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Glacier dynamics influenced carbon flows through lake food webs: evidence from a chironomid δ^{13}C-based reconstruction in the Nepalese Himalayas

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Abstract Using a sediment core covering the last 3,500 years, we analysed photosynthetic pigments' concentrations in lake sediments and carbon stable isotopic composition of chironomid (Diptera, Chironomidae) remains ($\delta^{13}C_{HC}$). We aimed to reconstruct temporal changes in aquatic primary productivity and carbon resources sustaining chironomid larvae in a high mountain lake (Lake Pyramid Inferior; 5,067 m a.s.l.) located in the Nepalese Himalayas. Both pigments and $\delta^{13}C_{HC}$ trends followed a similar fluctuating pattern over time, and we found significant positive relationships between these proxies, suggesting the strong reliance of benthic consumers on the aquatic primary production. Temporal trends matched well with main known climatic phases in the Eastern part of the Himalayan Mountains. Past glacier dynamics and associated in-lake solute concentrations appeared to be the main driver of autochthonous primary productivity, suggesting then the indirect impact of climate change on carbon processing in the benthic food web. During warm periods, the glacier retreat induced a rise in in-lake solute concentrations leading to an increasing primary productivity. Complementary investigations are still needed to strengthen our understanding about the response of past aquatic carbon cycling in CO_2-limiting environments.

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Introduction

Mountain areas play a key role in the regulation of climatic, hydrological and biogeographical processes at a global scale. The Himalayan Mountains are often considered as the "Third Pole" (Qiu, 2008), mainly because they exhibit the largest glacial area outside of the Polar Regions and they are located in the most remote region of the world (Yao et al., 2012a). Glacier dynamics in the Himalayan Mountains induces numerous feedbacks on regional climate, water supply and associated biogeochemical cycles (Yao et al., 2012b). However, numerous studies revealed dramatic

decreases in glacial area over the last decades due to climate warming (Salerno et al., 2008; Chudley et al., 2017). Since glacier retreat in the Himalayan Mountains is expected to dramatically increase in a near future due to global warming (Soncini et al., 2016); it has then become necessary to assess climate change impacts on ecosystems in this area.

Lakes are sentinels of climate change, and they are considered as relevant indicators of environmental quality in high mountain landscapes (Adrian et al., 2009; Williamson et al., 2009). Mechanisms by which climate affects lake ecosystems can be summarized by the conceptual energy–mass framework published by Leavitt et al. (2009; Appendix 1). They proposed to distinguish the energy (E effects; atmospheric heat, irradiance, etc.) and mass fluxes (m effects; precipitation patterns, nutrient run-off, etc.) affecting the different compartments of lake functioning. The broader catchment area and the lake itself play then a role of environmental filters to mediate climate change effects (Blenckner, 2005; Appendix 1). In the Nepalese Himalayas, lakes seem to be affected by both E (i.e. water temperature) and m (i.e. nutrients and solute dynamics) fluxes. However, climate-induced glacier dynamics (E effects) appear to play a major role in solute concentrations in lakes (indirect m effects; Salerno et al., 2016) leading to the control of aquatic primary production (Lami et al., 2010), and likely the associated pelagic food web (Nevalainen et al., 2014), but nothing is known about the whole ecosystem response.

The understanding of food web functioning, including all steps of exchange of energy and matter among aquatic consumers (Lindeman, 1942), is an essential knowledge to elucidate the impact of climate change on lakes at ecosystem level. Among the complexity of trophic interactions in lakes, the benthic food web represents an integrative proxy for the understanding of whole lake trophic functioning (Vadeboncoeur et al., 2002). Indeed, lake sediments play a central role in recycling processes of organic matter coming from aquatic ecosystems (i.e. autochthonous primary production) and the associated catchment (i.e. terrestrial organic matter; Meyers & Ishiwatari, 1993). Moreover, the functioning of the benthic food web involves numerous couplings with the surrounding terrestrial environment (Northington et al., 2010) and among in-lake compartments

