First lacustrine application of the diatom-bound nitrogen isotope paleo-proxy reveals coupling of denitrification and N$_2$ fixation in a hyper-eutrophic lake

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Abstract

Past changes in the input/output, and internal cycling, of bioavailable nitrogen (N) in marine and lacustrine environments can be reconstructed by analyzing the N isotopic composition (δ^{15}N) of organic matter in the sedimentary record. To verify, and eliminate, potential biases of bulk sedimentary δ^{15}N (δ^{15}N$_{bulk}$) signatures by diagenetic alteration and external N inputs, we applied, for the first time, the diatom-bound N isotope (δ^{15}N$_{db}$) paleo-proxy to lake sediments. By comparing δ^{15}N$_{bulk}$ and δ^{15}N$_{db}$ in a sedimentary record from eutrophic Lake Lugano (Switzerland), we demonstrate that changing redox conditions influence the degree of N-isotopic alteration of the bulk sediment, emphasizing the need for caution when interpreting δ^{15}N$_{bulk}$ in paleolimnological studies. Furthermore, in combining δ^{15}N$_{db}$ measurements with X-ray fluorescence scanning and state-of-the-art molecular biomarker analyses, we reconstruct nutrient cycling and paleoenvironmental conditions in the lake over the past ~ 125 yr. Coeval with the period of severe eutrophication in Lake Lugano in the 1960s, our proxy data indicate that export production, δ^{15}N$_{db}$, and the concentration of heterocyst glycolipids (a biomarker for N$_2$-fixing cyanobacteria) increased simultaneously. Together, these data suggest that the rise in δ^{15}N$_{db}$ is likely the result of enhanced water-column denitrification in response to increased phytoplankton productivity. We hypothesize that greater export production during eutrophication led to anoxic conditions in the hypolimnion as a result of enhanced organic matter remineralization, raising water-column denitrification. Enhanced N loss and remobilization of phosphorous (P) from the sediments under anoxic conditions lowered the N : P ratio in the lake, fostering cyanobacterial N$_2$ fixation in surface waters.

Nitrogen (N) is a major nutrient required by all phytoplankton for growth. In lakes, high concentrations of bioavailable ("fixed") N resulting from fertilizer or municipal waste inputs

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can accelerate eutrophication and loss of oxygen, reduce biodiversity, as well as stimulate toxic algal blooms (Carpenter et al. 1998). It is therefore crucial to understand the sources and sinks of fixed N in lakes, past and present. The analysis of stable N isotopes has proven a powerful tool to study the budget and internal cycling of fixed N in lakes. The isotopic composition (the ^{15}N/^{14}N ratio, or δ^{15}N, with δ^{15}N (in ‰ vs. Air) = (^{15}N/^{14}N$_{sample}$/^{15}N/^{14}N$_{Air}$ − 1) * 1000) of nitrate in the water column changes as a function of the N input/output, as well as internal N-cycling processes, which are all associated with distinct isotopic signatures.

The main sources of fixed N to lakes are atmospheric deposition, tributary rivers, groundwater, terrestrial runoff, and microbial N$_2$ fixation. The δ^{15}N of atmospheric deposition ranges from −6‰ to +6‰ (e.g., Freyer 1991), whereas N$_2$ fixation produces nitrate (NO$_3^-$) with a δ^{15}N of around −2‰ to 0‰ (Carpenter et al. 1997). The N isotopic composition of nitrate

inputs from surface waters and groundwater range from $-2‰$ to $2‰$ in regions where synthetic fertilizers are used, and from $3.5‰$ to $16‰$ in areas where manure is used (Kendall 1998; Bateman and Kelly 2007). Denitrification is the major loss term of fixed N in aquatic systems, and occurs both in the water column and in the sediments. While water-column denitrification leads to a clear $\delta^{15}N$ elevation, sedimentary denitrification results only in little $\delta^{15}N$ change in the nitrate pool of a lake, due to reduced nitrate exchange between the sediment pore water nitrate pool and the overlying water column (e.g., Lehmann et al. 2004b, 2007).

The nitrate isotopic signature of the surface water is recorded by phytoplankton when they assimilate the nitrate with an isotope effect of around $5‰$ (Waser et al. 1998). The phytoplankton biomass, and for diatoms also their frustule-bound organic matter, records the N isotopic composition of the assimilated nitrate, the signal of which is ultimately transferred to the sediment record after their death and burial.

Most paleoenvironmental and paleolimnological studies rely on the analysis of bulk sedimentary $\delta^{15}N$ to reconstruct past changes in the N cycle and/or the N budget of a lake. For example, bulk sedimentary $\delta^{15}N$ records have been used to infer the history of eutrophication, nitrate utilization, denitrification and changes in external N loading (Hodell and Schelske 1998; Teranes and Bernasconi 2000; Lehmann et al. 2004a). However, contamination by external particulate N and syn- and post-sedimentary diagenetic alteration can potentially compromise the use of bulk sedimentary N for paleoenvironmental reconstruction (Hodell and Schelske 1998; Talbot 2001; Gälman et al. 2009; Robinson et al. 2012; Brahney et al. 2014). In marine environments, outside of regions characterized by high organic matter accumulation, the $\delta^{15}N$ of the surface sediment is elevated by $2-5‰$ relative to that of sinking particles (Altabet and François 1994; Robinson et al. 2012). The isotopic alteration of the sedimentary organic N, which occurs at the sediment–water interface or in the shallow sediment during early burial, is caused by the preferential loss of a low-$\delta^{15}N$ fraction of the organic matter (Altabet and François 1994; Lehmann et al. 2002) and/or bacterial reworking and in situ biosynthesis (e.g., Lehmann et al. 2020). In addition, input of allochthonous N, such as terrestrial organic N or clay-bound N, can compromise the use of bulk sedimentary N as a recorder for paleoenvironmental change (Schubert and Calvert 2001; Talbot 2001). Because of these concerns, recent research has focused on the N isotopic analysis of organic N incorporated in the mineral matrix of fossils, which records the pristine, unbiased N isotope signature of water-column nitrate, and is thought to be unaffected by secondary alteration and allochthonous N contamination (King 1977; Swift and Wheeler 1992).

