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Abstract

Organic carbon (C_{org}) and bulk nitrogen (N_{bulk}) isotope compositions (respectively $\delta^{13}C_{org}$ and $\delta^{15}N_{bulk}$) of sedimentary rocks deposited in the Archean and Proterozoic eons are commonly used as proxies for the biomass $\delta^{13}C_{org}$ and $\delta^{15}N_{bulk}$ values in paleo-environment and paleo-ecosystem studies. Many sedimentary rocks from these eons were deposited under ferruginous conditions, but it remains unknown whether C and N isotope signatures are preserved during diagenetic degradation of organic matter under this chemical state. Lake Pavin, Massif Central, France, is a permanently stratified freshwater lake with anoxic and ferruginous deep waters and represents a unique natural site for evaluating the impact of organic matter decomposition predominantly through iron redox cycling and methanogenesis. Here we present C and N isotope compositions of sediment collected from traps placed at different depths in the water column and from cores recovered in the anoxic zone of the lake. The $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ values in the living biomass of Lake Pavin are estimated from the shallowest sediment traps. The biomass $\delta^{13}C_{org}$ values show a seasonally-controlled bimodal distribution, around -23.3 ± 1.7 ‰ (2σ) in September and November (organic matter produced in summer and autumn) and -28.0 ± 2.6 ‰ (2σ) in April and June (organic matter produced in winter and spring). These seasonal variations are attributed to variable $\delta^{13}C$ values in dissolved inorganic carbon, inherited from fluctuation in the relative rates of photosynthesis *versus* respiration. The $\delta^{15}N_{tot}$ values are near -1 ‰ for most seasons, indicating that the dominant N source to photosynthetic biomass is atmospheric N_2.

Along the water column profile, organic matter mineralization released about 20 % of C and 30 % of N, with only moderate effects on $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ (decreased by 1.3 and 0.4 ‰, respectively). Although the water column data show significant seasonal variations, global annual fluxes, estimated from regular trap collection over 15 months, are remarkably consistent with the top sediment composition. In the sedimentary column, microbial degradation through ferric iron reduction and methanogenesis induces a loss of 40-50% C_{org} and 50-60% N_{tot}, with negligible modification of $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$. We conclude that although the C/N ratio increases significantly as organic matter is degraded in the water column and in the first ~15 cm of the sedimentary column, $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ are well preserved, supporting the idea that C and N isotope signatures can be reliably interpreted as tracers of primary biomass in sedimentary rocks deposited in anoxic and ferruginous environments.

1. Introduction

Carbon and nitrogen are both central to life, representing the main elements in organic molecules from terrestrial and extra-terrestrial materials. The evolution of carbon and nitrogen biogeochemical cycles through time can be delineated from the sedimentary record back to the Archean period (3.8-2.5 Ga) (*e.g.* Thomazo and Papineau, 2013; Ader *et al*., 2016; Stüeken *et al*., 2016). Carbon and nitrogen isotope compositions of sedimentary organic matter have been widely used for reconstructing paleobiospheric evolution (Pinti *et al*., 2001; 2009; Stüeken *et al*., 2015a), variations in biological productivity (Karhu and Holland,1996; Godfrey and Falkowski, 2009; Kump *et al*., 2011; Hashizume *et al*., 2016), C and N sources (Beaumont and Robert, 1999; Stüeken *et al*., 2015b; Luo *et al*., 2018) as well as the redox state of the ocean (Beaumont and Robert, 1999; Garvin *et al*., 2009; Thomazo *et al*., 2011; Ader *et al*., 2014; Zerkle *et al*., 2017; Kipp *et al*., 2018; Cheng *et al*.,2019; Koehler *et al*., 2019).

The use of C and N isotope compositions as proxies of primary living organisms and/or sources relies on the assumption that they are not significantly modified during diagenesis, and eventually subsequent metamorphism (*e.g.* Ader *et al*., 2016; Stüeken *et al*., 2016; Quan and Adeboye, 2021). However, organic C and N isotope signatures might be altered during diagenesis through various aerobic or anaerobic microbial processes in the water column and sediments. For instance, based on laboratory experiments and natural samples, early diagenetic modification of organic matter was proposed to decrease $\delta^{13}C_{org}$ values by ~1.5‰, both in oxic and anoxic conditions (Lehmann *et al*., 2002), reflecting preferential removal of ^{13}C-enriched organic compounds. In contrast, other studies on natural sediments demonstrated roughly constant $\delta^{13}C_{org}$ values during diagenesis (*e.g.* Meyers, 1994; Freudenthal *et al*., 2001; Chen et al., 2008; Kohzu *et al*., 2011).

In the case of nitrogen, diagenetic alteration of its isotope composition has been shown to follow distinct patterns for either oxic or anoxic environmental conditions. Under oxic depositional conditions in the water column and/or top sediments and with long oxygen exposure-time, bulk $\delta^{15}N$ values are increased by +3 to +5 ‰, as observed in both natural sites and laboratory studies (Altabet *et al*., 1999; Freudenthal *et al*., 2001; Lehman *et al*., 2002; Prahl *et al*., 2003; Robinson *et al*., 2012; Gaye *et al*., 2009). The precise origin of this increase is not completely understood but is likely related to isotopic fractionation during N release, potentially through preferential degradation of ^{14}N-depleted amino acids (Gaye *et al*., 2009; Möbius *et al*., 2010), or redox reactions such as partial nitrification during diagenesis (Prokopenko *et al*., 2006). Under anoxic conditions or with high sediment depositional rates (with limited O_2 exposure), bulk $\delta^{15}N$ values

are generally either unmodified or slightly lowered, by less than 3 ‰ (Altabet *et al*., 1999; Lehmann *et al*., 2002; Thunell *et al*., 2004; Möbius *et al*., 2010; Chen *et al*., 2008). This suggests that the N isotope compositions of sediments deposited under anoxic conditions are preserved with good fidelity. However, while this has been extensively documented for environments where microbial anaerobic respiration is based on nitrate and sulfate, it may not be the case for other types of anoxic aquatic systems. In fact, an increase in $\delta^{15}N$ of up to 3‰ has been recently documented in the anoxic and alkaline Dziani Dzaha lake in which no electron acceptors are present at depth and most of the organic matter is mineralized by methanogenesis (Cadeau *et al*., 2021). The preservation of $\delta^{15}N$ during early diagenesis in anoxic and ferruginous systems where ferric iron is a dominant electron acceptor thus remains unknown.

The present work aims at filling this knowledge gap by evaluating the effect of diagenesis on C and N isotope compositions of organic matter under anoxic and ferruginous conditions, similar to those prevailing in the Archean and Paleoproterozoic oceans. Lake Pavin, France, has been selected as a modern analog for ancient oceans because it is permanently stratified, with Fe-rich deep waters where Fe(II) accumulates up to 1.2 mM (Busigny *et al*., 2014, 2016) and has been extensively studied over the past 40 years for its biodiversity (Amblard, 1986; Grami *et al*., 2011; Lehours *et al*., 2005, 2007; Biderre-Petit *et al*., 2011), geochemistry (Michard *et al*., 1994, Viollier *et al*., 1997; Assayag *et al*., 2008; Busigny *et al*., 2014) and mineralogy (Schettler *et al*., 2007; Cosmidis *et al*., 2014). Microbial degradation of organic matter in Lake Pavin is essentially based on iron-reduction and methanogenesis (Lehours *et al*., 2009; Biderre-Petit *et al*., 2011), two processes that were probably established and possibly major in Archean and Paleoproterozoic sedimentary environments (Vargas *et al*., 1998; Konhauser *et al*., 2005; Ueno *et al*., 2006). In the present contribution, four sediment traps were set up at different depths in the water column of Lake Pavin, and collected regularly over 15 months. Three sediment cores were collected in the anoxic zone to examine potential lateral variabilities. Carbon and nitrogen isotope compositions were measured in all samples, allowing assessment of possible diagenetic modifications in the water and sediment columns, as well as seasonal variations. A few plant fragments and litters collected from the lake border were also analyzed and demonstrate that detrital organic matter contribution to the lake sediment is negligible. Our data demonstrate for the first time that C and N isotope signatures of primary biomass are faithfully transferred to sediments in a modern anoxic and ferruginous lacustrine environment (although up to ~80% of organic matter is degraded). These results suggest that C and N isotopic tracers can be safely used as paleo-proxies in the Precambrian rock record if subsequent metamorphism is not extreme.

2. Description of Lake Pavin

Lake Pavin is a freshwater lake located in the Massif Central, France, 35 km to the southwest of Clermont-Ferrand city (45°29.740'N - 2°53.280'E; Fig. 1). It is a volcanic lake resulting from phreatomagmatism. Its geometry is quasi-circular, with a diameter of 750 m and a maximum depth of 92 m. The age of Lake Pavin has been estimated to 6970 ± 60 years BP from studies of volcanic products (Juvigné et Gewelt, 1987) and sediment core dating (Chapron *et al*., 2010). Lake Pavin is meromictic, *i.e.* permanently redox-stratified, with anoxic and ferruginous deep waters from *ca*. 60 to 92 m depth (*i.e*. the monimolimnion), topped by oxic shallow waters from 0 to *ca*. 60 m (*i.e*. the mixolimnion) (Michard *et al*., 1994; Viollier *et al*., 1995; Fig. 1c). Over the last ten years, the oxic-anoxic interface was shifted upward and is now located between 50 and 55 m depth (see Fig.S4 in Busigny *et al*., 2021; Bidaud *et al*., 2022). In the deep waters, ferrous iron, $Fe(II)_{aq}$, is the main dissolved cation, with concentrations up to 1.2 mM. Dissolved iron is efficiently confined below the oxic-anoxic boundary due to the formation of insoluble ferric iron species, $Fe(III)_s$, by oxidation with O_2 and other oxidants (*e.g.*, NO_3^-, Mn(IV)) at the redoxcline. This produces a significant peak of turbidity related to the formation of iron particles. The $Fe(III)_s$ particles settle and are effectively reduced in the anoxic waters and at the lake bottom by reaction with organic matter, yielding soluble $Fe(II)_{aq}$. It then diffuses upward in the water column and finally is re-oxidized to Fe(III) at the redox boundary (Busigny *et al*., 2016). This classic cycle is known as the "iron-wheel" (Campbell and Torgersen, 1980). The lake has remarkably low sulfate concentrations (< 20 µM), and shares some similarities with Archean oceans (Busigny *et al*., 2014) - that is, high dissolved Fe(II) concentrations and low levels of sulfate and sulfide. Mineral particles in the water column and sediments include mostly diatom frustules, Fe-phosphate, Fe-(oxy)hydroxide, detrital silicate (clays, sanidine), and siderite together with low amounts of iron sulfide (Michard *et al*., 1994, Viollier *et al*., 1997; Schettler *et al*., 2007; Bura-Nakic *et al*., 2013; Cosmidis *et al*., 2014; Busigny *et al*., 2016). The sedimentation rate estimated from radiocarbon dating on leaves from sediment cores is on average 0.5 mm yr^{-1} (Chapron *et al*., 2010).