(benthic–pelagic coupling, Wagner et al. 2012; pelagic–benthic coupling, Jónasson, 1972; Johnson & Wiederholm, 1992), making carbon processing in the benthic food web an excellent integrator, in both time and space, of organic matter cycling in lakes. First investigations of the Himalayan lakes were only focused on the characterization of biological communities (Manca et al., 1998). Carbon processing in the benthic food web is then poorly understood in this area. Fortunately, lake sediments and paleolimnological approach can be used to compensate for the lack of hydrobiological surveys in remote areas and to reconstruct the past dynamic of aquatic food webs (Catalan et al., 2013).

The study of lake sediments and archived biological remains is a promising approach to better understand the impact of climate change on aquatic ecosystems. Sedimentary pigments of former aquatic primary producers (from both planktonic and benthic populations) are usually well preserved in lake sediments (Leavitt & Hodgson, 2002). Then, sedimentary pigment analysis provides a quantitative reconstruction of the past dynamic of aquatic primary producers, illustrating temporal changes in lake trophic status (Guilizzoni et al., 2011). Moreover, remains of benthic consumers can be found in lake sediments at high concentrations (Walker, 2001). The larvae of Chironomidae (Arthropoda; Diptera; Nematocera) dominate benthic secondary production in high-elevation lakes in the Himalayan Mountains (Manca et al., 1998). In addition, the most sclerotized part of their exoskeleton (the head capsules, HC) shed after each moulting is morphologically and chemically well conserved in lake sediments (Verbruggen et al., 2009). Due to a negligible trophic fractionation (Goedkoop et al., 2006; Frossard et al., 2013) and significant differences in carbon stable isotopic composition ($\delta^{13}C$) of food sources (Rounick & Winterbourn, 1986), $\delta^{13}C$ analysis of subfossil chironomid is often used to track carbon sources and energy pathways fuelling chironomid biomass (Wooller et al., 2012; Belle et al., 2014, Frossard et al., 2015). Then, it is possible to study the relationships between phytoplankton dynamics (sedimentary pigment analysis), carbon sources at the base of the benthic food web ($\delta^{13}C$ analysis of subfossil chironomid) and past climate dynamics over long time scale.

Using combined analysis of sedimentary pigments and carbon stable isotopic composition of chironomid remains, we aimed to reconstruct the temporal changes in aquatic primary productivity and carbon resources sustaining chironomid larvae in a high mountain lake (Lake Pyramid Inferior; Nepalese Himalayas). We hypothesized that past climate fluctuations influenced the glacier dynamic leading to a control of in-lake solute concentrations and aquatic primary production. We expected to demonstrate the correlation between changes in aquatic primary productivity and $\delta^{13}C_{HC}$ values and then better understand the effects of climate change on high-elevation lakes in the Himalayan Mountains.

Methods

Site description

Lake Pyramid Inferior is situated in the Khumbu Valley (Sagarmatha National Park), Nepal (27°96′N, 86°81′E; Fig. 1A). It is a small lake (1.67 ha; Table 1) located above the tree limit in the Nepalese Himalayas (at 5,067 m a.s.l.), with a maximal water depth of 14.8 m (Fig. 1A). A survey conducted in 1998 (Tartari et al., 1998b) estimated that 11% of the entire catchment area was occupied by a glacier (Table 1). Lake Pyramid Inferior exhibits very low nutrient contents (TP = 3 µg.l^{-1}, TN = 204 µgN.l^{-1}; Tartari et al., 1998a) and can be characterized as an ultra-oligotrophic lake (Lami et al., 2010).