Diatoms (siliceous algae made of opal) are one of the fossil archives that have successfully been explored over the past 20 yr for reconstructing paleoenvironmental change by measuring their frustule-bound N isotope composition (e.g., Robinson et al. 2004; Studer et al. 2015; Ai et al. 2020). Culture studies as well as modern-ocean samplings and in-situ experiments have shown that the $\delta^{15}N$ of the organic matter contained and protected within the silica frustules of diatoms ("diatom-bound" $\delta^{15}N$) reliably records the $\delta^{15}N$ of the nitrate source to the diatoms (Horn et al. 2011; Robinson et al. 2020; Jones et al. 2022). However, the application of the diatom-bound nitrogen isotope paleo-proxy has so far been limited to the marine realm. The overarching goal of this study was to test the applicability and establish the diatom-bound N isotope paleo-proxy in lake sediments. Using a sediment core from eutrophic Lake Lugano (Switzerland), we investigated the N budget and internal N cycling of the lake's recent past, and explored the use of diatom-bound N isotope ratios to track the (well-documented) trophic evolution of the lake, without bias from diagenetic alteration and allochthonous N input. In order to assess the role of N_2 fixation as natural N input, we also measured the concentration of heterocyst glycolipids in the sediments, which is a diagnostic biomarker for N_2-fixing cyanobacteria. Finally, we reconstructed past export production in Lake Lugano using a multiproxy approach, with the goal to provide a holistic reconstruction of past paleoenvironmental conditions over the past century.

Materials and methods

Study area

Our study focuses on the South Basin of Lake Lugano (Switzerland and Italy), which is 95 m deep, has a catchment area of 290 km^2, and a water renewal time of 1.4 years (Supplementary Fig. S1) (Barbieri and Simona 2001). It is mono-mictic and turns over during late winter. During spring and summer, the basin stratifies thermally, and primary productivity increases. The phytoplankton biomass is typically dominated by diatoms during spring and by cyanobacteria and green algae during summer (e.g., Lehmann et al. 2004b; Lepori et al. 2022a). The bottom waters of the South Basin are seasonally anoxic from summer to the end of the following winter (Lepori and Capelli 2021). The main inflows to the South Basin are from the Lake Lugano North Basin and from the Vedeggio River, with the external nutrient load mostly coming from anthropogenic sources (i.e., household and sewage 85%; industrial 10%; agricultural 5%) (Barbieri and Simona 2001). Lake Lugano became severely eutrophic in the 2nd half of the 20th century. In the South Basin, varve sedimentation started in the 1930s, indicating the onset of seasonally anoxic bottom-waters (Niessen et al. 1992). In the 1960s, total phosphorous and N concentrations continued to rise, and the South Basin reached a hypertrophic state in the 1970s (Barbieri and Mosello 1992). Since the early 1980s, Lake Lugano is being monitored continuously, and a lake restoration program has been put in place (Imboden 1992; Lepori et al. 2022b).

Sediment coring and age determination

We collected two sediment cores using a custom-built gravity corer, one in 2018 (Figino18-2) and one in 2019 (Figino19-2),

from the deepest part (95 m) of the South Basin of Lake Lugano (Supplementary Fig. S1). The two cores were split lengthwise, photographed, and visually correlated to each other at 31 tie points spread over ∼ 65 cm (Supplementary Fig. S2). Samples for gamma spectrometry dating were freeze-dried, ground, weighted into sealed plastic sampling tubes, and left to equilibrate in order to measure radon-222 (^{222}Rn) and its daughter elements for 20 d. Radionuclide activities of lead-210 (^{210}Pb), radium-226 (^{226}Ra), americium-241 (^{241}Am) and cesium-137 (^{137}Cs) were determined by γ-spectrometry in a High-purity Germanium (HPGe) Well Detector (gamma spectrometer; Mirion Technologies [Canberra] Inc., Meriden, CT, USA) at Eawag (Dübendorf, Switzerland).

Paleoproductivity (export production) reconstructions

There are different ways to reconstruct past primary productivity in lakes, and every proxy has its own strengths and potential biases. For this reason, here, we follow a multi-proxy approach to provide a robust measure of past changes in lake primary productivity through time, combining measurements of the barium-to-titanium (Ba/Ti) ratio of the sediment, with analyses of the mass accumulation rate of organic carbon (C_{org} MAR), and the carbon isotopic composition of organic matter ($\delta^{13}C_{TOC}$). In the paleolimnological context of this study, we will refer to past primary productivity as the accumulation of organic matter in the sediment after settling through the water column. In the paleoceanographic context, this is typically referred to as "export production," and we will adopt this terminology in the following. It differs from the limnological definition of primary productivity (i.e., the rate at which carbon is fixed by autotrophs) in that it excludes the fraction of organic matter that is remineralized already in the water column.

Ba/Ti ratio determination by XRF scanning

The archive-half of core Figino19-2 was scanned longitudinally at a 1-mm resolution using an integration time of 20 s with an ITRAX x-ray fluorescence (XRF) element core scanner equipped with a Cr-tube set to 30 kV and 50 mA at the University of Bern. We use the relative intensity ratio of Ba/Ti obtained from the XRF scanning as a proxy for past export production in Lake Lugano. The content of biogenic barium or barite in sediments is widely used as a paleo-productivity indicator (Dymond et al. 1992; Horner et al. 2017), and normalization to a lithogenic element such as Ti allows to correct for the contribution of nonbiogenic, detrital input (Thomson et al. 2006). We complement the Ba/Ti data with other productivity proxies to account for re-precipitation as barite at the transition to the sulfate-bearing zone (McManus et al. 1998).

Isotopic composition and mass accumulation rate of organic carbon

Increases in C_{org} MAR and the $\delta^{13}C_{TOC}$ in lake sediments have been linked to enhanced in-lake primary productivity (Hollander and McKenzie 1991; Hodell and Schelske 1998). Here, we measured the weight-percent of total organic carbon

(TOC) and the $\delta^{13}C$ of organic carbon using a Flash EA coupled to a Delta V Plus isotope ratio mass spectrometer (EA-IRMS) on 12 mg aliquots of freeze-dried and ground bulk sediment, from which carbonate has been removed by fumigation with 12 N HCl. The $\delta^{13}C_{TOC}$ data were referenced against the internationally calibrated standards USGS40 ($\delta^{13}C = -26.39‰$), IAEA-CH6 ($\delta^{13}C = -10.45‰$), and an in-house EDTA standard ($\delta^{13}C = -30.25‰$), and are reported in ‰ vs. V-PDB. Based on replicate analyses of samples and standards, the average standard deviation for $\delta^{13}C_{TOC}$ measurements was < 0.1‰. C_{org} MAR was calculated by multiplying the weight–% of TOC with the dry bulk density of the sediment and with the sedimentation rate derived from the linear interpolation between the depth-age pointers 2019 CE, 1986 CE, 1963 CE, 1954 CE and the core bottom age 1895 CE (*see* age model below), and has a propagated error ranging from $\pm\,0.19$ to 1.35 mg cm^{-2} yr^{-1} (error bars in Fig. 1c).