The biomass of the lake is essentially composed of phytoplankton and zooplankton in shallow waters, but Bacteria, Archaea, viruses and fungi are present in the whole water column (Grami *et al*., 2011). The density of the microbial community is elevated in the anoxic layer, and Archaea represented the largest microbial group in the water column, with a mean

Archaea/Bacteria ratio around 1.5 (Lehours *et al*., 2005). The dominant phytoplankton species are alternatively diatoms (bloom in spring) and cyanobacteria (bloom in summer) (Amblard, 1986, 1988; Stebich *et al*., 2005). Several studies report variations in the dominant phytoplankton species with depth (Amblard, 1986) and with the seasons (Bourdier and Amblard, 1987; Amblard, 1988). These are summarized in Tables S1 and S2 (supplementary materials). Other microorganisms have been identified below the chemocline which enable organic matter mineralization under anoxic conditions including methanogenic Archaea (Lehours *et al*., 2005), iron-oxidizing or -reducing Bacteria (Lehours *et al*., 2007, 2009) and sulfate-reducing Bacteria (Biderre-Petit *et al*., 2011). The organic matter content of Lake Pavin sediments is high and ranges from 4 to 18 wt% (Restituito, 1984a; Schettler *et al*., 2007; Cosmidis *et al*., 2014).

3. Methods

3.1. Samples collection

3.1.1. Water column sampling for dissolved N species analysis

The carbon biogeochemical cycle in the water column of Lake Pavin was previously studied through dissolved inorganic carbon (DIC) concentrations (Michard *et al*., 1994) and isotope compositions (Assayag *et al*., 2008), as well as methane concentrations (Lopes *et al*., 2011; Jézéquel *et al*., 2016). In contrast, the nitrogen biogeochemical cycle has never been investigated, although dissolved NO_3^- and NH_4^+ concentrations were reported for the oxic and anoxic zones respectively (Michard *et al*., 1994). A sampling of the water column was therefore conducted along a depth profile in June 2011 for measurement of N isotope compositions and concentrations of nitrate (NO_3^-) and ammonium (NH_4^+). Water samples were collected using a 5L Niskin bottle and were subsequently filtered under N_2 atmosphere (O_2-free), at 0.7 µm using GF/F filters. The samples were stored in clean Nalgene bottles at 4°C for geochemical and/or isotope analyses. For isotope analysis of NH_4^+, water samples were acidified in the field to pH < 6 with sulfuric acid (H_2SO_4) for later precipitation of ammonium sulfate salt (see section 3.2.1 below).

3.1.2. Sediment traps

199 Sediment traps (Uwitec, each made up of a pair of PVC tubes 60 cm long and 90 mm in diameter
200 terminated in 2L Nalgene bottles) were deployed near the center of the lake along a cable at 4
201 different depths in the water column (Fig. 1c): 25 m (oxic zone), 56 m (just above the
202 chemocline), 67 m (below the turbidity peak in the anoxic zone), and 86 m (near the lake
203 bottom). The traps were set up from March 2011 to June 2012. Five sampling campaigns were
204 organized in June 2011, September 2011, November 2011, April 2012 and June 2012. Since
205 the traps were left in place for more than 2 months, organic matter could have been partially
206 degraded within the traps. Accordingly, all traps were filled with 1 g of $CuCl_2$ to kill any
207 microorganisms and avoid degradation in the trap, which would represent a sampling artifact
208 (a $CuCl_2$ > 2 mg/L is lethal). In brief, the trap was filled with lake surface water, $CuCl_2$ powder
209 was dispersed in the trap and immediately dissolved, then the trap was gently moved down until
210 reaching the desired depth. The use of $CuCl_2$ as a poison induced dark mineral precipitation in
211 the two traps of the anoxic zone (*i.e.* 67 and 86 m depth), likely reflecting copper sulfide
212 precipitation. Since this precipitate modified the mass of sediments recovered in the anoxic trap,
213 we have not used the bulk carbon and nitrogen concentrations (in wt%) but rather used the mass
214 flux of organic carbon and nitrogen (in mg m^{-2} day^{-1}), which is independent of the mass of
215 copper sulfide precipitate. It is worth noting that a few traps were settled without $CuCl_2$. For
216 those one, we observed that organic matter contents were always lower than in the
217 corresponding poisoned traps while C/N ratios were higher, confirming a degradation effect
218 and thus a sample bias due to temporal evolution. This motivated the unique use of poisoned
219 traps in the present study.
220 During each sampling campaign, the traps were collected and immediately transferred to a
221 glove bag under anoxic N_2 atmosphere. The oxygen content was monitored with an Oxi 340i
222 WTW oxygen meter and was always below the detection limit (*i.e.* < 0.1 mg/L). The uppermost
223 water of the traps was removed with a syringe. The residual water and sediment were mixed
224 together and homogenized before being transferred to 50 mL centrifuged tubes. The tubes were
225 shortly removed from the glove bag and immediately centrifuged on the field at 5300 rpm for
226 10 min, before being placed back in the glove bag to remove the overlying water under anoxic
227 conditions. Finally, the sediment samples were sealed under anoxic conditions and brought back
228 to the laboratory.
229
230 *3.1.3. Anoxic sediment cores*
231

Three different cores collected in the anoxic zone of Lake Pavin were studied to evaluate potential lateral variability in the sediment depending on the depth of emplacement (Fig. 1). The detailed collection procedure and sample treatment have been described in a previous contribution (Busigny *et al*., 2014). The first core (C1) was sampled at 60 m depth, near but just below the redox transition zone (Michard *et al*., 1994; Viollier *et al*., 1995). The second core (C2) was collected at 65 m depth, around the peak of sulfate reduction zone (Bura-Nakic *et al*., 2009). The last core (C6) was collected at the lake bottom, *i.e.* depth of ~ 92 m. Sediment cores were transferred into a glove bag and placed under anoxic conditions (pure N_2 atmosphere) immediately after collection, on the field. They were processed and split into cm-scale fractions along the core's vertical axis in the glove bag. The pore waters were separated from the solid phases using Rhizon samplers (cut-off threshold ≈ 0.15 μm). The solid phases were then frozen and transferred to the laboratory. The total thickness of sediments in each core represents 10 to 14 cm. It records a time span of ~280 years, assuming a mean sedimentation rate of 0.5 mm yr^{-1} (Chapron *et al*., 2010).

3.1.4. Plants and soil samples

Potential detrital sources in Lake Pavin sediment may derive from plant fragments and soils surrounding the lake. Two main types of trees are found in Lake Pavin: beech and spruce (*e.g.* Stebich *et al*., 2005). We collected leaves, thorns and soils in June 2011, on the south-eastern edge of Lake Pavin crater. All samples were placed in 50 mL Falcon® tubes and frozen immediately after collection until further treatments in the laboratory. Brown beech leaves were taken from broken branches on the ground. Spruce thorns were sampled directly from the trees by scraping branches. Litters from two different locations on the southeast flank of the crater were collected using a clean plastic spatula. They are supposed to be representative mixtures of plant fragments, soil and roots.

3.2. Analytical methods

3.2.1. Analysis of dissolved N species: nitrate and ammonium

Nitrate and ammonium concentrations were measured at IPGP by colorimetry (AxFlow Quaatro continuous flow autoanalyzer). The limit of quantification is *ca*. 0.1 μM for both species.

The $\delta^{15}N$ of nitrate was measured by chemical reduction of NO_3^- to NO_2^- with spongey cadmium followed by reduction of NO_2^- to N_2O with azide (McIlvin and Altabet, 2005). Naturally occurring NO_2^- present in the samples was previous removed by reaction with sulfamic acid prior to cadmium reduction (Granger and Sigman, 2009). $\delta^{15}N$ was determined on the N_2O gas produced by mass spectrometry on a Delta V Plus. Standards IAEA-N3, USGS-34 and USGS-35 were used for calibration and reproducibility on replicate measurements was better than 0.3 ‰.

For isotope analysis of NH_4^+, water samples previously acidified with sulfuric acid were evaporated at 80°C for seven days in order to precipitate ammonium sulfate (method modified after Li *et al*., 2012). $(NH_4)_2SO_4$ salts were recovered and loaded in quartz tubes, sealed under vacuum, with a mix of Cu-CuO wires. The tubes were then heated to 800°C for 6 hr and slowly cooled down to transform nitrogen to N_2. Finally, the tubes were cracked under vacuum, N_2 was purified and analyzed in dual-inlet mode on a Delta+XP mass spectrometer. This method was tested and optimized by dissolving IAEA-N1 and IAEA-N2 international standards in NH_4-free Lake Pavin water (from 25 m depth). The results demonstrated a good yield of ammonium recovery between 87 and 105 %, and $\delta^{15}N$ values of 0.3 ± 0.5‰ and 20.0 ± 0.5 ‰ for IAEA-N1 and -N2 standards, in good agreement with the values recommended for these two standards (Gonfiantini *et al*., 1995; Busigny *et al*., 2005).

3.2.2. Solid samples preparation (drying, grinding)

Once in the laboratory, sediment traps and sediment core samples, as well as the two litters, were dried and ground. The sealed sediment traps and the litters were loaded in a Jacomex® glove box under Ar (Alphagaz 1, Air Liquide) and free of O_2 (<5 ppm), and were dried in a desiccator under a vacuum of ~30 mbar. The sediment samples from cores C1 and C2 were fully dehydrated by freeze-drying, while those from the core C6 were dried by evaporation at 50°C in an oven for 48 hr. All dried sediment samples (traps and cores) were then finely crushed and homogenized in an agate mortar, while any visible plant fragments such as leaves or stems were discarded. The two litter samples, brown beech leaves and spruce thorns were also crushed and carefully homogenized in an agate mortar.

