarce (François et al. 1996; Teranes and Bernasconi 2000). Besides the N-isotope fractionation during nitrate uptake, the N-isotope composition of the nitrogen species being incorporated represents an additional control on the $\delta^{15}\text{N}$ value of PON. Given a constant N-isotope fractionation between the source nitrate and bulk phytoplankton, autochthonous PON reflects the $\delta^{15}\text{N}$ of surface water nitrate minus the associated N-isotope effect. Particularly in eutrophic lakes with relatively short residence times, rapid changes in the N-isotope composition of external loads may modulate the N-isotopic composition of the surface-water nitrate pool (Teranes and Bernasconi 2000), thus obscuring variations in $\delta^{15}\text{N}_{\text{PON}}$ values related to the degree of nitrate utilization. The N-isotopic composition of the nitrate pool may also be affected by other N-cycling reactions, in particular by denitrification, which results in an enrichment in ^{15}N in the nitrate reservoir. Hence, the interpretation of $\delta^{15}\text{N}$ values for organic matter is strongly dependent on the availability of data on the $\delta^{15}\text{N}$ values of ambient nitrate. Ostrom et al. (1997) reported on $\delta^{15}\text{N}$ values for organic matter collected from a cold coastal environment that were higher than those for corresponding nitrate, indicating that the assimilation of nitrate was not the primary mechanism controlling the isotopic composition of phytoplankton. The authors proposed that, among other processes, the assimilation of ^{15}N -enriched ammonium may have contributed to the high $\delta^{15}\text{N}$ values. If N_2 serves as a N source, the $\delta^{15}\text{N}$ value of the bulk organic matter may be significantly lowered because isotope fractionation associated with N_2 fixation is small compared to photosynthesis (Fogel and Cifuentes 1993 and references therein) and the $\delta^{15}\text{N}$ of dissolved N_2 is close to 0‰. Lastly, the $\delta^{15}\text{N}$ value for POM can be altered by microzooplankton grazing, microbial decompositional processes, and protein hydrolysis (Silfer et al. 1992; Hoch et al. 1996). On the basis of several marine studies (e.g., Altabet 1988; Freudenthal et al. 2001), microbial degradation has traditionally been considered to enrich ^{15}N in the residual organic matter; the evidence, however, is conflicting (Libes and Deuser 1988; Lehmann et al. 2002).

During winter, PON was highly enriched in ^{15}N with respect to ambient nitrate, indicating that isotopic composition of organic matter was not controlled by nitrate assimilation. Bernasconi et al. (1997) attributed the high winter values observed in the southern basin of Lake Lugano to the contribution of ^{15}N -enriched organic matter originating from an adjacent water treatment plant. Indeed, riverine sediments and aquatic vegetation from the Vedeggio River, sampled downflow from the water purification plant (discharging into the Vedeggio River) in 1997, displayed exceptionally high $\delta^{15}\text{N}$ values of +16.2‰ and +18.0‰, respectively (data not shown). In winter 1999 and 2000, however, the $\delta^{15}\text{N}$ values

of PON from the Vedeggio River were significantly lower (<5‰, data not shown). Very heavy winter $\delta^{15}\text{N}_{\text{PON}}$ values have been reported from other lakes (Hodell and Schelske 1998; Teranes and Bernasconi 2000), but the causes for the observed ^{15}N -isotope enrichment remain enigmatic.