Bulk sediment and diatom-bound N isotope analysis

Twelve milligrams of nonfumigated, freeze-dried, and ground sediments were packed into tin capsules and analyzed for their N content and $\delta^{15}N$ using a Delta V Plus EA-IRMS, and referenced against the internationally calibrated standards IAEA-N1 ($\delta^{15}N = 0.4‰$), IAEA-N$_2$ ($\delta^{15}N = 20.3‰$) and EDTA#2 (from A. Schimmelmann, Indiana University, $\delta^{15}N = -0.83‰$), as well as an in-house EDTA standard ($\delta^{15}N = -1.1‰$). Replicate analyses of samples and standards indicate an average standard deviation of better than 0.1‰ for bulk $\delta^{15}N$.

Diatom-bound $\delta^{15}N$ was measured with the persulfate-denitrifier technique, after physical separation and chemical cleaning of the diatom fraction from the bulk sediment, following, and adapting, the protocols of Studer et al. (2018), Morales et al. (2013), and Horn et al. (2011) (for a detailed description of the separation and cleaning procedure, *see* Supplementary Section 1). After the chemical cleaning, 2–5 mg of dried clean diatom opal was weighed into precombusted 4-mL vials, and 1.5 mL of a 1.25 M sodium hydroxide and 0.18 M potassium persulfate solution was added, using four times recrystallized potassium persulfate. The samples were autoclaved for 90 min at 121°C in order to dissolve the diatom opal, and to liberate and oxidize the organic N contained within the frustules to nitrate. Ten procedural blanks and 15 international reference amino acid standards of known isotopic composition (USGS64, $\delta^{15}N = 1.76‰$; USGS65, $\delta^{15}N = 20.68‰$, USGS40, $\delta^{15}N = -4.5‰$; USGS41, $\delta^{15}N = 47.6‰$) were run in every batch of 30 samples to monitor the oxidation, and to correct for the contribution of the reagent blank to the sample. The nitrate concentration of the oxidized samples was determined by reduction to nitric oxide (NO) in a solution of acidic vanadium (III) at 90°C and chemiluminescent detection of the NO (Braman and Hendrix 1989), and aliquots equivalent to 20 nmol of N were converted to nitrous oxide using the denitrifier technique (Sigman et al. 2001). The N isotopic composition of the nitrous oxide gas was determined by gas
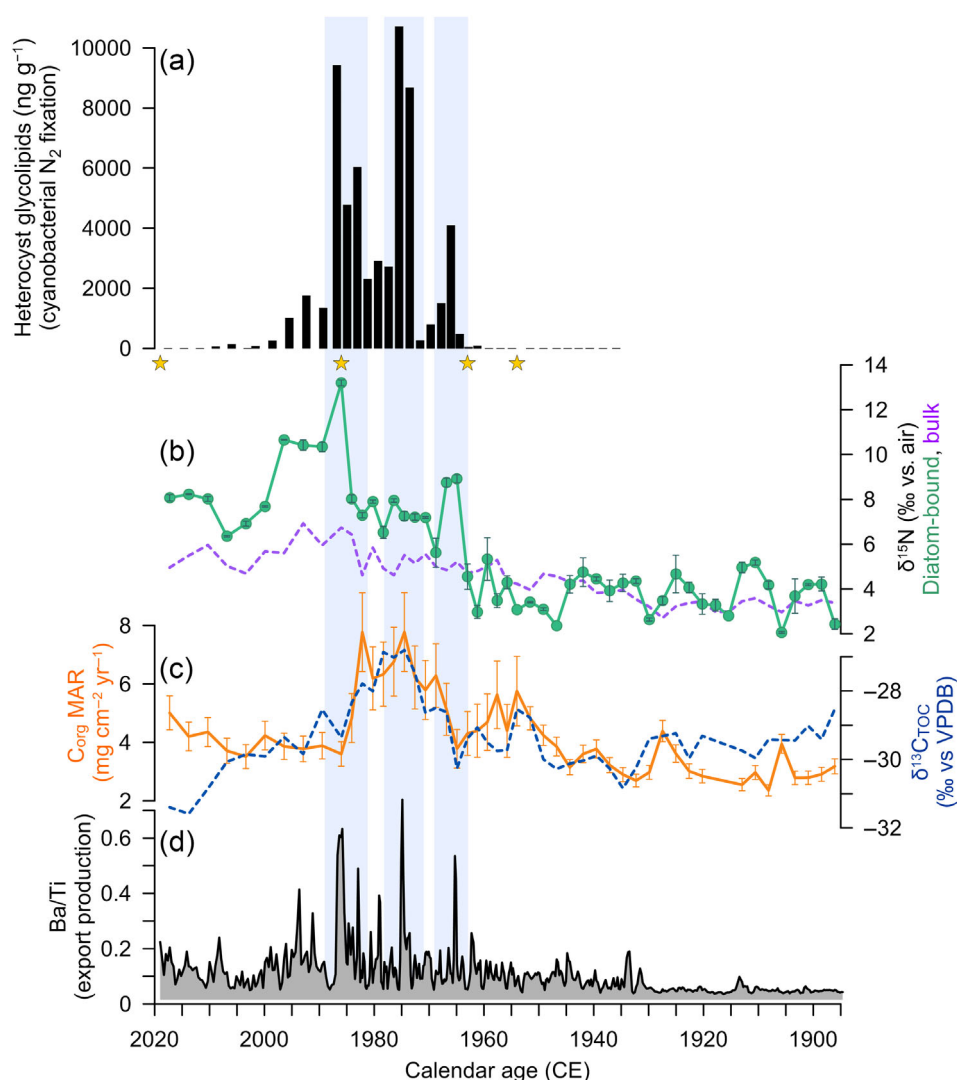
Fig. 1. Compilation of down-core records from the Lake Lugano South Basin vs. calendar age (CE, common era). (**a**) Concentration of heterocyst glyco-lipids (HGs) in the sediment, a biomarker for cyanobacterial N_2 fixation. (**b**) Diatom-bound (green) and bulk sediment (dashed purple) $\delta^{15}N$. (**c**) Mass accumulation rate (MAR) of C_{org} (orange) and the $\delta^{13}C$ of TOC (dashed blue), proxies for past export production. (**d**) Ba/Ti ratio of the sediment as measured by XRF scanning, another indicator for past export production. Light blue background shadings highlight peaks in HG concentrations and Ba/Ti ratios. The four yellow stars indicate calendar depth-age pointers that were used for the construction of the age model (2019 CE, 1986 CE, 1963 CE, and 1954 CE).

chromatography isotope ratio mass spectrometry (GC-IRMS) using a Thermo DeltaV Plus instrument coupled to a Gasbench used for N_2O purification. The average size of the N blank associated with the wet chemical oxidation was 0.34 μM (which is typically 1–2% of the sample size) and the average standard deviation for replicate sample analyses was $\pm$ 0.25‰.