3.2.3. Isotopic measurements by elemental analyzer

Carbon and nitrogen concentrations and the isotope compositions of solid sediment samples (traps and cores), leaves, thorns and litters were determined using a Flash EA1112 elemental analyzer coupled to a Thermo Finnigan Deltaplus XP mass spectrometer via a Conflo IV interface (Thermo Fisher Scientific, Waltham, MA, United States) (Cheng *et al*., 2019; Cadeau *et al*., 2021). Sample powders were weighed and loaded into tin capsules for analysis. Blanks estimated based on empty capsules show a negligible contribution (lower than 0.5 % of the total C and N amounts in the samples). Four internal standards (*i.e.* organic-rich soils and sediments), calibrated against international standards, were used to calculate the $\delta^{13}C$ and $\delta^{15}N$ values of all samples. The external precision on $\delta^{13}C$ and $\delta^{15}N$, determined from replicate measurements of standards, was better than ±0.34 ‰ and ±0.30 ‰ (2σ), respectively. The long-term reproducibility obtained on C and N contents, and C/N ratio, for internal standards was always better than ± 6,7 and 6 % relative to the measured values (2σ), respectively. Since siderite, an iron carbonate, can be locally present in some layers of Lake Pavin sediments (Schettler *et al*., 2007), our sample powders were decarbonated using 0.5 M HCl at room temperature for four days, rinsed four times with distilled water and dried in an oven at 40°C for two days. Carbon and nitrogen concentrations and isotope compositions were measured in both untreated and decarbonated samples. The C concentration, $\delta^{13}C$ and $\delta^{15}N$ were undistinguishable in both cases but the N concentration of decarbonated was always lower in decarbonated samples (resulting in higher C/N ratios). Eight tests on sediment traps showed that decarbonation induced a loss comprised between 3 to 31 % of the total nitrogen, with a mean value of 22 ± 11 % (1σ; n=8). Twelve tests on sediment samples randomly selected from the three cores gave N loss between 16 and 37, with a mean value of 28 ± 7 % (1σ; n=12). Similar N alteration due to HCl treatment has already been observed by Brodie *et al*. (2011a, b). Therefore, in the following sections, we will only present and discuss the data obtained on untreated samples.

4. Results

4.1. Dissolved nitrate and ammonium in the water column

The concentrations and isotope compositions of nitrate and ammonium in Lake Pavin water column are reported in Table 1 and illustrated in Figure 2. Ammonium is virtually absent (<0.2 µmol L^{-1}) in the oxic zone, but its concentration increases progressively below 50 m depth, from 2.2 to 1750 µM (Table 1). Its isotope composition is remarkably constant in the anoxic zone, with $\delta^{15}N$ values near 0‰, except a positive value of 4.7 ‰ near the redox boundary (56

m; Fig. 2). Nitrate concentrations show a very distinct behavior with a peak up to 18 µmol/L between 40 and 50 m, but very low concentrations (<0.2 µM) in shallow (0-20 m) and deep (below 60 m) waters. Only four water samples yielded nitrate N isotope ratios and showed $\delta^{15}N_{NO3-}$ values increasing with depth (30 to 50 m), from -2.8 to 5.5 ‰. Similar profiles of ammonium and nitrate were described in other redox stratified systems like the Black Sea, Saanich Inlet, Framvaren Fjord (Velinsky *et al*., 1991; Velinsky and Fogel, 1999) or Cariaco Basin (Thunell *et al*., 2004).

4.2. Carbon and nitrogen fluxes and isotope compositions in sediment traps

The abundances of C and N are reported as a mass flux integrated over the time (in mg m^{-2} day^{-1}). The fluxes and isotope compositions of organic C and N deposited in the sediment traps are illustrated in Figure 3 (data in Table 2). The fluxes of C and N show significant seasonal variabilities, with a total range from 27 to 91 and 3.2 to 13 mg m^{-2} day^{-1} respectively. The depth profile variations of C and N are similar for the various seasons. Both C and N fluxes show a marked decrease with depth in the water column in June 2011 and June 2012. In contrast, September and November 2011 display a flux increase between the oxic and anoxic zones, but a decrease at the bottom of the anoxic zone (Fig. 3). Fluxes from April 2012 were roughly constant along the depth profile. Interestingly, the fluxes in June 2012 are substantially higher compared to other seasons. This could reflect a bloom of diatoms subsequent to a mixing of the mixolimnion (due to nutrients supply to the lake surface).

Carbon isotope composition shows a strong seasonal variability (Fig. 3c), with more negative values in June 2011, April 2012 and June 2012 (avg. $\delta^{13}C = -28.0 \pm 2.6$ ‰, 2σ, n=12) compared to September and November 2011 (avg. $\delta^{13}C = -23.3 \pm 1.6$ ‰, 2σ, n=8). Nitrogen isotope variations differ from those of carbon isotopes. Most $\delta^{15}N$ values in sediment traps range between -2 and -1 ‰, except a single value near 0‰ at 25 m depth in April 2012, and positive values around +0.7‰ at all depth in June 2012 (Fig. 3d). This finding also supports a mixing of the mixolimnion in April 2012, then fully recorded in the traps of June 2012. C/N ratios show a moderate increase with depth for all seasons, and a total range between 6.8 and 9.7 (Fig. 3e).

4.3. Carbon and nitrogen concentrations and isotope compositions in sediment cores

The C and N data of the three sediment cores are reported in Table 3 and illustrated in Figure 4. The organic carbon concentration in sediment samples from core C1 (collected at 60 m depth, near the redox transition zone) decreases with depth from 13.8 to 6.9 wt% (Fig. 4a, Table 3). In

365 contrast, organic C isotope compositions do not show any specific trend with depth and are
366 characterized by $\delta^{13}C_{org}$ values between -27.7 and -26.1 ‰ (avg. -26.8 ± 1.1 ‰, 2σ). Total N
367 concentration decreases significantly with depth from 1.9 to 0.8 wt% (avg. 1.2 ± 0.8 wt%, 2σ)
368 while $\delta^{15}N_{tot}$ values exhibit modest variability from -1.3 to -0.9 ‰ (avg. -1.2 ± 0.4 ‰, 2σ). The
369 C/N ratio increases moderately from 8.6 to 9.9 (avg. 9.4 ± 1.1) along the depth profile (Fig. 4e).
370 Sediments from core C2 (~65 m depth, near the peak of sulfate reduction) are remarkably
371 similar to those of the core C1 (Fig. 4). Organic carbon content decreases with depth from 13.8
372 to 6.8 wt%. The $\delta^{13}C_{org}$ values vary between -27.4 and -26.2 ‰ (avg. -26.8 ± 0.7 ‰, 2σ). Total
373 N content and isotope composition range from 0.8 to 1.9 wt% (avg.1.2 ± 0.6 wt%, 2σ) and from
374 -1.3 to -0.6 ‰ (avg. -1.0 ± 0.4 ‰, 2σ) respectively. The C/N ratio increases with depth from
375 8.6 to 10.5 (avg. 9.8 ± 1.3).
376 Sediment from the core C6 collected at the lake bottom (92 m) is globally similar to cores C1
377 and C2 (Fig. 4a-d), except for C/N ratio (Fig. 4e). Organic C and total N concentrations in core
378 C6 both decrease with depth from 11.0 and 6.8 wt% and 1.1 to 0.6 wt%, respectively. The
379 $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ show limited variability, with values between -27.3 to -25.2‰ (avg. -26.6 ±
380 1.9 ‰) and -1.0 to 0.07 ‰ (avg. -0.6 ± 0.8 ‰) respectively. The C/N ratios in core C6 sediments
381 are significantly higher than those of the two other cores, with values from 11.3 to 13.2 (Fig.
382 4e).
383
384 **4.4. Carbon and nitrogen concentrations and isotope compositions in plant fragments**
385 **and soils**
386 The C and N concentrations and isotope compositions of the beech leaves, spruce thorns and
387 litters are given in Table 4. The mean C concentrations and $\delta^{13}C_{org}$ values are 45.9 ± 3.3 wt%
388 and -30.8 ± 0.8 ‰ for leaves, 26.5 ± 2.8 wt% and -29.8 ± 1.4 ‰ for thorns, and near 14 wt%
389 and -28 ‰ for the two litters (Table 4). The N concentrations and $\delta^{15}N_{tot}$ values average 1.3 ±
390 0.4 wt% and -6.7 ± 0.5 ‰ for leaves, 11 ± 0.3 % and -1.2 ± 0.8 ‰ for spruce thorns, and a
391 range from 0.9 to 1 wt% and -2.4 to -3.2 ‰ for the two litters. The C/N molar ratios are high
392 compare to Lake Pavin traps and core sediments, with values averaging 40.5, 27.7 and 17 for
393 leaves, thorns and litters, respectively (Table 4). Moreover, it must be noted that these values
394 do not take into account possible degradation happening once the samples have fallen into the
395 lake.
396

5. Discussion

5.1. Assessing the contribution of detrital carbon and nitrogen to Lake Pavin sediments

Lake Pavin is not a closed system and allochtonous detrital components may contribute to the C and N budget in the lake sediments. It is essential to determine the importance of these sources before discussing the processes at play in the water column and sediments. The bedrock of Lake Pavin consists of volcanic rocks, essentially rhyolites and basalts, which both contain negligible amount of C and N relative to the high flux of organic matter carried by primary producers thriving in the water column. Here we have not analyzed volcanic rocks from Lake Pavin but for comparison, N contents in other basalts and rhyolite are in a range from 3 to 23 ppm, respectively (Boocock *et al*., 2023), about three orders of magnitude lower than N concentrations in Lake Pavin sediments (*i.e.* 0.6 to 1.9 wt%, Table 3). This is thus unlikely that they contribute significantly to the C and N budget in Lake Pavin sediment. A possible source of allochtonous C and N could be provided by plants and/or soils surrounding the lake. Although any visible leaves or stems were discarded from the sediment samples before analyses, the presence of micrometer-scale fragments can hardly be ruled out. The data obtained on beech leaves, spruce thorns and litters (Table 4) can be compared to those of core sediments (Table 3) to explore if they can be considered as a source of organic matter in our samples. $\delta^{13}C$ and $\delta^{15}N$ of leaves, thorns and litters are slightly more negative than those of lake sediments, with average values from -30.8 to -27.9 ‰ and -6.7 to -1.2 ‰ *versus* -26.8 to -26.5 ‰ and -1.2 to -0.6 ‰, respectively (Tables 3 and 4). The greatest difference is observed for C/N ratio, with values of 9.4 to 12.3 in core sediments (Table 3) *versus* 16.3 to 40.5 (Table 4). The low C/N ratios of lake sediments are better explained by a dominant contribution of organic matter from the water column, as also recorded in sediment traps (Table 2). The sediments from the lake bottom (core C6, 92m, Table 3) has a C/N ratio (12.3) relatively close to those of litters (16.3-17) but the $\delta^{15}N$ values of these sediments are markedly heavier (-0.6 ‰) and contrast with the lighter values measured in litters (-2.4 to -3.2 ‰). Accordingly, we can reasonably assume that our lake sediment samples contain only a minor proportion of allochthonous organic matter but a dominant biomass from primary producers of the water column. This conclusion is also consistent with further calculation showing that global annual sediment particles recorded in traps are consistent with top core sediment values (see section 5.4 below).