Of all the dissolved N species, nitrate is the main nitrogen source during photosynthetic assimilation in productive aqueous systems. During spring and summer, the $\delta^{15}\text{N}$ of surface-water PON was always lower than that of nitrate (Fig. 7B). However, clear reservoir effects following Rayleigh distillation kinetics were not recognizable. Only in July, in association with peak-time primary productivity, a significant drop in $[\text{NO}_3^-]$ may have been related to an increase in the $\delta^{15}\text{N}$ value for the photic zone nitrate and, correspondingly, bulk organic matter. At the beginning of the productive period, before significant nitrate depletion took place in the surface water, the N-isotope enrichment factor (ϵ) for nitrate uptake by diatoms could be readily estimated from the difference in $\delta^{15}\text{N}$ values between the source NO_3^- and the product particulate nitrogen ($\Delta\delta^{15}\text{N}$). Using March and April N-isotope data (0.4 to 5 m), we assessed an ϵ of $-1.0 \pm 0.9\%$ (Fig. 7B). This apparent N-isotope fractionation is low compared to estimates from marine and culture studies (compilation in Fogel and Cifuentes 1993), but is consistent with results from a sediment trap study in Lake Baldegg, which revealed ϵ values for phytoplankton nitrate assimilation that ranged between -1.9% and -2.7% (Teranes and Bernasconi 2000). $\delta^{15}\text{N}_{\text{PON}}$ values as low as 2.1‰ were measured in June. The significant depletion in ^{15}N in the bulk organic matter may be attributed to a distinct bloom of N_2 -fixing cyanobacteria (*Aphanizomenon flos-aquae*) with an anticipated $\delta^{15}\text{N}$ of $\sim 0\%$. *A. flos-aquae* is the only cyanobacteria species in Lake Lugano in which heterocysts, indicators of the potential to fix nitrogen (Fogg 1974), are observed on a regular basis and at significant amounts. During the June bloom, *A. flos-aquae* accounted for approximately 30% of the total phytoplankton biomass in the epilimnion at the end of June (Fig. 4). The low $\delta^{15}\text{N}_{\text{PON}}$ provides some indication for substantial N_2 fixation in the southern basin, yet it remains to be addressed why phytoplankton would prefer N_2 over nitrate, which was never biolimiting. During July and August, the $\Delta\delta^{15}\text{N}$ was significantly higher than during spring, indicating that the N-isotope fractionation ($\epsilon = -3.4\% \pm 0.4\%$) associated with nitrate uptake by green algae and cyanobacteria, the dominant groups of algae during this time, is larger than for nitrate uptake by diatoms (Fig. 7B). It is known that N-isotope fractionation factors can vary with the phylogenetic group (Montoya and McCarthy 1995). However, in culture experiments, Montoya and McCarthy observed that the N-isotope fractionation associated with nitrate uptake by green algae was lower than that during uptake by diatoms.

The significant decrease in the $\delta^{15}\text{N}$ of surface-water nitrate observed between August and November coincides with an increase in the nitrate concentration (Figs. 3B and 7B), indicating nitrate replenishment exceeding nitrate consumption. In the lower epilimnion, isotopically light nitrate was regenerated through the combined processes of mineralization and nitrification, leading to the development of a $\delta^{15}\text{N}_{\text{NO}_3^-}$ minimum zone between July and September (Fig.

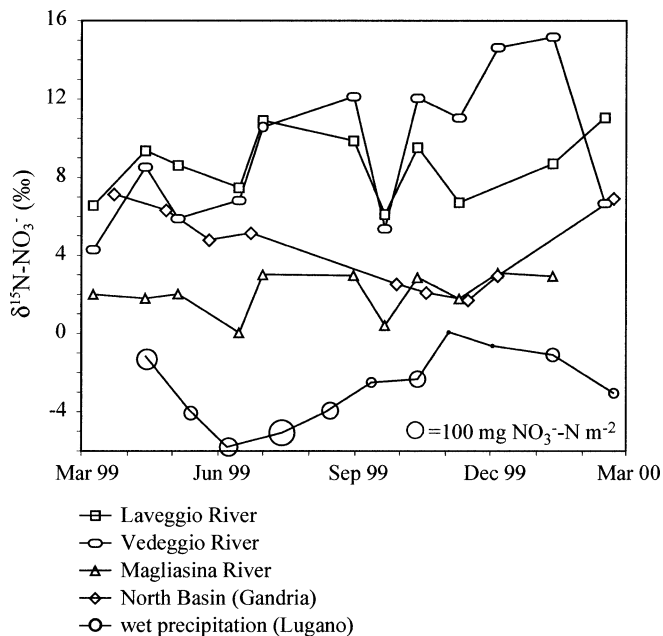


Fig. 9. Time series of $\delta^{15}\text{N}$ values for nitrate from external sources to the southern basin of Lake Lugano. Riverine and lacustrine data represent discrete water samplings. Rainwater was continuously collected. The size of the circles indicates the amount of nitrate being precipitated during each time interval.