Analysis of heterocyst glycolipids

Heterocyst glycolipids (HGs) are a diagnostic biomarker for N_2 fixation by heterocystous cyanobacteria (Bauersachs et al. 2010; Wörmer et al. 2012). To track their abundance over time, sediment samples ($\sim$ 0.3 g) from core Figino18-2 were spiked with 1 μg of an internal standard (PC-DAG$_{42:0}$)

and ultrasonically extracted in a solvent mixture (v : v) of methanol, dichloromethane and aqueous buffer following standard procedures in polar lipid analysis (Wörmer et al. 2017). One percent of the obtained total lipid extract was then analyzed on a maXis quadrupole time-of-flight mass spectrometer (Bruker Daltonics) equipped with an electrospray ionization source, and coupled to a Thermo Ultimate 3000RS UHPLC system. Chromatographic separation was performed on a Waters Acquity BEHC$_{18}$ column (Wörmer et al. 2017), with a slightly modified gradient to improve detection of HGs. Mass spectrometric characterization was performed in positive ionization mode, while scanning a mass-to-charge (m/z) range from 150 to 2000. MS^2 scans were obtained in data-dependent

mode; for each MS full scan up to three MS^2 experiments, targeting the most abundant ions, were performed. Active exclusion limits the times a given ion is selected for fragmentation (three times every 0.5 min), and thus allowed us to also obtain MS^2 data of less abundant ions. Lipid identification was achieved by monitoring exact masses of possible parent ions (present as either H^+, NH_4^+, or Na^+ adducts) in combination with characteristic fragmentation patterns, as outlined by Wörmer et al. (2012). HGs were quantified by comparing their peak area to the one of the internal standard, and assuming a response factor of 1.

Results

Sedimentological description and age model

Sediment core Figino19-2 is characterized by laminations that are intercalated with seven turbidites (Supplementary Fig. S2). The turbidites have a different appearance and geochemical composition (e.g., elevated zirconium content and C/N ratios) relative to the background sediment, and are mainly composed of terrigenous material deriving from the catchment. The ^{137}Cs peak from the 1986 Chernobyl accident, and the ^{137}Cs and ^{241}Am maxima from the peak-time 1963 atmospheric nuclear weapon testing fallout were identified at depths of 9.5 and 21.5 cm downcore, respectively (turbidites > 1 cm have been "removed" from the composite depth for age determination). Furthermore, ^{137}Cs was first detected at a depth of 26.5 cm, which can be ascribed to the very beginning of the nuclear weapon testing in 1954 (e.g., Foucher et al. 2021). These time markers yield average sedimentation rates of 0.29 cm yr^{-1} from 1986 to 2019, 0.52 cm yr^{-1} from 1963 to 1986 and 0.56 cm yr^{-1} from 1954 to 1963, respectively. The identification of the 1986, 1963, and 1954 events in the sedimentary record provide excellent age control for the onset and the end of the peak eutrophication period in Lake Lugano. Ages were linearly interpolated between the core surface (2019 common era, CE) and the time markers mentioned above, and ages were then extrapolated to the base of the core using an average sedimentation rate of 0.41 cm yr^{-1} computed from the excess ^{210}Pb, yielding a calendar age of 1895 CE for the core bottom.

Changes in export production

Past primary productivity increased in Lake Lugano at the beginning of the 1960s, which is evidenced by the rise of the Ba/Ti ratio in the sediment, the rise of C_{org} MAR, and the increase in the $\delta^{13}C_{TOC}$ (Fig. 1). While all three proxies indicate highest export production during the mid-1970s, Ba/Ti ratios reveal a more nuanced temporal pattern, with three distinct periods of enhanced productivity (light blue background shading in Fig. 1). Export production remained high until the mid-1980s, and started to decline thereafter, without returning to baseline values observed prior to the 1930s.

Bulk sediment $\delta^{15}N$ and diatom-bound $\delta^{15}N$

Both bulk sediment $\delta^{15}N$ ($\delta^{15}N_{bulk}$) and diatom-bound $\delta^{15}N$ ($\delta^{15}N_{db}$) values are lower during the period prior to eutrophication, and fluctuate around an average of 3.6‰ for $\delta^{15}N_{bulk}$ and 3.8‰ for $\delta^{15}N_{db}$ (excluding turbidite layers), with $\delta^{15}N_{bulk}$ showing lower-amplitude fluctuations than $\delta^{15}N_{db}$ (Fig. 1b). At the beginning of the 1960s, concomitant with the intensification of eutrophication in Lake Lugano, both $\delta^{15}N_{bulk}$ and $\delta^{15}N_{db}$ increase. During the mid-1980s, $\delta^{15}N_{bulk}$ reaches maximum values of 6.9‰, and $\delta^{15}N_{db}$ peaks at 13.2‰ in 1986. Thereafter, following P management, both $\delta^{15}N_{bulk}$ and $\delta^{15}N_{db}$ decline towards present-day values (5.0‰ for $\delta^{15}N_{bulk}$, 8.1‰ for $\delta^{15}N_{db}$), which are still higher than pre-eutrophication levels. Prior to eutrophication, $\delta^{15}N_{bulk}$ and $\delta^{15}N_{db}$ were quite similar to each other, yet in the vicinity of turbidites, there seems to be a tendency of $\delta^{15}N_{db}$ to be lower than $\delta^{15}N_{bulk}$ (which is not statistically significant; $p > 0.05$ for a one-tailed Mann–Whitney test) (Fig. 2a, blue fill). Since the mid-1960s, $\delta^{15}N_{db}$ is significantly higher than $\delta^{15}N_{bulk}$ ($p < 0.01$ for a one-tailed Mann–Whitney test) (Fig. 2a, orange fill). The two distinct $\delta^{15}N_{db}$ peaks at the beginning of the 1960s and during the mid-1980s correlate with the peaks in export production, as inferred from the Ba/Ti ratio during that time (Fig. 1d, light blue shading).

Heterocyst glycolipid concentrations in sediments

The concentration of the sum of all measured HGs (HG_{26}-diol, HG_{26}-keto-ol, HG_{28}-diol, HG_{28}-keto-ol, and HG_{28}-triol) is very low in the older part of the sedimentary record, and rapidly increases at the beginning of the 1960s (Fig. 1a), indicating a rise in N_2 fixation during the eutrophication period. HG concentrations show three distinct peaks during the eutrophication period that are coeval to the peaks observed in the Ba/Ti export production record, as well as in $\delta^{15}N_{db}$. HG concentrations decreased again to values below 1000 ng g^{-1} at the end of the 1980s.