5.2. Carbon and nitrogen isotope signatures of primary biomass in Lake Pavin

In this section, we examine the origin of C and N isotope compositions of the primary biomass of Lake Pavin. These isotope compositions depend essentially on the composition of the chemical compounds that are assimilated by living organisms (*i.e.* sources of C and N), as well as isotope fractionations related to the assimilation processes. The primary biomass in Lake Pavin is essentially produced between 10 and 15 m depth in the water column (Amblard, 1986, 1988), identified on depth profile by the peaks of O_2 production and turbidity (Assayag *et al*., 2008; Busigny *et al*., 2016). Thus, the most appropriate and best preserved samples recording the primary biomass are the sediment traps collected at 25 m depth.

5.2.1. Origin of the C isotopic composition of organic matter in Lake Pavin

The C isotope composition of organic matter in sediment traps from 25 m depth shows a bimodal distribution, closely tied to seasonal variations, with mean $\delta^{13}C$ values at -23.3 ± 1.6 ‰ (2σ, n=8) in September and November (organic matter produced in summer and autumn) and -28.0 ± 2.6 ‰ (2σ, n=12) in April and June (organic matter produced in winter and spring) (Fig. 3c). This seasonal difference may reflect a change in (i) the carbon isotope fractionation between dissolved CO_2 and organic matter (noted as ε_p, the subscript "p" standing for photosynthesis; *e.g.* Hayes, 2001) and/or (ii) the $\delta^{13}C$ of dissolved CO_2 (*i.e.* source of C in biomass). These two possibilities are examined below.

We first estimate the values of ε_p for the two distinct periods (summer-autumn *vs*. winter-spring) to evaluate if they differ significantly and could explain the $\delta^{13}C$ offset of ~4.7 ‰ (Fig. 3c). Variable ε_p values could for instance arise due to differences in the primary producing organisms between the seasons. The biomass in the upper water column of Lake Pavin is composed of phytoplankton and zooplankton. The dominant phytoplankton species are diatoms (present all year long, and characterized by a bloom in spring) and cyanobacteria, which bloom in summer (Amblard, 1986, 1988, Stebich *et al*., 2005). Previous studies demonstrated a vertical stratification of phytoplankton species living during a single period of time (Amblard, 1986; see compilation in Table S1), as well as a seasonal variability of phytoplankton (Bourdier and Amblard, 1987; Amblard, 1988; see Table S2). The determination of ε_p values in Lake Pavin is not straightforward. While C isotope data of organic matter are available from the sediment traps collected at 25 m depth, the isotope composition of CO_2 dissolved in the water column ($\delta^{13}C_{CO2}$) has not been determined in the present work. An approximation $\delta^{13}C_{CO2}$ can be calculated from previously published isotope compositions of dissolved inorganic carbon (DIC)

(Assayag *et al*., 2008). In Lake Pavin water column, DIC is dominated by the chemical species HCO_3^- because the pH is comprised between 6 and 9 (Assayag *et al*., 2008). Thus, the isotope compositions of DIC and HCO_3^- can reasonably be considered as identical (*i.e.* $\delta^{13}C_{DIC} = \delta^{13}C_{HCO3-}$). Then, the isotope composition of dissolved CO_2 ($\delta^{13}C_{CO2}$) in equilibrium with HCO_3^- can be estimated from the relation between the isotope fractionation $\varepsilon_{CO_2-HCO_3^-}$ and temperature (Mook *et al*., 1974):

$$\varepsilon_{CO_2-HCO_3^-} = 24.12 - \frac{9866}{T+273} \sim \delta^{13}C_{CO_2} - \delta^{13}C_{HCO_3^-} \quad (1)$$

where the temperature T is expressed in °C. Equation 1 can also be written as:

$$\delta^{13}C_{CO_2} \sim \delta^{13}C_{HCO_3^-} + 24.12 - \frac{9866}{T+273} \quad (2)$$

The isotope composition of dissolved CO_2 can be assessed for any temperature and isotope composition of HCO_3^- (equal to DIC). Using the temperature and $\delta^{13}C_{DIC}$ values measured previously in the zone of maximum primary productivity (surface to 15-20 m), we estimated a range of $\delta^{13}C_{CO2}$ for summer and winter based on data from July 2004 and November 2002 respectively (Assayag *et al*., 2008). The considered $\delta^{13}C_{HCO3-}$ and temperatures ranged from -2.7 to 0.5‰ and 4.8 to 9.7°C in November 2002, and from 1.0 to 4.4‰ and 4.5 to 16.6°C in July 2004, respectively (Table S3). The calculated $\delta^{13}C_{CO2}$ values are on average -8.3 ± 0.8 ‰ (n=8) for summer and -11.7 ± 1.1 ‰ (n=7) for winter (Table S3). If these values can be extended in time (an assumption confirmed from unpublished DIC data monitored over years by IPGP teams), the C isotope fractionation (ε_p) between dissolved CO_2 and organic matter can be calculated by combining these $\delta^{13}C_{CO2}$ values with our data on organic matter from the sediment traps at 25 m as:

$$\varepsilon_p = \delta^{13}C_{CO_2} - \delta^{13}C_{o.m.} \quad (3)$$

The estimated isotope fractionation ε_p associated to photosynthesis is about 14.0 ± 1.0 ‰ in summer and 15.0 ± 2.1 ‰ in winter, both values being in the range determined for diatoms under various experimental conditions (*e.g.* Riebesell *et al*., 2000). The similarity between these two estimates (within uncertainty) implies that the seasonal variability observed in organic matter $\delta^{13}C$ (a ~4.7 ‰ offset between -23.3 ‰ in summer-autumn *vs*. -28.0 ‰ in winter-spring) cannot be simply explained by different C isotope fractionation related to temperature variations and/or differences in primary producing organisms. Therefore, it more likely reflects a seasonal change in the C isotope composition of DIC. This change might be related to variable photosynthetic activity in time, with higher activity in summer and lower activity in winter. Seasonal control of C isotope composition in organic matter has already been observed (*e.g.*

Gu and Schelske, 1996), with sometimes large variations like in Lake Lugano, where $\delta^{13}C_{org}$ values are around -35 and -25 ‰ in winter and summer respectively (Bernasconi *et al.*, 1997). Large isotopic variations between seasons were interpreted as resulting from a combination of primary productivity level, changes in the carbonate chemistry (specifically controlled by pH variations) and isotope fractionation related to photosynthesis (Bernasconi *et al.*, 1997). It was not possible to establish a dominant parameter because of the strong vertical and temporal variability in the water column. Our variations observed in Lake Pavin can be fully explained by seasonal changes in the isotope compositions of the CO_2 assimilated during primary productivity, itself probably resulting from changes in the seasonal changes in the ratio of photosynthesis to respiration.

5.2.2. Origin of the N isotope signature of organic matter in Lake Pavin

In the sediment traps collected at Lake Pavin, the $\delta^{15}N_{tot}$ values are near -1 ‰ for all seasons (Fig. 3d), except in April 2012 (0.1 ‰ at 25 m depth) and June 2012 (~ +1 ‰ at all depth). The N isotope composition of organic matter can help to decipher the N sources assimilated by living organisms. Autotrophic organisms can assimilate four different sources of inorganic N in the form of either dinitrogen (N_2), nitrite (NO_2^-), nitrate (NO_3^-) or ammonium (NH_4^+). Nitrate and ammonium are usually the first chemical species to be used because their assimilation requires less energy than N_2, for which the triple bound is hard to break, and nitrite concentrations are generally very low in natural system (Quan and Falkovski, 2009). In Lake Pavin, NH_4^+ is present in anoxic deep waters, with an increasing concentration from the redox boundary to the lake bottom. Similar profiles have been observed in other redox stratified aquatic systems like the Black Sea, Saanich Inlet or Framvaren Fjord (*e.g.* Velinsky *et al.*, 1991; Velinsky and Fogel, 1999). Nitrate is virtually absent (< 0.2 µM) in surface waters, down to 20 m depth. It then shows a moderate peak (up to 18 µM) between 40 and 50 m depth (Fig. 2), likely reflecting some degree of organic matter mineralization by oxygen in this interval of the water column. This could explain the progressive increase of $\delta^{15}N$ with depth from -2.8 to +5.5 (Fig. 2b). Near the redox boundary, both NO_3^- and NH_4^+ concentrations decrease, which may indicate a consumption by denitrification and anammox processes (Galbreith *et al.*, 2008; Sigman *et al.*, 2009). Part of the nitrate accumulating between 50 and 56 m may result from the oxidation of ammonium diffusing upward in the water column (Fig. 2). Recent studies suggested that dissimilatory nitrate reduction to ammonium (DNRA) could be an important biological mechanism in ferruginous systems, where aqueous Fe(II) could act as an electron