6E). A reduction of the surface-water temperatures weakened the thermal stratification, allowing for vertical mixing of high- $[\text{NO}_3^-]$ and ^{15}N -depleted nitrate from the lower epilimnion into surface waters. As the density-related water column stability was further reduced, nitrate from the hypolimnion may have become more and more influencing. The upward advection of mid-water nitrate, characterized by $\delta^{15}\text{N}_{\text{NO}_3^-}$ values of $\sim +7.5\text{‰}$, and, after holomixis, the homogenization with bottom-water nitrate, which was enriched in ^{15}N because of microbial nitrate reduction (see below), may have contributed to the increase in surface water $\delta^{15}\text{N}_{\text{NO}_3^-}$ values observed after November.

Variations in the $\delta^{15}\text{N}$ of nitrate from external sources also strongly influence the N-isotopic composition of surface-water nitrate. The $\delta^{15}\text{N}$ of nitrate from riverine sources and wet precipitation, as well as from surface water of the northern basin of Lake Lugano, was measured to evaluate the impact of external NO_3^- on the isotopic composition of the South Basin dissolved inorganic nitrogen pool. Stream water nitrate from the Magliasina River was characterized by comparatively low mean $\delta^{15}\text{N}$ ($+1.4\text{‰}$) and, thus, differed quite strongly from the mean $\delta^{15}\text{N}$ of the Vedeggio and Laveggio Rivers ($+9.8$ and $+8.8\text{‰}$, respectively) (Fig. 9, Table 1), indicating differing nitrogen sources (soil N vs. sewage N). The Vedeggio, the Laveggio, and the Magliasina Rivers represent the major N-inputs to the southern basin of Lake Lugano, accounting for 50% of the total nitrogen load in 1999. The nitrate $\delta^{15}\text{N}$ of wet precipitation had a weighted annual mean of -3.3‰ . This is consistent with $\delta^{15}\text{N}$ values reported for nitrate in atmospheric precipitation (e.g., Freyer 1991). The N-isotopic composition of surface-water nitrate from the

Table 1. Nitrate loading and isotopic composition (weighted means) for most important nitrate inputs to the southern basin of Lake Lugano.

	NO_3^- -N load in 1999 ($\text{kg} \times 10^3$)	$\delta^{15}\text{N}-\text{NO}_3^-$ (‰)
Laveggio River	133	+8.8
Vedeggio River	340	+9.8
Magliasina River	64	+1.4
North Basin	104	+4.2
Wet precipitation	22	-3.3
Total	663	+7.5

North Basin varied between $+7.1\text{‰}$ and $+1.7\text{‰}$, with a weighted annual mean of $+4.2\text{‰}$. Accounting for 10% to 20% of the overall N input, the northern basin of Lake Lugano represents a significant nitrate source to the South Basin. The weighted average $\delta^{15}\text{N}$ value of total inflowing nitrate is $\sim +7.5\text{‰}$ (Table 1), and, thus, indistinguishable from the $\delta^{15}\text{N}_{\text{NO}_3^-}$ value for the mixed water column. This suggests that the N inputs from external sources control the isotopic composition of the whole-basin nitrate pool. It is reasonable to assume that a variable partitioning of NO_3^- inputs from different sources (e.g., rain with low $\delta^{15}\text{N}_{\text{NO}_3^-}$ vs. sewage runoff with high $\delta^{15}\text{N}_{\text{NO}_3^-}$) is likely to have short-term effects on the N-isotopic composition of the surface-water nitrate. Especially during summer, when the thermocline is well developed and the exchange between the epilimnion and hypolimnion is sluggish, the impact of external nitrate on epilimnetic $\delta^{15}\text{N}_{\text{NO}_3^-}$ values is amplified, and the effects of internal N-cycling reactions (e.g., nitrate assimilation) may be obscured.