Two sediment cores were retrieved from the deepest part of Lake Pyramid Inferior in 1993 (Pinf-93-01; Lami et al., 1998) and 2002 (Pinf-02-03; Lami et al., 2010) using a gravity corer (6 cm in diameter). Magnetic susceptibility was measured on both cores every 1 cm using a Bartington Instruments Magnetic Susceptibility Meter (model MS2) and MS2C Core Logging Sensor. Cross-correlation between cores was performed using a new set of identified lithological boundaries (Fig. 1B). Seven radiocarbon dates ($n = 4$ for Pinf-93-01 and $n = 3$ for Pinf-02-03) were obtained from bulk sediment samples by accelerator mass spectrometry performed at the Poznan Radiocarbon Laboratory (Poland) and ANSTO AMS Centre (Australia). The number, name

and non-calibrated age of each radiocarbon date are summarized in Table 2. Radiocarbon dates were re-calibrated using the most recent atmospheric cali-bration dataset (IntCal13; Reimer et al. 2013). Age–depth modelling of the core collected in 2002 (Pinf-02-03) was then obtained by combining all radio-carbon dates (according to the new cross-correlation shown in Fig. 1B), and two different models were produced with linear interpolation using the *Clam* package for R (Blaauw, 2010). New age–depth models were slightly different than those produced by Lami et al. (2010). Without new radiocarbon dates, it was impossible to select one of these models (Fig. 1B), and the chronology should then be treated cautiously. However, both models shown that 70 cm of sediments from Lake Pyramid Inferior covered the last ca. 3,500 years, corresponding to an average sedimentation rate of about 0.2 mm.year^{-1} (Fig. 1B). All the following proxies were analysed on the same core collected in 2002 (Pinf-02-03). All sample depths were converted to calibrated year BP (year cal. BP, with 0 cal. BP = AD 1950) according to the two age–depth models (Fig. 1B).