528 donor for nitrate reduction (*e.g.* Michiels *et al*., 2017). Although we cannot exclude that this
529 mechanism is present in Lake Pavin, it is unlikely a dominant process near the redox interface
530 for the following reasons: first, nitrate is present in the depth zone where oxygen is still available
531 and no ammonium is detected, and second, Fe(II) is oxidized and trapped well below the depth
532 of nitrate accumulation.
533 The major biomass production in surface waters of Lake Pavin cannot be sustained by NO_3^-
534 and/or NH_4^+ because none is present. They may only occasionally be brought to surface waters
535 by mixing with deep waters (*e.g.* from 30 to 60 m; Fig. 2a). Nitrogen is thus likely a limiting
536 nutrient in Lake Pavin surface waters (like phosphorus, which is trapped and sequestered in the
537 monimolimnion by Fe-oxyhydroxide precipitation; *e.g.* Cosmidis *et al*., 2014; Busigny *et al*.,
538 2016) and N_2 represents the main N source for this biomass. The biological fixation of N_2 by
539 autotrophic organisms under either oxic or anoxic conditions is associated with low N isotope
540 fractionations (< 3‰; Minagawa and Wada, 1986; Fogel and Cifuentes, 1993). Consequently,
541 organic matter produced by N_2-fixation generally shows $\delta^{15}N$ close to 0‰, similar to
542 atmospheric values. Laboratory cultures of N_2-fixing organisms in Fe-rich medium
543 demonstrated slightly negative N isotope compositions of organic matter down to -3 ‰ (Zerkle
544 *et al*., 2008), and in some cases with extreme values down to -7 ‰ when other metal co-factors
545 are present (Zhang *et al*., 2014). In Lake Pavin, the $\delta^{15}N$ of organic matter collected in sediment
546 traps (~ –1‰; Fig. 3d) suggests that the dominant N source for biomass corresponds to
547 atmospheric N_2 dissolved in surface waters. Rare mixing events of surface waters with waters
548 from 30 to 60 m depth, like in January 2012, can inject some NO_3^- in shallow waters, and
549 potentially also some NH_4^+. Nitrate and ammonium can then be assimilated and produce
550 positive $\delta^{15}N$ values in biomass (*e.g.* +1‰ in June 2012). Such assimilation would require to
551 be complete (or almost) since nitrate or ammonium assimilation processes both favor ^{14}N over
552 ^{15}N, and a partial assimilation would impart negative $\delta^{15}N$ values in biomass. A complete
553 assimilation is not reasonable given the high primary productivity in Lake Pavin. The average
554 $\delta^{15}N$ value of dissolved NO_3^- is 2.4‰, suggesting active denitrification/anammox near the
555 redox interface. Ammonium measured near the redox interface (56 m depth) has a $\delta^{15}N$ value
556 of +4.7‰. A complete consumption of NO_3^- and NH_4^+ after mixing of the upper waters,
557 together with some degree of N_2-fixation, could easily account for the positive $\delta^{15}N$ values
558 observed in April (at 25 m) and June 2012 (all depths) (Fig. 3d). Changes of N sources (from
559 NH_4^+ to NO_3^- and/or to N_2) have already been described for other systems like Lake Kinneret,
560 Israel (Hadas *et al*., 2009), where strong seasonality effects are the main cause of these changes.

However, N isotope signature dominantly controlled by N_2-fixation is rarely observed in modern environments but more common in the sedimentary record for instance during oceanic anoxic events (OAE; Rau *et al.*, 1987; Jenkyns *et al.*, 2007; Junium and Arthur, 2007) and some Archean periods (Stüeken *et al.*, 2015; Stüeken and Buick, 2018).

5.3. Diagenetic modifications in the water column

The impact of organic matter degradation in the water column of Lake Pavin can be evaluated by comparing the fluxes of C and N, and the $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ values recorded in the different sediment traps along the depth profile. Although these four parameters show relatively consistent values, they are characterized by significant seasonal variabilities (Fig. 3). Rather than describing detailed seasonal variations, we present here a global perspective based on annual fluxes and isotope compositions in order to convey the main trends with respect to diagenetic modifications. This approach has the advantage of providing average values to be compared to sediment core data (see section 5.3 below).

We calculated the annual fluxes of C, N, as well as average $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ values (weighted-average considering the respective fluxes of C and N) for the sediment traps at 25 m (oxic zone), 56 m (just above the chemocline), 67 m (below the turbidity peak in the anoxic zone), and 86 m depth (near the lake bottom). The data are summarized in Table 5. The annual fluxes of C and N show a decrease from shallow to deep sediment traps, with values from 17.8 to 14.3 and 2.7 to 1.9 g m^{-2} yr^{-1}, respectively (Table 5). This decrease corresponds to a loss of 23.8 ± 1.9 % of organic carbon and 29.6 ± 2.3 % of nitrogen, indicating that N is more efficiently mineralized than C (translated to an increase of C/N ratio of 8.2 ± 0.5 %; Fig. 3). The mean annual $\delta^{13}C_{org}$ decreases with depth by ~1.3 ‰ (-25.2 to -26.5 ‰), while $\delta^{15}N_{tot}$ also decreases but by only 0.4 ‰ (-0.5 to -0.9 ‰; Table 5). In detail, the isotopic modifications appear to take place mostly in the shallow oxic zone, while the deep sediment traps of the anoxic zone have identical values. Intra-seasonal variabilities are not discussed here for simplicity, but if considered, they lead to similar conclusions for the evolution of C and N isotope compositions. Previous comparison of $\delta^{15}N$ values in sediment traps and surface sediments of the modern well-oxygenated ocean shows variably altered signatures, with enrichment in heavy isotope up to 6 ‰ (Altabet and François, 1994). Alteration of $\delta^{15}N$ values is a function of water depth and likely reflects oxygen exposure time (see review in Robinson *et al.*, 2012). In the open ocean under low sedimentation rate, the alteration can be significant. In contrast, under high sedimentation rate, high export production, low O_2, and good organic matter preservation, for instance in settings near continental margins, the alteration of the N isotope composition is minimal. In the stratified

595 euxinic Cariaco basin, Thunell *et al*. (2004) observed a moderate decrease of $\delta^{15}N$ (by 0.7‰)
596 between sinking particles and surface sediments. The direction and magnitude of this variation
597 is similar to the one observed here in Lake Pavin (~0.4‰). A larger decrease of $\delta^{15}N$ (3‰) was
598 sometimes reported in sinking particles like at the North Atlantic Bloom Experiment site, which
599 was interpreted as selective removal of ^{15}N-enriched compounds, or addition of ^{14}N-enriched
600 organic material (Altabet *et al*., 1991). In the case of Lake Pavin particulate matter, the
601 combined loss of C and N with depth is better explained by a removal than an addition of
602 organic compounds.
603
604 **5.4. Diagenetic modifications in sediments**
605 Previous studies of diagenetic evolution in oceanic or lacustrine environments, under the
606 influence of O_2, NO_3^- and SO_4^{2-}, illustrated limited modification of C isotope composition *(e.g.*
607 Freudenthal *et al*., 2001; Kohzu *et al*., 2011a) but contrasted N isotope results (Sachs and
608 Repeta, 1999; Freudenthal *et al*., 2001; Lehmann *et al*. 2002; Kohzu *et al*., 2011b). Analysis of
609 a 30 cm long sediment core collected near the Canary Islands in eastern Atlantic Ocean revealed
610 that $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ were poorly modified during diagenesis with variations lower than 1‰
611 (Freudenthal *et al*., 2001). Detailed analysis of C isotope composition in sediments from Lake
612 Kasumigaura, Japan, also show a good preservation, with $\delta^{13}C_{org}$ of 15 cm-long sediment cores
613 varying within ±0.6‰ (Kohzu *et al*., 2011a). In contrast, a companion study on the same
614 samples determined significant N isotope modifications, with increasing $\delta^{15}N_{tot}$ values in the 4
615 cm top sediments but decreasing values in deeper sediments (Kohzu *et al*., 2011b). The
616 diagenetic modifications of sediments are usually believed to increase $\delta^{15}N$ values under well-
617 oxygenated conditions, but decrease under anoxic conditions (Sachs and Repeta, 1999;
618 Lehmann *et al*. 2002). The $\delta^{15}N$ decrease in Lake Kasumigaura sediments may reflect either
619 preferential degradation of ^{15}N-enriched components (like the chitin fraction; *e.g.* Brahney *et*
620 *al*., 2006) or addition of ^{14}N-enriched organic matter by bacterial growth from dissolved
621 ammonium in porewater sediments (Kohzu *et al*., 2011b).
622 In Lake Pavin, the diagenetic modifications of organic C and N can be addressed from the depth
623 profile of the three sediment cores collected at 60, 65 and 92 m depth (Fig. 4). Before discussing
624 the chemical and isotope evolutions within the cores, it is interesting to compare the sediment
625 surface (top core) to the global annual values calculated in section 5.2. The average annual
626 sediment deduced from sediment traps have $\delta^{13}C_{org}$, $\delta^{15}N_{tot}$ and C/N values of -26.5 ‰, -0.9 ‰
627 and 8.4 (Table 5). These values are undistinguishable from those of the top core sediments (Fig.

4, Table 5), which validates both measurements and calculations. Moreover, it provides a reliable assessment of the starting material, before diagenetic modifications in the sedimentary pile. Part of the total nitrogen in solid sediment is possibly carried as NH_4^+ substituting for K^+ in clay minerals (*e.g.* Müller, 1977). However, clay content in Lake Pavin is particularly low. The sediment is dominated by diatom frustules (> 40% SiO_2 in bulk; Schettler *et al.*, 2007), vivianite (iron phosphate), and organic matter (>10 % in the top sediment; Table 3). Aluminum content is always lower than 2.5 wt% (Schettler *et al.*, 2007) and generally around 1 wt% (Busigny *et al.*, 2014), which translates to a maximum K content of 1.3 wt% (considering K/Al molar ratio of 0.37 ± 0.07, 2SD, n=14; data from Busigny *et al.*, 2014). Assuming a relation between K^+ and NH_4^+ similar to the one established for oceanic sediments (Müller, 1977), the maximum NH_4^+ content in clay minerals of Lake Pavin sediments can be estimated to 100 ppm. This value is two orders of magnitude lower than the ~1 wt% measured for total N in bulk (Table 3), demonstrating that organic matter is the main N carrier in Lake Pavin sediments.