Discussion

Rise in export production during eutrophication

The rise in export production in the Lake Lugano South Basin at the beginning of the 1960s that is reflected in all three productivity proxies (Fig. 1) is a direct consequence of the increasing population and industrial activity in the catchment area and the resulting enhancement of wastewater nutrient inputs (Barbieri and Mosello 1992; Niessen et al. 1992). The decline in export production since the mid-1980s likely reflects a parallel reduction in primary productivity (measured as carbon assimilation) and in phytoplankton biomass (measured as chlorophyll *a* or biovolume) that occurred mainly between 1988 and 1989 (Barbieri and Simona 2001; DACD-SUPSI 2022). The reduction of P loadings due to restoration measures may have had an impact, although we note that, even after 1989, P concentrations and loadings remained high
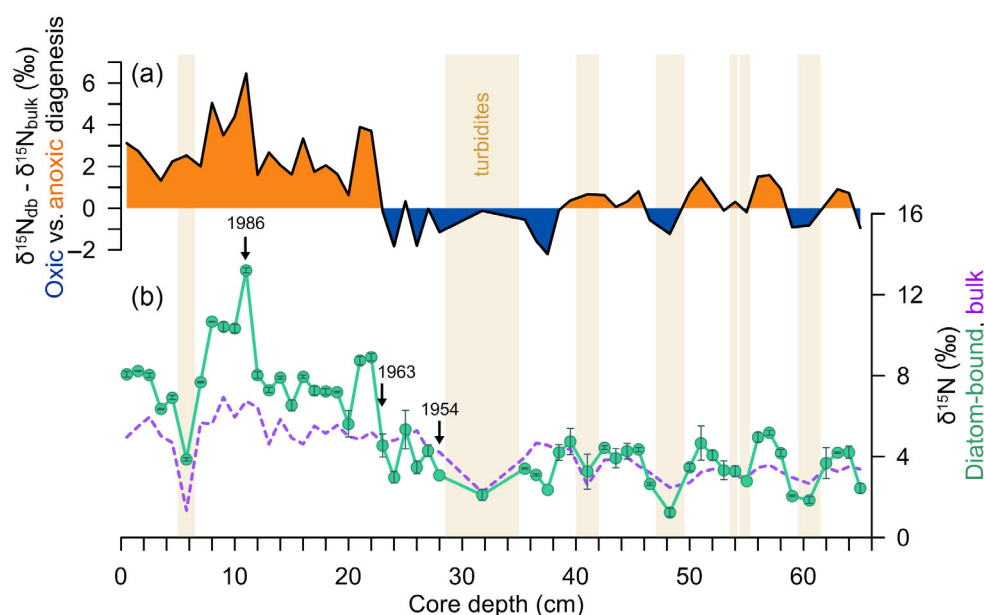
Fig. 2. Nitrogen isotope ratios of the down-core record from the Lake Lugano South Basin. (**a**) Difference between diatom-bound and bulk sediment $\delta^{15}N$. Blue fill highlights negative offsets and orange fill highlights positive offsets, which we interpret as indicative of oxidative vs. anoxic preservation conditions/diagenesis, respectively (*see* the text). (**b**) Diatom-bound (green) and bulk sediment (dashed purple) $\delta^{15}N$. Beige-colored background shadings highlight turbidites in the sedimentary sequence.

and well above literature-based thresholds of P limitation (Smith 1979; Lepori and Capelli 2021; Lepori et al. 2022*b*). In addition, the decline of phytoplankton biomass coincided with a sudden increase in zooplankton biomass, which suggests that top-down processes (increased grazing) also contributed to that change (Barbieri and Simona 2001; Lepori et al. 2022*b*).

Difference between bulk and diatom-bound $\delta^{15}N$: Diagenesis or external N input?

Given the concerns associated with $\delta^{15}N_{bulk}$ raised above, we wanted to explore the mechanisms that may bias $\delta^{15}N_{bulk}$ in the Lake Lugano sediments, which give rise to the observed difference between bulk and diatom-bound $\delta^{15}N$. First, we investigated the effect of allochthonous N contribution, such as inorganic N or terrestrial organic N, to the $\delta^{15}N_{bulk}$ signature. In a crossplot of total nitrogen (TN) against total organic carbon (TOC), the nonzero y-intercept at 0% TOC indicates that 16% ± 8% of the average TN is inorganic (Fig. 3a). The $\delta^{15}N$ of inorganic N, such as ammonium bound in clay minerals, is generally low (− 2‰ to 4‰; Wada et al. 1987; Schubert and Calvert 2001), whereas terrestrial organic N has a $\delta^{15}N$ around 3–4‰ (Wada et al. 1987; Kendall 1998). Therefore, if external N input were to modulate $\delta^{15}N_{bulk}$ in Lake Lugano, a negative correlation between TN and $\delta^{15}N_{bulk}$ would be expected, but the opposite is observed here, with higher $\delta^{15}N_{bulk}$ associated with high TN (Fig. 3b). It is thus unlikely that external N input is driving and biasing the overall $\delta^{15}N_{bulk}$ signal. This finding is consistent with the fact that

$\delta^{15}N_{bulk}$ shows a similar temporal pattern as $\delta^{15}N_{db}$, although with a muted amplitude (Fig. 2b).