Clearly decoupled from the trend in surface-water $\delta^{15}\text{N}_{\text{NO}_3^-}$ values, the $\delta^{15}\text{N}$ values of PON displayed an increasing trend during late summer and fall. Sometimes, $\delta^{15}\text{N}$ values for PON were slightly higher than for ambient nitrate (Fig. 7B). This may be attributed to microzooplankton grazing, release of nitrogenous compounds during microbial degradation, or the regeneration of inorganic nitrogen in the euphotic layer. These microbial-loop processes all have the net effect of concentrating ^{15}N in the seston (Ostrom et al. 1997). Recycling of organic carbon and nitrogen within the epilimnion amounts to 65% to 85% of the primary production (Bernasconi et al. 1997; Lehmann et al. 2002). Hence, one can assume that regenerated nitrogen may have served as a N source for phytoplankton during late summer and fall. The relatively high surface-water $\delta^{15}\text{N}_{\text{PON}}$ values measured between August and December may be attributable to the utilization of ^{15}N -enriched ammonium, which accumulated in the subsurface and may, in parts, have served as a N source for algae (the enrichment of ^{15}N in NH_4^+ is likely to have occurred as a result of N-isotope fractionation associated with microbial nitrification). However, it is questionable whether ammonium concentrations were high enough to drive the observed shifts toward higher $\delta^{15}\text{N}_{\text{PON}}$. Another and probably more likely explanation for those comparatively high $\delta^{15}\text{N}_{\text{PON}}$ values observed in the epilimnion is the episodic admixture of high- ^{15}N material from the upper hypolimnion.

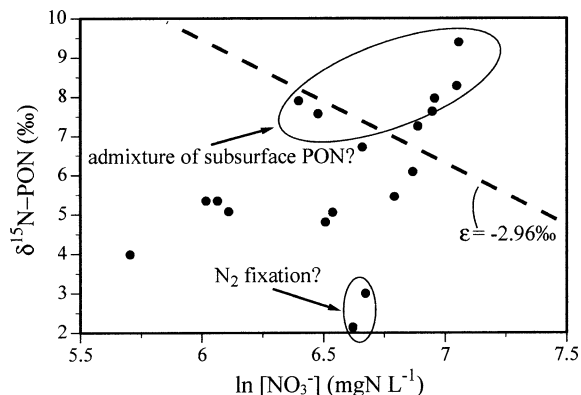


Fig. 10. Plot of $\delta^{15}\text{N}_{\text{PON}}$ data vs. the natural logarithm of near-surface $[\text{NO}_3^-]$. High nitrogen isotope values (+13‰ to +22‰) from the small amounts of PON collected in January and February were not considered, as they probably do not represent a phytoplankton organic matter source. The dashed line indicates the linear regression line that is expected, if nitrate utilization with an isotope effect of $\epsilon = -2.96\text{‰}$ (taken from Teranes and Bernasconi 2000) is the main control on the N-isotope composition of Lake Lugano PON. The discrepancy between expected and measured data is due to the fact that mechanisms other than Rayleigh distillation kinetics during nitrate assimilation were involved in biosynthesis (e.g., variable isotope fractionation, N_2 fixation, admixture of subsurface PON, external nitrate loading). See text for discussion.

The lack of correlation between the $[\text{NO}_3^-]$ and $\delta^{15}\text{N}_{\text{PON}}$ values, as summarized in Fig. 10, indicates that, in the South Basin of Lake Lugano, $\delta^{15}\text{N}$ values of POM do not reflect the degree of nitrate utilization. The lack of coupling can be attributed to the fact that the isotope fractionation factor for nitrate uptake by phytoplankton was not constant, that nitrogen species other than nitrate (NH_4^+ , N_2) may have temporarily served as a N source for autochthonous organic matter, or that the surface-water nitrate pool was continuously replenished through the input from external N sources with variable isotopic composition. In addition, the $\delta^{15}\text{N}$ values of surface water nitrate and PON may have been influenced by N-cycling reactions other than nitrate assimilation (e.g., nitrification, organic matter decomposition, mixing with organic matter from allochthonous sources or from the deep hypolimnion). All these processes equally possess the potential to suppress typical N-isotope reservoir effects associated with nitrate assimilation. Therefore, rather than recording the degree of nitrate consumption, Lake Lugano $\delta^{15}\text{N}_{\text{PON}}$ values reflect the partly superimposed effects of changes in algal population and N species being incorporated, N-cycling reactions within the microbial loop, and the admixture of particulate organic material not produced in situ.