The three sediment cores, C1 (60 m, near the redox transition zone), C2 (65 m, near the peak of sulfate) and C6 (92 m, lake bottom), show consistent evolution in the sedimentary column, with a decrease in C and N concentrations but no specific trends in $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ values (Fig. 4). Overall 40-50 % C and 50-60 % N were lost during this diagenetic evolution. The $\delta^{13}C_{org}$ values of cores C1, C2 and C6 average -26.8 ± 1.1 ‰ (2σ, n=6), -26.8 ± 0.7 ‰ (2σ, n=12), -26.5 ± 1.9 ‰ (2σ, n=5), while $\delta^{15}N_{tot}$ values are -1.2 ± 0.4 ‰ (2σ, n=6), -1.0 ± 0.4 ‰ (2σ, n=12), -0.6 ± 0.8 ‰ (2σ, n=5), respectively (Table 3). In each core, both $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ can be regarded as constant within uncertainty. Since oxygen, nitrate and sulfate are absent in the sediment, the decrease of particulate C and N concentrations with depth can only be attributed to organic matter mineralization by microbial reduction of Fe(III) phases such as iron (oxy)hydroxides and ferric phosphate (Busigny *et al.*, 2014; Cosmidis *et al.*, 2014), and methanogenesis (Lehours *et al.*, 2005; Borrel *et al.*, 2011; Biderre-Petit *et al.*, 2011; Lopes *et al.*, 2011). Importantly, the relative constancy of $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ observed here indicates that these metabolic degradation pathways (Fe(III)-based and methanogenesis) do not impact the C and/or N isotope compositions of organic matter, even though ~50 % of the initial organic matter have been lost. Nevertheless, it must be pointed out that our samples represent only the 12 cm top-sediments and this conclusion will need to be tested further in longer sediment cores. An intriguing result in Lake Pavin sediments is the difference between C/N ratios of 8.6-10.5 in cores C1 and C2 and 11.3-13.2 in core C6. The origin of this difference is not clear but may reflect a specific degradation mechanism of organic matter at the lake bottom. A possibility is

for instance that sediments from C1 and C2 cores were more impacted by Fe(III) reduction, due to the proximity to the redox boundary, while the lake bottom sediments mostly involved methanogenic degradation. Indeed, ferric iron particles are partly reduced in the water column and are thus less abundant in the deepest sediments, after a longer travel when sinking in the water column (Cosmidis *et al*., 2014; Busigny *et al*., 2016). The fate of ammonium produced by organic matter degradation in sediment can hardly be discussed from our Lake Pavin data since no ammonium concentrations and/or isotope compositions were determined in the sediment porewater profiles. However, we note that $\delta^{15}N_{NH4+}$ in the deepest sample of the water column sample (90 m) is -0.36‰, a value undistinguishable from the average (-0.6 ± 0.8‰) of lake bottom sediment (92 m, Table 3). This suggests that most ammonium released from sediment is unchanged and simply diffuses towards the water column although a minor part of it might be oxidized to N_2 or NO_2^- by Fe(III)-user microorganisms, as observed in wetland soils (Clement *et al*., 2005) or tropical upland soil slurries (Yang *et al*., 2012).

5.5. Implications for paleo-environment and –ecosystem reconstruction

In Lake Pavin water column, organic matter mineralization reached ~20%, while $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ decrease by only ~1.3 and 0.4 ‰, respectively (Table 5). In sediments, about 50% of the initial organic matter was mineralized (on the top of the 20% that already happened in the water column) but $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ remained roughly constant, within uncertainty. This indicates that organic matter degradation based on ferric minerals and/or methanogenesis does not modify significantly C and N isotope compositions in anoxic and ferruginous environments. Such limited modifications are consistent with previous results obtained for other anoxic settings where organic matter degradation was based on nitrate or sulfate reduction processes (*e.g*. Altabet *et al*., 1999; Lehmann *et al*., 2002; Thunell *et al*., 2004; Möbius *et al*., 2010; Chen *et al*., 2008). Therefore, while strong isotopic effects are observed for oxic degradation of organic matter (*e.g.* Robinson *et al*., 2012), sediments deposited under any type of anoxic environment should preserve their C and N isotope compositions, and be safely used as paleo-recorders of past C and N biogeochemical cycles. This conclusion is important specifically for isotope studies of sedimentary rocks from the Archean and Proterozoic eons, as well as anoxic periods from the Phanerozoic (*e.g.* oceanic anoxic events, sapropels, thermal maximum periods like PETM or MECO).

In the rock record, metamorphic processes can eventually modify $\delta^{13}C$ and $\delta^{15}N$ values. However, previous studies on coals of variable metamorphic grades demonstrated that $\delta^{15}N$ is

well preserved although N content decreases and C/N ratio increases (Ader *et al*., 1998; Boudou *et al*., 2008). In the case of N present as ammonium (NH_4^+) substituting for K^+ in the structure of silicate minerals, metamorphism can lead to a partial N loss associated to an increase in $\delta^{15}N$ values by a few per mil in the residual rock (Bebout and Fogel, 1992; Mingram and Brauer, 2001; Busigny and Bebout, 2013). The potential effect of metamorphism can generally be avoided by studying rocks of low metamorphic grade, such as prehnite–pumpellyite or greenschist facies (*e.g.* Stüeken *et al*., 2015, 2016; Luo *et al*., 2018).

The isotope composition of different N species in Lake Pavin are consistent with a typical model of redox stratified system, where the dissolved NO_3^- and NH_4^+, respectively in the oxic and anoxic zones, are largely lost at the redox interface via denitrification and anammox processes. Diazotrophic organisms living in the photic zone therefore consume dissolved N_2 to counterbalance the absence or limited availability of NO_3^-. The low $\delta^{15}N$ values observed in biomass (~ -1‰) are similar to those measured in organic matter-rich sediments deposited during anoxic marine periods such as Oceanic Anoxic Event (OAE) and sapropels (Rau *et al*., 1987; Jenkyns *et al*., 2007; Junium and Arthur, 2007) and in some sedimentary rocks deposited in the Archean and Paleoproterozoic (Stüeken *et al*., 2015; Luo *et al*., 2018; Koehler *et al*., 2019). These results are however distinct from redox stratified marine systems like the Black Sea or Cariaco basin, where positive $\delta^{15}N$ values of sediments (essentially between 3 and 7‰) can be attributed to a biomass assimilating partially-denitrified NO_3^- (Thunell *et al*., 2004; Fulton *et al*., 2012; Quan *et al*., 2013). In the specific case of the Black Sea, redox conditions have changed with time due to rise and fall of sea level related to glacial-interglacial periods. The Black Sea is known to have alternated between anoxic, saline waters in high level (*i.e*. connection with the Mediterranean Sea), and oxic, less saline conditions (dominated by freshwaters) in low sea level. Quan *et al*. (2013) showed that $\delta^{15}N$ in sediments are similar under oxic and anoxic periods (3 to 4‰) but increase to higher values (~6‰) during glacial-interglacial transition periods. This increase in $\delta^{15}N$ was attributed to partial denitrification under suboxic conditions, and subsequent transfer of the isotope signature of residual nitrate to biomass. Although the lowest $\delta^{15}N$ values of the Black Sea sediment can be explained by a stronger influence of N_2-fixing organism, these values are still positive and require some degrees of ^{15}N-enriched nitrate or ammonium consumption. The Black sea and Cariaco basin are often considered as analogues for stratified oceanic systems and taken as references for interpretation of paleo-environments. However, they are not really suitable analogues for the global N cycle because they are fed by external sources of NO_3^-. Lake Pavin may represent a

727 better analog and illustrates, in a natural system, some of the theoretical concepts developed for
728 OAE and Precambrian ocean.
729

730 ## 6. Conclusion

731 We present the first assessment of organic matter C and N isotope evolution during diagenesis
732 in anoxic and ferruginous sedimentary system, as illustrated by the case of Lake Pavin. Apart
733 from significant seasonal variability affecting C isotope compositions, $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ in
734 sediment traps along the water column profiles are only moderately different by 1.3 and 0.4 ‰,
735 respectively. Moreover, the average sediment traps collected in the anoxic zone are remarkably
736 similar to the top sediments at the lake bottom. In the sedimentary column, $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$
737 do not show any specific evolution with depth and can be regarded as constant. Lake Pavin data
738 therefore demonstrate that organic matter degradation in anoxic and ferruginous conditions
739 preserve $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ of the primary biomass, with only minor modifications. It confirms
740 previous assumptions of Archean and OAE studies that $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ can be used as
741 reliable paleo-proxy of biomass and environmental conditions.
742

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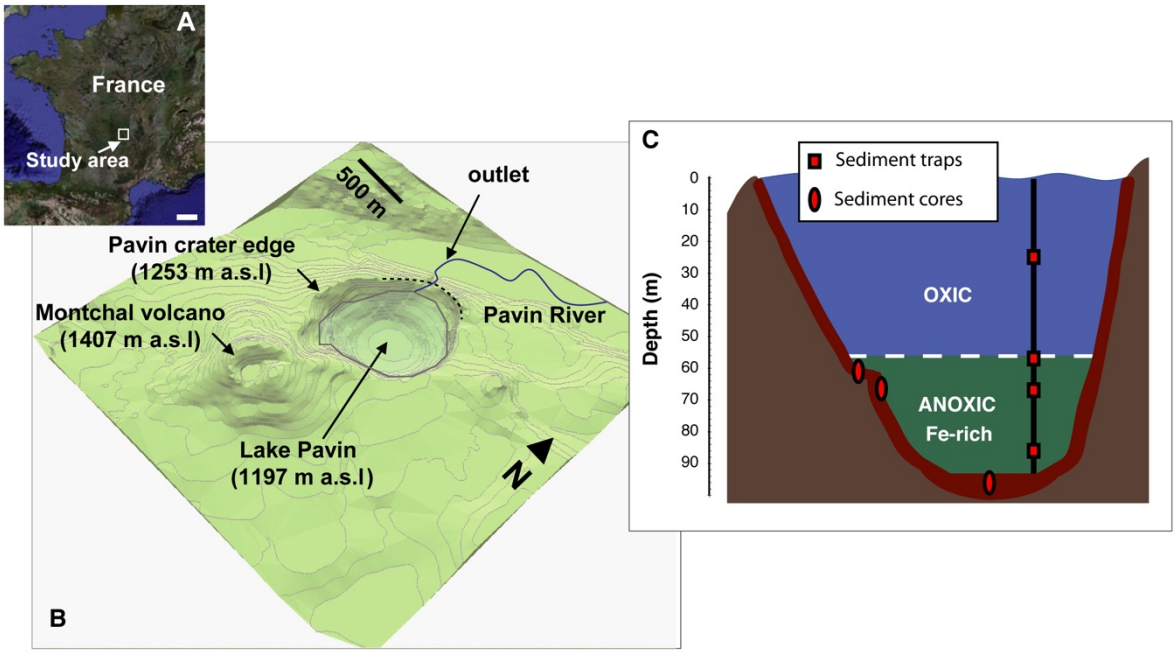

Fig. 1. (A) Location of Lake Pavin, France (scale given by the white bar, representing 100 km). (B) Elevation/bathymetric map showing the main features of Lake Pavin area. (C) Schematic representation of Lake Pavin and the samples studied here: 4 sediment traps in the oxic (25 and 56 m depth) and anoxic (67 and 86 m) zones, as well as 3 sediment cores from the anoxic zone. Figures A and B are modified from Chapron *et al*. (2010).