Second, we investigated the potential effect of early diagenesis on $\delta^{15}N_{bulk}$. The alternating temporal offset between $\delta^{15}N_{db}$ and $\delta^{15}N_{bulk}$ (Fig. 2) could point to a role of sediment oxygenation in the diagenetic alteration of $\delta^{15}N_{bulk}$, because turbidite deposition is associated with the oxygenation of sediment porewaters within the turbidites and the adjacent background sediment (Span et al. 1992, *see* below). Ample support for the role of oxygen in driving nitrogen isotopic alteration of organic matter comes from observations in the sediment, the water column, and incubation experiments. In a comprehensive data compilation, Robinson et al. (2012) showed that marine surface bulk sediment $\delta^{15}N$ is elevated relative to sinking particle $\delta^{15}N$, and that oxygen exposure time at the seafloor is an important modulating control. Similarly, elevated $\delta^{15}N$ was found in the oxic layers of sediment cores (Freudenthal et al. 2001; Möbius et al. 2010; Brahney et al. 2014), whereas $\delta^{15}N$ depletion was found in anoxic sediments (Freudenthal et al. 2001; Gälman et al. 2009). In the water column, the $\delta^{15}N$ of suspended particulate organic nitrogen (PON) increased with increasing depth due to oxic degradation (Altabet 1988; Libes and Deuser 1988), whereas the $\delta^{15}N$ of PON from anoxic environments was depleted in ^{15}N relative to PON from the oxic water column (Libes and Deuser 1988; Voss et al. 1997). Finally, particulate organic matter incubated under oxic conditions showed an increase in $\delta^{15}N$ over time in both marine and freshwater settings (Holmes et al. 1999; Lehmann et al. 2002), whereas the $\delta^{15}N$ of organic matter decreased by more than
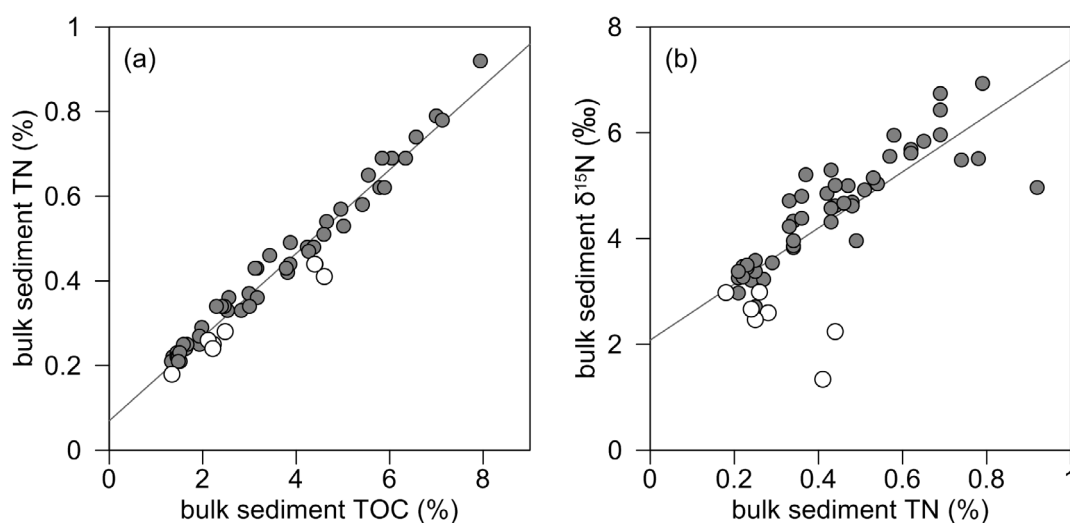
Fig. 3. (**a**) Crossplot of bulk sediment total N (TN) against total organic carbon (TOC). (**b**) Crossplot of bulk sediment $\delta^{15}N$ against bulk sediment TN. Filled symbols represent background sediment, open symbols represent turbidites.

3‰ in anoxic incubations (Lehmann et al. 2002). Overall, increasing $\delta^{15}N$ during oxic diagenesis has been attributed to the excretion of isotopically light ammonium during deamination (Macko and Estep 1984) or due to isotopic fractionation associated with protein hydrolysis (e.g., Bada et al. 1989). On the other hand, N-isotopic depletion under anoxic conditions has been explained by either (1) the bacterial incorporation of ^{15}N-depleted ammonium (Libes and Deuser 1988; Voss et al. 1997; Lehmann et al. 2002), or (2) the removal of a ^{15}N-enriched fraction (Lehmann et al. 2002). Given the above reasoning, we can therefore expect that the Lake Lugano background sediments, which are perennially anoxic, display a $\delta^{15}N_{bulk}$ that has been lowered during anoxic diagenesis. This is consistent with the positive offset between $\delta^{15}N_{db}$ and $\delta^{15}N_{bulk}$ that we observe during the eutrophication period and the earlier nonturbidite sedimentation (orange fill in Fig. 2). The situation is different in turbidite deposits, which are at least transiently characterized by oxygenated porewaters (Span et al. 1992). Turbidity currents are important mechanisms for oxygenating deep lacustrine basins (Hsü and Kelts 1985), and the oxygen within the porewaters of turbidite deposits can diffuse into the adjacent layers of the sediment (Span et al. 1992). In the vicinity of turbidites, oxic diagenesis is therefore expected to raise $\delta^{15}N_{bulk}$, consistent with the observed negative offset between $\delta^{15}N_{db}$ and $\delta^{15}N_{bulk}$ in the Lake Lugano sedimentary record (blue fill in Fig. 2).

To summarize, it is likely that $\delta^{15}N_{bulk}$ in the Lake Lugano sedimentary record has been biased by diagenetic alteration rather than external N input. Importantly, the magnitude of $\delta^{15}N$ alteration was not constant and changed through time in the sedimentary sequence, likely due to changing sediment porewater oxygenation. This contributed to the overall muted response of $\delta^{15}N_{bulk}$ relative to $\delta^{15}N_{db}$ across the entire sedimentary record. This example clearly shows that in paleolimnological reconstructions, $\delta^{15}N_{bulk}$ records need to be regarded with caution when being interpreted in terms of in-lake primary productivity and nutrient cycling, particularly in lakes with changing preservation conditions due to short-term fluctuations in the redox conditions.

Temporal coupling between denitrification and N_2 fixation

The absolute values of both $\delta^{15}N_{bulk}$ and $\delta^{15}N_{db}$ are significantly lower within the turbidites compared to the background sediment ($p < 0.01$ for both for a one-tailed Mann–Whitney test). For $\delta^{15}N_{bulk}$, this is due to the fact that the largest part of the material that is comprised within the turbidite deposits (mainly terrigenous material) was transported into the lake from the catchment during heavy rainfall or flood events, which is likely N-isotopically different from the background sediment that is predominantly produced within the lake. We cannot provide an explanation for the relatively low $\delta^{15}N_{db}$ values within the turbidites, but diatom frustules were likely resuspended and redeposited from elsewhere in the lake during turbidite deposition, and therefore lack any clear temporal context. For the remainder of this manuscript, we thus focus on $\delta^{15}N_{db}$ in the nonturbidite sediment as an un-biased indicator for changes in the N cycle in the Lake Lugano South Basin. Concomitant with the increase in export production during the intensification of eutrophication in Lake Lugano at the beginning of the 1960s, $\delta^{15}N_{db}$ rose by more than 4‰ (Fig. 1c). Increasing $\delta^{15}N_{db}$ in the Lake Lugano South Basin could either be the result of (1) enhanced nitrate utilization, (2) a rise in the $\delta^{15}N$ of the nitrate input to the lake, or (3) enhanced water-column denitrification. Each of these possibilities will be discussed in the following.