Bacterial biosynthesis in the BNL—The development of a BNL, zones of high suspended-particle density, is a common feature in lakes (e.g., Mudroch and Mudroch 1992; Wieland et al. 2001). They have often been associated with resuspension of sedimented material or sediment focusing (Hawley and Lee 1999; Wieland et al. 2001). The exact controls on the origin of BNLS, however, remain enigmatic. In

Lake Lugano, resuspension of bottom sediments cannot explain the isotope composition of near-bottom suspended particles. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for surface sediment layers range between +4‰ and +7‰ and between -28‰ and -31‰, respectively (Bernasconi et al. 1997; Lehmann et al. 2002), much higher than those for POM from the BNL (~-5‰ and ~-60‰, respectively). The strongly depleted nitrogen and carbon isotope ratios determined for near-bottom suspended organic matter indicate a chemosynthetic origin for the suspended organic matter. High concentration of organic matter and nutrients at chemoclines leads to enhanced microbial activity resulting in high concentrations of chemotrophic bacteria, and, under suitable conditions, the formation of bacterial layers on top of the sediment or in the water column (e.g., Overmann et al. 1991). In situ biosynthesis by chemotrophs as a likely explanation for the high near-bottom particle concentration observed in the southern basin of Lake Lugano is further supported by low C/N ratios of POM (as low as 5), typical for bacterial biomass (Fenchel et al. 1998). The very low $\delta^{13}\text{C}$ values provide evidence that the bacteria utilized methane as their carbon source. Methanogenesis is the major pathway of organic matter degradation in the sediments of Lake Lugano. Methane diffuses up into the water column, where it can serve as an energy source for methanotrophic bacteria. Strongly ^{13}C -depleted biogenic methane is the only possible substrate leading to $\delta^{13}\text{C}$ values for synthesized organic matter as low as the ones observed in Lake Lugano. A $\delta^{13}\text{C}$ value of approximately $-70\text{‰} \pm 15\text{‰}$ for lacustrine methanotrophic bacteria can be inferred from typical $\delta^{13}\text{C}$ ranges (-65‰ to -50‰) for biogenic methane from freshwater sediments (Whiticar et al. 1986) and from reported isotope effects (-2‰ to -20‰) associated with the microbial oxidation of CH_4 in aquatic environments (Whiticar and Faber 1986 and references therein; Happell et al. 1994). Given the maximum bulk $\delta^{13}\text{C}$ values of ~-60‰ measured in the BNL and -30‰ to -35‰ for “background” organic matter supplied by the settling of particles from the epilimnion and resuspension of sediments (Fig. 5), we could assess that, during fall, the contribution of CH_4 oxidizing bacterioplankton to the near-bottom POM was more than 80%. Hence, the population of methanotrophic bacteria could alone account for the formation of the BNL. Methane oxidizers are known to utilize different sources of nitrogen for cellular growth, including ammonium, N_2 , and dissolved organic nitrogen (DON) (e.g., amino acids, urea) (Whittenbury and Dalton 1981; Murrell and Dalton 1983; Chu and Alvarez-Cohen 1998). We assume that, in Lake Lugano, the methanotrophic bacteria use ammonium from remineralization of sedimentary organic matter as their main N source. We cannot exclude the bacterial assimilation of labile DON compounds or N_2 ; however, NH_4^+ is the most common N source for methanotrophic bacteria (Whittenbury and Dalton 1981) and, if available in high concentrations, it is most likely preferred to other N compounds (C. Murrell pers. comm.). The $\delta^{15}\text{N}$ value for ammonium in suboxic near-bottom waters in Lake Lugano measured in July and October 1999 was ~+12.5‰ (Lehmann et al. 2001). An apparent enrichment factor for bacterial ammonium metabolism can be estimated, if ammonium is available in excess, and the N-isotope composition of the bacterioplankton is

known. Given that methane oxidizers account for at least 80% of the biomass in the BNL, the N-isotopic composition of the chemotrophs can be inferred to range between -5.3‰ and -7.8‰ , on the basis of the peak bulk $\delta^{15}\text{N}$ (-5.3‰). Accordingly, if NH_4^+ is indeed the main N source for methanotrophic bacteria in the deep hypolimnion, the approximate apparent isotope fractionation for ammonium uptake amounts to $-19 \pm 1.2\text{‰}$.