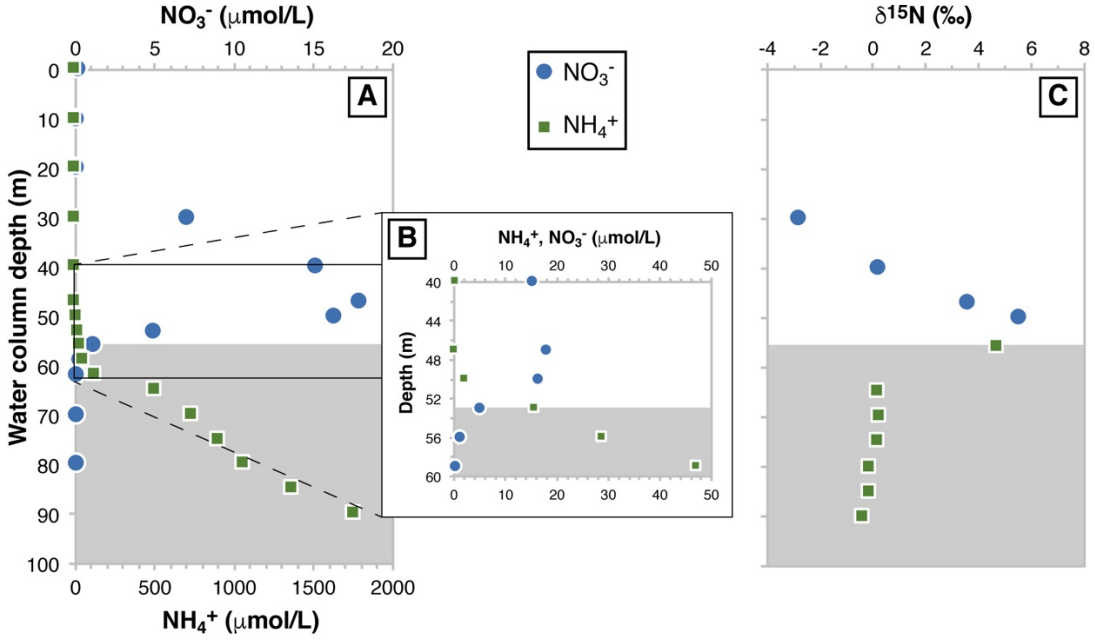

Fig. 2. Dissolved nitrate (circles) and ammonium (squares) concentrations (A) and isotope compositions (B) in lake Pavin water column. (A) nitrate and ammonium concentrations, (B) zoom in the redox transition zone, (C) nitrogen isotope compositions. Note the different scales for nitrate and ammonium concentrations in figure A. The grey area represents the anoxic zone.

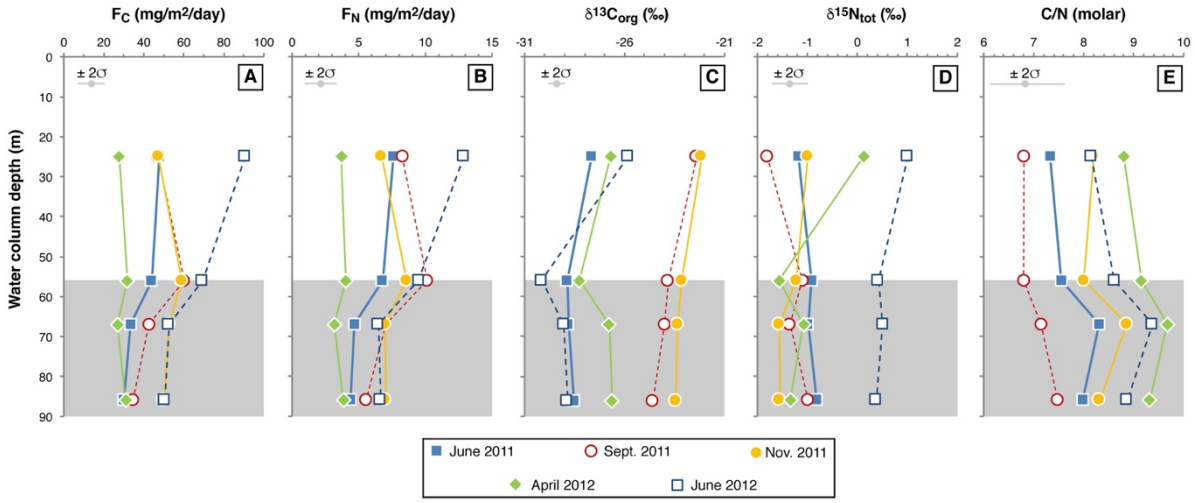

Fig. 3. Organic carbon and nitrogen fluxes (F_C and F_N) and isotope compositions ($\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$), as well as C/N ratios measured in sediment traps collected in Lake Pavin at 5 different periods, from June 2011 to June 2012. The grey area represents the anoxic zone. The error bars correspond to ±2σ.

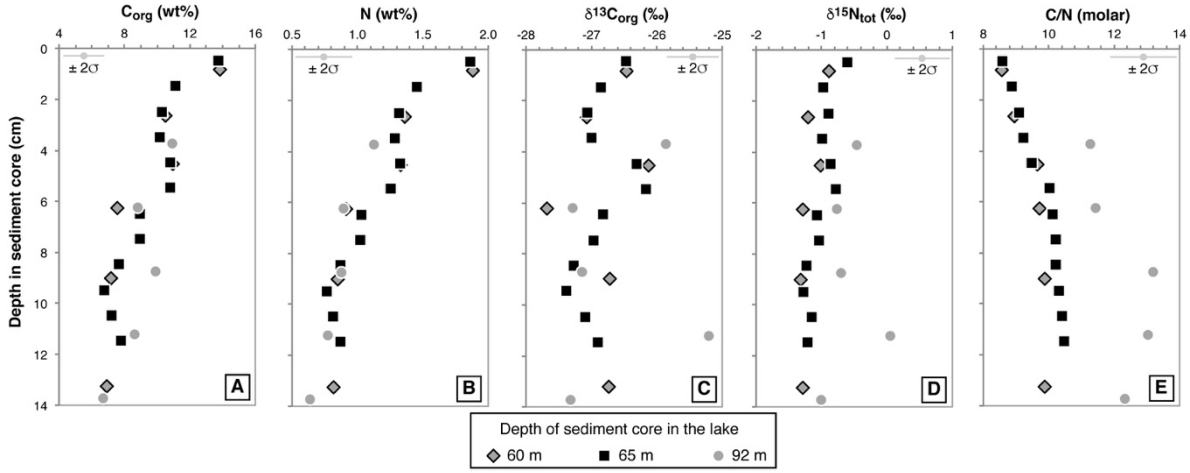

Fig. 4. Organic carbon and nitrogen concentrations and isotope compositions, as well as C/N ratios measured in the three sediment cores from the anoxic zone of Lake Pavin (see core location in Fig. 1). The three cores were collected near but just below the redox transition zone (60 m), near the peak of sulfate reduction zone (65 m), and at the lake bottom (92 m). The error bars correspond to ±2σ.

Table 1

Dissolved ammonium and nitrate concentrations and isotope composition in Lake Pavin water column.

Depth* (m)	NH_4^+ (μM)	$\delta^{15}N_{NH4+}$ (‰)	NO_3^- (μM)	$\delta^{15}N_{NO3-}$ (‰)
0	0.2		0.20	
10	0.2		0.01	
20	0.1		0.01	
30	0.1		7.00	-2.8
40	0.2		15.18	0.2
47	0.0		17.85	3.6
50	2.2		16.30	5.5
53	15.7		4.92	
56	28.8	4.70	1.16	
59	47.1		0.29	
62	118		0.09	
65	500	0.22		
70	731	0.27	0.06	
75	900	0.17		
80	1057	-0.13	0.06	
85	1366	-0.12		
90	1753	-0.36		

* Depth in the water column.

Table 2

Organic carbon and nitrogen fluxes (F_C and F_N) and isotope compositions ($\delta^{13}C_{org}$ and $\delta^{15}N_{org}$). as well as C/N ratios. in sediment traps from lake Pavin. All data correspond to the average values and 2SD (standard deviation) of two or three replicate analyses.

Sample	Depth* (m)	F_C mg.m^{-2}.day^{-1}	F_N mg.m^{-2}.day^{-1}	$\delta^{13}C_{org}$ (‰)	± (2σ)	$\delta^{15}N_{org}$ (‰)	± (2σ)	C/N	± (2σ)
March-June 2011									
MX33-T23P	25	47.8	7.6	-27.6	*0.4*	-1.2	*0.1*	7.3	*0.3*
MX33-T58P	56	43.9	6.8	-28.9	*0.1*	-0.9	*0.2*	7.6	*0.3*
MX33-T70P	67	33.4	4.7	-28.8	*0.1*	-1.0	*0.1*	8.3	*0.1*
MX33-T88P	86	30.0	4.4	-28.5	*0.1*	-0.8	*0.2*	8.0	*0.3*
Mean		**38.8**	**5.9**	**-28.5**		**-1.0**		**7.8**	
SD (2σ)		**16.8**	**3.1**	**1.1**		**0.3**		**0.9**	
June-September 2011									
MX34-T23P	25	47.0	8.3	-22.4	*0.2*	-1.8	*0.2*	6.8	*0.3*
MX34-T58P	56	60.2	10.1	-23.8	*0.1*	-1.1	*0.1*	6.8	*0.1*
MX34-T70P	67	42.6	7.0	-24.0	*0.2*	-1.3	*0.1*	7.2	*0.3*
MX34-T88P	86	34.5	5.6	-24.6	*0.2*	-1.0	*0.1*	7.5	*0.3*
Mean		**46.1**	**7.8**	**-23.7**		**-1.3**		**7.1**	
SD (2σ)		**21.5**	**3.9**	**1.9**		**0.7**		**0.6**	
September-November 2011									
MX35-T23P	25	47.3	6.7	-22.1	*0.3*	-1.0	*0.1*	8.2	*0.3*
MX35-T58P	56	58.9	8.6	-23.1	*0.3*	-1.2	*0.1*	8.0	*0.1*
MX35-T70P	67	52.9	7.0	-23.3	*0.2*	-1.6	*0.1*	8.9	*0.2*
MX35-T88P	86	50.1	7.0	-23.4	*0.3*	-1.5	*0.0*	8.3	*0.2*
Mean		**52.3**	**7.3**	**-23.0**		**-1.3**		**8.3**	
SD (2σ)		**9.9**	**1.7**	**1.2**		**0.6**		**0.7**	
November 2011-April 2012									
MX37-T23P	25	27.5	3.7	-26.7	*0.1*	0.1	*0.1*	8.8	*0.4*
MX37-T58P	56	31.8	4.0	-28.2	*0.1*	-1.6	*0.2*	9.2	*0.0*
MX37-T70P	67	26.8	3.2	-26.8	*0.1*	-1.1	*0.2*	9.7	*0.2*
MX37-T88P	86	30.8	3.9	-26.6	*0.0*	-1.3	*0.1*	9.3	*0.0*
Mean		**29.2**	**3.7**	**-27.1**		**-1.0**		**9.2**	
SD (2σ)		**4.9**	**0.7**	**1.6**		**1.5**		**0.7**	
April-June 2012									
MX38-T23P	25	90.7	12.9	-25.8	*0.1*	1.0	*0.1*	8.2	*0.3*
MX38-T58P	56	69.4	9.5	-30.1	*0.2*	0.4	*0.3*	8.6	*0.2*
MX38-T70P	67	52.7	6.5	-29.0	*0.1*	0.5	*0.2*	9.4	*0.1*
MX38-T88P	86	50.5	6.6	-28.9	*0.0*	0.4	*0.0*	8.9	*0.2*
Mean		**65.8**	**8.9**	**-28.5**		**0.6**		**8.8**	
SD (2σ)		**37.3**	**6.0**	**3.7**		**0.6**		**1.0**	

* Depth in the water column.