In marine environments, where N is a limiting nutrient, it has been shown that the $\delta^{15}N$ of sedimenting organic matter records surface nitrate utilization due to the preferential

incorporation of ^{14}N into the biomass of phytoplankton (e.g., Altabet and François [1994]). With progressing degree of surface nitrate depletion, the organic matter produced in the photic zone will increase in δ^{15}N following Rayleigh distillation systematics (Mariotti et al. [1981]). In the lacustrine environment, where P rather than N limits primary productivity, evidence for a correlation between the δ^{15}N of PON and surface nitrate concentration is sparse (Correll [1999]). In the epilimnion of Lake Lugano, nitrate concentrations declined in 1994 by 75% during the growth season, and yet, showed no correlation with the δ^{15}N of PON (Bernasconi et al. [1997]). Similarly, no consistent relationship was found among surface nitrate concentration, the δ^{15}N of nitrate, and the δ^{15}N of PON during the 1999 growth season (Lehmann et al. [2004b]). Apparently, processes other than nitrate utilization control the δ^{15}N of POM in the South Basin of Lake Lugano (Bernasconi et al. [1997]; Lehmann et al. [2004b]). There is no lake monitoring data available for the mid-1970s to prove that this was also the case during the eutrophication period. Moreover, we cannot fully exclude that, in the presence of excess P during peak eutrophication, N was limiting primary productivity. Yet, in light of the N isotope studies conducted in the 2000s (e.g., Lehmann et al. [2004a], [2004b]), which do not indicate any obvious links between surface water nitrate δ^{15}N and nitrate availability on interannual and seasonal timescales, we disregard enhanced nitrate utilization as the prime mechanism for the rise in diatom-bound δ^{15}N during that time.

Another process that could raise the δ^{15}N of nitrate in Lake Lugano and with that, δ^{15}N$_{db}$, is the input of nitrate from external sources. High-δ^{15}N farmyard manure can enter lakes through direct runoff from land, but the greatest part is probably transported from the catchment into the lake through discharge into the rivers. In 1999, the weighted average δ^{15}N of total inflowing nitrate from precipitation, the main rivers and the inflow from the North Basin into the South Basin of Lake Lugano have been measured to be 7.5‰ (Lehmann et al. [2004b]). This value was identical to the average nitrate δ^{15}N of the water column after winter holomixis (7.5‰ ± 0.6‰), leaving Lehmann et al. ([2004b]) to conclude that catchment runoff and sewage input are more important controls on the δ^{15}N of organic matter than other processes in the Lake Lugano South Basin. If external N input were the main controlling factor for whole-lake nitrate δ^{15}N, one would expect major changes in the sewage/household/catchment runoff into the South Basin to be imprinted in the δ^{15}N$_{db}$ record. For example, in 1976, 80% of the wastewaters generated in the North Basin were diverted into the South Basin of Lake Lugano, and eight wastewater treatment plants were gradually put into operation (Barbieri and Simona [2001]). In 1995, the 4th stage of wastewater treatment (fixed-N removal through denitrification, a process that raises the δ^{15}N of the residual nitrate) was introduced in one of the treatment plants in the lake's catchment. Yet, these changes are not reflected in the δ^{15}N$_{db}$ record. That said, without monitoring data available prior to 1984, we cannot exclude

that the input of high-δ^{15}N manure or sewage during the 1960–1986 period contributed to an overall increase in whole-lake nitrate δ^{15}N. However, given the very high δ^{15}N$_{db}$ values observed (13.2‰), we believe that external nitrate input was not the sole driver responsible for the rise in δ^{15}N$_{db}$ during eutrophication.

Last, we consider the possibility of enhanced water-column denitrification to explain the increase in δ^{15}N$_{db}$ during the eutrophication period. During water-column denitrification, bacteria reduce nitrate to N$_2$ gas under suboxic conditions, a process that leaves the residual nitrate heavily enriched in ^{15}N, with a majority of data from marine environments suggesting an isotope effect between 15‰ and 25‰ (Sigman and Fripiat [2019], and references therein), and somewhat lower N isotope effects reported for lacustrine denitrification zones (Lehmann et al. [2004a]; Wenk et al. [2014]). In previous work, it has been shown that the δ^{15}N of bottom water nitrate in the Lake Lugano South Basin increases up to 27‰ as nitrate concentrations decline (e.g., Lehmann et al. [2004b]; Wenk et al. [2014]). This increase is associated with active microbial denitrification in the deep hypolimnion during the summer months, where due to water-column stratification anoxic conditions prevail. As the stability of the water column decreases during the following fall/winter overturn, the ^{15}N-enriched nitrate from the hypolimnion is mixed in the water column and may contribute to the rise in surface nitrate δ^{15}N that is generally observed from November onwards (e.g., Lehmann et al. [2004b]). We suggest that the rate of water-column denitrification in the hypolimnion of the Lake Lugano South Basin was enhanced and/or water column denitrification was vertically expanded during the peak-time eutrophication period, and was partially responsible for raising the δ^{15}N of the whole-lake nitrate inventory. Our line of reasoning is based on the tight temporal coupling between peaks in δ^{15}N$_{db}$, export production and N$_2$ fixation, the latter inferred from the concentration of HGs in the sediment (Fig. [1], light blue shading).

The logic is as follows (Fig. [4]): During the peak eutrophication period between the 1960s and mid-1980s, enhanced productivity in the lake resulted in higher organic matter fluxes and, in turn, higher organic matter remineralization and oxygen consumption rates, leading to an expansion of the anoxic zone in the lake's water column. Under anoxic conditions, water-column denitrification increased, removing fixed N from the system, while leaving the residual nitrate in the lake heavily enriched in ^{15}N, raising the whole-lake nitrate δ^{15}N. At the same time, phosphorous is remobilized from the sediment into the water column (Span et al. [1992]). Under oxic conditions, inorganic P is adsorbed onto particulate Fe- and Mn-oxyhydroxides, whereas under anoxic/reducing conditions, with the dissolution of the metal oxy-hydroxides, P is released from the P-Fe/Mn complexes into the overlying water (Span et al. [1990], [1992]). Both of these processes, N loss through denitrification and P release from the sediment, caused the N : P ratio in the Lake Lugano
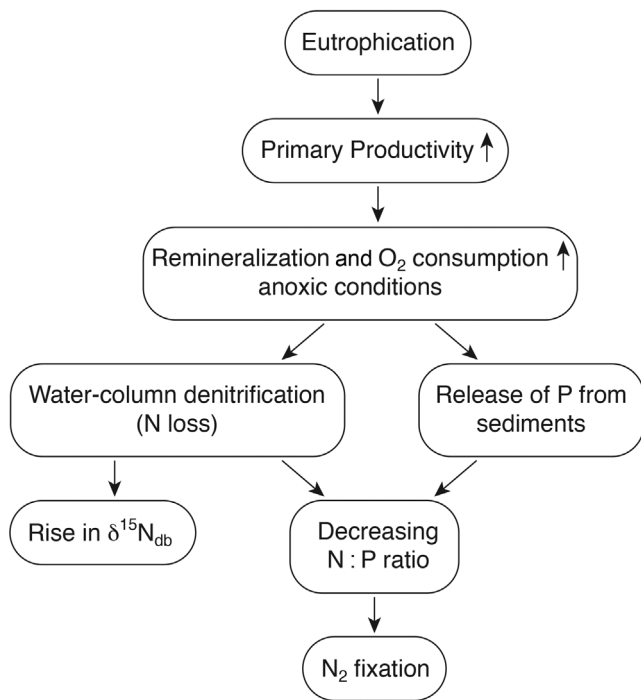
Fig. 4. Flow chart of the processes in the Lake Lugano South Basin during eutrophication.