Notwithstanding some biogeochemical evidence for anaerobic methane oxidation (Hoehler et al. 1994; Borowski et al. 1996), methane oxidizers generally only occur where oxygen and methane coexist. Both the carbon and nitrogen isotopic ratios of the near-bottom SPM traced a “bacteriocline”, which rose with the expanding volume of bottom-water suboxia and, therefore, corroborate the confinement of methanotrophic organisms to the oxic–suboxic interface.

Denitrification in the deep hypolimnion—The distribution of hypolimnetic $\delta^{15}\text{N}_{\text{NO}_3^-}$ has been comprehensively discussed and modeled in Lehmann et al. (2003). In brief, the strong ^{15}N -isotope enrichment can be ascribed to the isotope fractionation associated with active denitrification in anaerobic waters. During dissimilatory consumption of nitrate, $^{14}\text{NO}_3^-$ is preferentially removed from waters below the redoxcline, leaving a strong imprint upon the nitrogen isotope composition of the residual nitrate. Previously, using the Rayleigh equation (closed-system approach), we obtained an apparent N-isotope enrichment factor of $\sim -11.2\text{‰}$ for denitrification in the deep hypolimnion of the South Basin of Lake Lugano. On the basis of reaction-diffusion model results, we reported that the N isotope fractionation is lower when denitrification rates were high, possibly in association with high organic carbon availability. In addition, variations in the N-isotope effect could be attributed to the variable importance of sedimentary denitrification having only a small isotope effect on the water column (Brandes and Devol 1997).

Denitrification potentially affects the N-isotopic composition of the whole-lake nitrogen inventory. Nitrogen mass balance calculations show that the total net elimination of nitrogen in the South Basin amounted to $336 \times 10^3 \text{ kg N}$ in 1999 (Table 2), corresponding to approximately 22% of the total nitrogen inventory. Net organic nitrogen burial generally does not contribute much to the total N removal in eutrophic lakes (e.g., Mengis et al. 1997). For example, Meile (1996) has shown that, in the northern basin of Lake Lugano, N burial accounts for less than 13% of total annual N loss from this basin. We assume that denitrification is also responsible for most of the fixed N eliminated from the southern basin in 1999. The $\delta^{15}\text{N}$ value for dissolved nitrate of the chemically homogenized water column collected at the beginning of the sampling campaign was indistinguishable from that determined one year later (Fig. 6D–F). Accordingly, although a significant amount of nitrate was removed with a marked nitrogen isotope effect, the N-isotopic composition of the overall nitrate pool did not change. N_2 fixation could counteract the isotope effect of denitrification; however, it does not play an important role in the annual N budget of Lake Lugano. Nitrification as a closed-system process, in which ammonium is completely turned over, cannot

Table 2. Nitrogen budget for the southern basin of Lake Lugano in 1999. The total N input includes riverine N, rainwater N, and N from the northern basin. In- and outputs are calculated from flow/precipitation rates and corresponding N concentrations determined by the L.S.A. Lugano (L.S.A. 1980–2000). N elimination is calculated from mass balance considerations ($N_{\text{elim.}} = N_{\text{in}} - \Delta\text{N} - N_{\text{out}}$). It can be assumed that denitrification accounts for most of the total N elimination from the system.

Total reservoir N ($\text{kg} \times 10^3$)	1526
Δ reservoir N ($\text{kg} \times 10^3 \text{ yr}^{-1}$)	101
Total N input ($\text{kg} \times 10^3 \text{ yr}^{-1}$)	1471
N output ($\text{kg} \times 10^3 \text{ yr}^{-1}$)	1034
N elimination ($\text{kg} \times 10^3 \text{ yr}^{-1}$)	336