Table 3

Organic carbon and nitrogen concentrations and isotope compositions. as well as C/N ratios. in the samples of the three sediment cores from Lake Pavin. All data correspond to the average values and 2SD (standard deviation) of two or three replicate analyses.

Sample	Depth* (cm)	C_{org} %	± (2σ)	N_{tot} %	± (2σ)	$\delta^{13}C_{org}$ (‰)	± (2σ)	$\delta^{15}N_{tot}$ (‰)	± (2σ)	C/N	± (2σ)
Core C1 60m											
B2	0.9	13.8	*1.3*	1.9	*0.1*	-26.5	*0.0*	-0.9	*0.0*	8.6	*0.5*
D2	2.7	10.5	*0.8*	1.4	*0.0*	-27.1	*0.1*	-1.2	*0.1*	9.0	*0.4*
F2	4.6	11.0	*0.8*	1.3	*0.0*	-26.1	*0.1*	-1.0	*0.1*	9.6	*0.6*
H2	6.3	7.6	*0.9*	0.9	*0.1*	-27.7	*0.2*	-1.3	*0.2*	9.7	*0.5*
I2	9.0	7.2	*0.4*	0.8	*0.0*	-26.7	*0.0*	-1.3	*0.2*	9.9	*0.3*
L2	13.3	6.9	*0.2*	0.8	*0.0*	-26.7	*0.2*	-1.3	*0.1*	9.9	*0.2*
Mean		**9.5**		**1.2**		**-26.8**		**-1.2**		**9.4**	
SD (2σ)		**5.5**		**0.8**		**1.1**		**0.4**		**1.1**	
Core C2 65m											
C2-1-1	0.5	13.8	*0.9*	1.9	*0.0*	-26.5	*0.1*	-0.6	*0.0*	8.6	*0.6*
C2-2-1	1.5	11.1	*0.9*	1.5	*0.0*	-26.8	*0.1*	-1.1	*0.1*	8.9	*0.6*
C2-3-1	2.5	10.3	*0.9*	1.3	*0.1*	-27.0	*0.0*	-0.9	*0.0*	9.1	*0.8*
C2-4-1	3.5	10.2	*0.7*	1.3	*0.0*	-27.0	*0.1*	-1.0	*0.1*	9.2	*0.7*
C2-5-1	4.5	10.8	*1.0*	1.3	*0.0*	-26.3	*0.1*	-0.8	*0.0*	9.5	*0.8*
C2-6-1	5.5	10.8	*0.2*	1.3	*0.1*	-26.2	*0.0*	-0.8	*0.1*	10.0	*1.2*
C2-7-1	6.5	9.0	*0.1*	1.0	*0.1*	-26.8	*0.2*	-1.1	*0.0*	10.1	*1.1*
C2-8-1	7.5	9.0	*0.2*	1.0	*0.1*	-27.0	*0.0*	-1.0	*0.0*	10.2	*1.2*
C2-9-1	8.5	7.7	*0.1*	0.9	*0.1*	-27.3	*0.1*	-1.2	*0.0*	10.2	*0.7*
C2-10-1	9.5	6.8	*0.9*	0.8	*0.2*	-27.4	*0.4*	-1.3	*0.1*	10.3	*0.8*
C2-11-1	10.5	7.3	*0.3*	0.8	*0.1*	-27.1	*0.0*	-1.1	*0.1*	10.4	*0.7*
C2-12-1	11.5	7.8	*0.1*	0.9	*0.1*	-26.9	*0.1*	-1.2	*0.2*	10.5	*1.0*
Mean		**9.6**		**1.2**		**-26.8**		**-1.0**		**9.8**	
SD (2σ)		**4.0**		**0.6**		**0.7**		**0.4**		**1.3**	
Core C6 92m											
1-1	3.75	11.0		1.1	*0.3*	-25.8	*0.1*	-0.5	*0.1*	11.3	*0.6*
2-1	6.25	8.8	*1.3*	0.9	*0.0*	-27.3	*0.5*	-0.8	*0.1*	11.5	*0.4*
3-1	8.75	9.9	*1.5*	0.9	*0.2*	-27.1	*0.3*	-0.7	*0.1*	13.2	*0.7*
4-1	11.25	8.7	*2.3*	0.8	*0.2*	-25.2	*0.2*	0.1	*0.2*	13.0	*0.5*
5-1	13.75	6.8	*1.3*	0.6	*0.0*	-27.3	*0.3*	-1.0	*0.2*	12.3	*0.3*
Mean		**9.0**		**0.9**		**-26.5**		**-0.6**		**12.3**	
SD (2σ)		**3.1**		**0.4**		**1.9**		**0.8**		**1.8**	

***** Depth in the sediment core.

Table 4

Organic carbon and nitrogen concentrations and isotope compositions, as well as C/N ratios, in the beech leaves, spruce thorns and two litters samples collected on the flanks of Pavin crater lake.

Sample	C_{org} (wt.%)	N_{tot} (wt.%)	$\delta^{13}C_{org}$ (‰)	$\delta^{15}N_{tot}$ (‰)	C/N molar
brown leaves	46.3	1.4	-30.3	-6.8	37.7
brown leaves	46.3	1.6	-30.5	-6.8	33.7
brown leaves	44.4	1.1	-31.0	-6.6	46.7
brown leaves	44.2	1.3	-31.3	-6.4	41.2
brown leaves	48.3	1.3	-30.7	-7.0	43.4
Avg (±2SD)	*45.9 (±3.3)*	*1.3 (±0.4)*	*-30.8 (±0.8)*	*-6.7 (±0.5)*	*40.5 (±10.1)*
Spruce thorns	26.2	1.3	-30.3	-1.6	23.0
Spruce thorns	27.2	1.2	-28.9	-0.7	27.4
Spruce thorns	24.6	1.0	-30.4	-1.2	29.6
Spruce thorns	27.8	1.1	-29.6	-1.5	30.7
Avg (±2SD)	*26.5 (±2.8)*	*1.1 (±0.3)*	*-29.8 (±1.4)*	*-1.2 (±0.8)*	*27.7 (±6.8)*
Litter 1	13.5	0.8	-27.9	-3.1	19.2
Litter 1	14.6	1.2	-27.5	-3.2	14.5
Litter 1	15.6	1.0	-28.3	-3.3	18.5
Litter 1	10.8	0.8	-28.1	-3.1	15.3
Litter 1	12.9	0.9	-27.6	-3.2	17.4
Avg (±2SD)	*13.5 (±3.7)*	*0.9 (±0.3)*	*-27.9 (±0.6)*	*-3.2 (±0.1)*	*17.0 (±4.1)*
Litter 2	14.0	1.1	-28.3	-2.6	14.9
Litter 2	15.3	1.0	-28.2	-2.3	17.8
Litter 2	15.0	1.1	-28.7	-2.4	15.8
Litter 2	14.1	1.0	-28.8	-2.4	16.1
Litter 2	15.1	1.0	-28.1	-2.6	16.9
Avg (±2SD)	*14.7 (±1.3)*	*1.1 (±0.1)*	*-28.4±0.6*	*-2.4 (±0.3)*	*16.3 (±2.2)*

Avg (±2SD): average values and uncertainties expressed as two standard deviations.

Table 5

Global annual fluxes of organic carbon and nitrogen. their isotope compositions. and C/N ratios in Lake Pavin (data calculated from the sediment traps placed at 4 different depths in the water column).

Depth* (m)	F_C $g.m^{-2}.year^{-1}$	F_N $g.m^{-2}.year^{-1}$	$\delta^{13}C_{org}$ (‰)	$\delta^{15}N_{org}$ (‰)	C/N
25	17.8 ± 0.8	2.7 ± 0.1	-25.2 ± 0.2	-0.5 ± 0.1	7.9 ± 0.3
56	18.2 ± 1.0	2.7 ± 0.2	-26.9 ± 0.2	-0.8 ± 0.2	8.1 ± 0.1
67	14.3 ± 0.2	1.9 ± 0.1	-26.4 ± 0.1	-0.9 ± 0.1	8.9 ± 0.2
86	13.6 ± 0.7	1.9 ± 0.1	-26.5 ± 0.1	-0.9 ± 0.1	8.6 ± 0.2

* Depth in the water column.

Supplementary materials

Excel file attached

Table S1

Vertical variability of phytoplankton species in Lake Pavin water column in June 1984 (data compiled from Amblard. 1984. 1986). A cross indicates the presence of the species. while empty space corresponds to an absence.

Table S2

Seasonal variability of phytoplankton species in the first meters of Lake Pavin upper water column. in June 1984 (data compiled from Bourdier et al., 1987; Amblard, 1988). A cross indicates the presence of the species. while empty space corresponds to an absence.

Table S3

Measured temperature, dissolved O_2, pH, specific conductivity (at 25°C), and C isotope composition of dissolved inorganic carbon in November 2002 and July 2004 (Assayag et al., 2008). The isotope composition of CO_2 is calculated at every depth from isotope composition of DIC and temperature using Mook (1974).