South Basin to decline. When the N : P ratio in lakes drops below a threshold of $\sim 16:1$, cyanobacteria begin to dominate blooms, stimulating N_2 fixation in surface waters (Flett et al. 1980; Howarth et al. 1988; Funkey et al. 2014). Monitoring data from the Lake Lugano South Basin show that the dissolved inorganic nitrogen to total phosphorous (DIN : TP) ratio in the surface waters was around $16:1$ to $17:1$ in spring in 1984 and 1985 and thus near the threshold for N_2 fixation, and that it increased thereafter. These data thus support our interpretation of enhanced N_2 fixation prior to 1986. In summary, the combined productivity, $\delta^{15}N_{db}$, and HG biomarker data suggest that (1) the elevated $\delta^{15}N_{db}$ values during the peak eutrophication period can be explained by enhanced water-column denitrification, which raised the whole-lake nitrate $\delta^{15}N$ (and in turn, the $\delta^{15}N$ of plankton biomass) and (2) that N_2 fixation is closely coupled, and responsive, to water-column denitrification (and thus, N loss) in this lake basin. Given that both $\delta^{15}N_{bulk}$ and $\delta^{15}N_{db}$ values remained high during episodes of highest N_2 fixation implies that water-column denitrification must have overwhelmed any effect of N_2 fixation on the whole-lake nitrate $\delta^{15}N$ signature (Supplementary Section 2).

Partial recovery from (hyper-)eutrophic conditions

The input and in-lake concentrations of P into the Lake Lugano South Basin gradually decreased following the restoration measures (Barbieri and Mosello 1992; Lepori and Capelli 2021; Lepori et al. 2022b). On the other hand, inputs and

in-lake concentrations of N into the Lake Lugano South Basin increased, due to an increasing population density, an increase in nitrate and ammonium in atmospheric deposition until 2005, and due to the input of sewage from the North Basin into the South Basin (Barbieri and Mosello 1992; Rogora et al. 2016), leading to an increase in the N : P ratio. Our export production reconstructions indicate that organic matter accumulation in the sediment decreased in the mid-to-late-1980s (yet, without returning to baseline values observed prior to the 1930s, Fig. 1), which is expected to decrease the amount of organic matter remineralization at depth, and, therefore, reduce overall bottom-water anoxia. This in turn is expected to decrease water-column denitrification rates, leading to an overall reduction in N loss in the hypolimnion, and to a reduced release of P from the sediments. As a result, the N : P ratio in the lake further increased, causing N_2 fixation rates to decline, which is consistent with both the HG concentration data, as well as the increasing DIN : TP ratio observed in the monitoring data after 1986. Weaker water-column denitrification also resulted in a decline in the whole-lake nitrate $\delta^{15}N$ signature, as recorded in the declining $\delta^{15}N_{db}$ data since the mid-1980s, but a decline in the input of high-$\delta^{15}N$ manure and sewage might have contributed as well. Overall, $\delta^{15}N_{db}$ after the mid-1980s remained well above the values prior to the 1930s. Taken together, our paleo-proxy reconstructions agree well with the monitoring data, which indicate that the Lake Lugano South Basin has partially recovered from hyper-eutrophic conditions, without reaching yet the oligotrophic state that characterized the lake prior to the 1930s (Barbieri and Mosello 1992; Lepori et al. 2018, 2022b).

Conclusions and outlook

This study reports, for the first time, on the successful application of the diatom-bound nitrogen isotope paleo-proxy to lacustrine sediments. Based on $\delta^{15}N_{db}$ measurements in a sediment core from eutrophic Lake Lugano (Switzerland), we reconstructed the nitrogen cycle and paleoenvironmental conditions in the studied lake over the past 125 years. Comparison between bulk sediment and diatom-bound $\delta^{15}N$ data indicates that $\delta^{15}N_{bulk}$ has been biased by diagenetic N-isotope alteration rather than external N input in Lake Lugano. The magnitude of the observed $\delta^{15}N$ alteration was not constant and changed over time, probably as a function of transient sediment porewater oxygenation in association with sporadic turbidite sedimentation events (with increased $\delta^{15}N_{bulk}$ occurring in oxygenated sediments and decreased $\delta^{15}N_{bulk}$ occurring in anoxic sediments). Consequently, caution is advised in paleolimnological studies, when interpreting bulk sedimentary $\delta^{15}N$ as an indicator for past changes in the N sources and sinks, trophic state, primary productivity, or other lake-internal N cycling processes. On the other hand, the diatom-bound N isotope dataset provides an unbiased record of past changes in the N cycle of the Lake Lugano South Basin. During peak-time eutrophication

between the 1960s and the mid-1980s, export production increased, leading to an expansion of anoxia in the water column. Higher water-column denitrification caused a rise in the $\delta^{15}N$ of the whole-lake nitrate inventory (and thus, $\delta^{15}N_{db}$) as nitrate concentrations declined, but input of high-$\delta^{15}N$ manure and sewage might also have contributed to increasing $\delta^{15}N_{db}$. Concomitantly with a release of P from the sediments under anoxic conditions, this led to the decline in the N:P ratio of the lake, stimulating N_2 fixation in the surface waters. Our dataset thus provides evidence of a temporal coupling of water-column denitrification and N_2 fixation in a hyper-eutrophic lake. Such a coupling between N_2 fixation and denitrification has been described previously for oxygen-depleted zones in the ocean (Deutsch et al. 2007) and for the water column of the meromictic Lake Cadagno (Halm et al. 2009).

In this study, the diatom-bound N isotope paleo-proxy has proven a faithful indicator of the change in the trophic status of a lake as it transitioned first from oligotrophic to hyper-eutrophic conditions, and then partially recovered to mesotrophic conditions. We thus propose $\delta^{15}N_{db}$ as a promising new isotope proxy for future paleolimnological studies to explore the paleoenvironmental history and nutrient cycling of lakes with different trophic states, without bias by diagenetic alteration and external N input.

Data availability statement

The data that support the findings of this study are openly available at the Pangaea archive (www.pangaea.de), at https://doi.pangaea.de/10.1594/PANGAEA.967003.

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Conflict of Interest

None declared.