compensate for the gross loss of light nitrogen via denitrification. The loss of ^{15}N -depleted nitrate from the lake may have been effectively balanced by the input of light nitrogen from external sources. The weighted mean $\delta^{15}\text{N}$ of inflowing nitrate, however, was also $\sim +7.5$ (Table 1); that is, it had the same N-isotope composition as the whole-lake nitrate pool. Hence, nitrogen must be removed from the basin with an overall N-isotope effect of $\sim 0\text{‰}$. The deep hypolimnion may be regarded as a partly closed system where nitrate is almost completely consumed. Consequently, when mixed to the rest of the water volume, ^{15}N enriched deep-hypolimnetic water masses barely affect the whole-lake $\delta^{15}\text{N}_{\text{NO}_3^-}$ value. Another explanation for the unchanged whole-basin $\delta^{15}\text{N}_{\text{NO}_3^-}$ values may be that most of the nitrate is dissimilated with negligible isotope fractionation in the anoxic sediments above the water column redoxcline. In contrast to water-column denitrification, sedimentary denitrification in highly reactive sediments does not necessarily influence the N-isotope composition of water-column nitrate (Brandes and Devol 1997; Lehmann et al. in press).

Summary and concluding remarks—This study documents the large annual variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for SPOM and inorganic carbon and nitrogen species in the southern basin of Lake Lugano. Supplemented by hydrochemical and biological data, stable carbon and nitrogen isotope data could be effectively used to trace distinct carbon and nitrogen species transformation reactions, which include carbon and nitrogen assimilation, nitrogen fixation, nitrification, microbial nitrate reduction, and bacterial biosynthesis.

We conclude that the most important process controlling the C-isotopic composition of autochthonous organic matter in Lake Lugano is the concentration of CO_2 in surface waters as a function of phytoplankton growth. Hence, our data corroborate that $\delta^{13}\text{C}$ values are reliable indicators of surface water pCO_2 . The nitrogen isotope composition of organic matter sampled during one seasonal cycle barely reflected the primary productivity in the surface waters. The applicability of organic matter $\delta^{15}\text{N}$ values as indicators of the degree of nitrate utilization is particularly hampered by the fact that variations in the N-isotope ratios can be the result of numerous reactions and processes. The epilimnion of Lake Lugano cannot be considered a closed system. In Lake Lugano, changes in nitrogen species uptake and vari-

ations in the $\delta^{15}\text{N}$ value of the nitrogen source were probably more important controls on the N-isotopic composition of organic matter from the photic zone than the reservoir effect associated with N-isotope fractionation during nitrate assimilation. In the deep hypolimnion, the stable carbon and nitrogen isotope composition of organic matter permitted us to assess the origin of organic matter in the BNL. Nepheloid layer particles were highly depleted in both ^{13}C and ^{15}N , indicating an origin from methanotrophic microorganisms utilizing ammonium as their nitrogen source. Methanotrophs accounted for more than 80% of the near-bottom POC. Consequently, we conclude that bacterial biosynthesis can play a very important role in the genesis of BNLS. N-isotope fractionation associated with microbial nitrate reduction had a strong effect on the $\delta^{15}\text{N}$ of dissolved nitrate in anaerobic bottom waters in the southern basin of Lake Lugano. The impact of nitrate reduction on the N-isotopic composition of the whole-lake nitrate inventory, however, appeared to be negligible.

This study provides evidence that, in modern lacustrine environments, C and N stable isotope ratios can offer an excellent means to trace different biogeochemical processes and may allow for a detailed understanding of the origin and cycling of organic matter. The successful use of N isotopes in tracing sources and cycling of nitrogen has direct implications for the management practices to preserve the water quality of a reservoir. A thorough knowledge of the causes for the variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in actual systems may be used to evaluate the potential of C and N stable isotope ratios in sedimentary organic matter to record changes in environmental conditions in the past. Our findings support the hypothesis that the $\delta^{13}\text{C}$ values of organic matter in lake sediments have high potential to reflect the history of lacustrine primary productivity and trophic evolution. Yet, compared to the C cycle, the N cycle is much more complex. N-isotope signals archived in lake sediments are likely to have integrated the effects of multiple N-cycling processes. Therefore, caution is advised when interpreting organic matter $\delta^{15}\text{N}$ values in lake sediments.

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Received: 7 January 2003

Accepted: 23 October 2003

Amended: 14 November 2003