Sedimentological and sedimentary pigment analysis

Sediment cores were sliced into continuous 5-mm-thick samples ($n = 140$). Organic matter contents in sediments (OM) were analysed in each sample using loss-on-ignition method following recommendations of Heiri et al. (2001). Results were expressed in terms of percentage of dry weight (% of dry weight). To track the temporal changes in aquatic primary pro-ductivity, sedimentary pigments were extracted from about 1 g of fresh sediments for the same 140 samples, overnight under nitrogen, with a solution of acetone and water (90:10), and were then centrifuged at ca. 2,700 g for 10 min. Total carotenoids (TC) and chlorophyll derivatives (CD) were then quantified from the extract via spectrophotometry and expressed in milligrams per gram of organic matter (mg g^{-1} OM) and in Units per gram of organic matter (U g^{-1}OM), respectively, as described in Guilizzoni et al. (2011). Three outliers were detected in sedimentolog-ical data (OM = 46.4%; TC = 15.4 mg g^{-1} of OM, and CD = 632.1 mg g^{-1} of OM), and their extreme
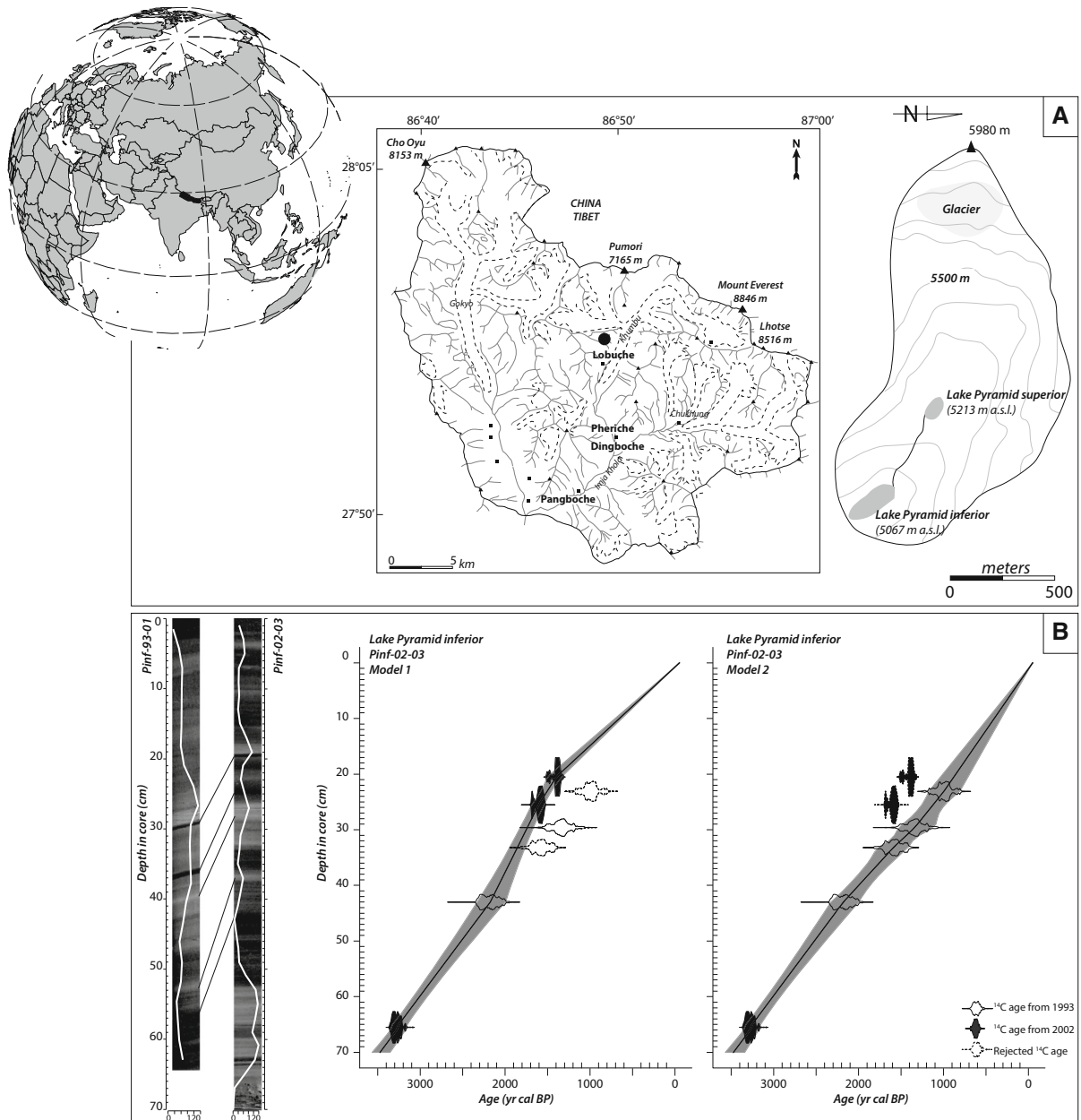
Fig. 1 A Geographical location of Lake Pyramid Inferior in the Sagarmatha National Park (Khumbu valley; Nepal) and elevation map of its catchment area. Sediment cores were retrieved from the centre of Lake Pyramid Inferior at ca. 14.5 m of water depth. **B** Pictures of cores collected in 1993 (Pinf-93-01) and 2002 (Pinf-02-03), magnetic susceptibility signals and indication of cross-correlation applied using sedimentological markers. Age–depth models (linear interpolation) of core collected in Lake Pyramid Inferior in 2002 (Pinf-02-03). All sample depths were converted to calibrated year BP (year cal. BP, with 0 cal. BP = AD 1950) according to the age–depth models

values were much higher than previously reported from another study conducted on the same lake (Lami et al., 1998). Thus, these samples were removed for

further statistical analysis. Results of OM and sedimentary pigment analyses were previously published and discussed by Lami et al. (2010).

Table 1 Morphological characteristics of Lake Pyramid inferior

Parameters	Lake Pyramid Inferior
Latitude (°N)	27.96
Longitude (°E)	86.81
Elevation (m a.s.l.)	5,067
Max. elevation (m a.s.l.)	5,980
Catchment area (km^2)	1.08
Glaciated area AD 1998 (%)	11
Lake area (ha)	1.67
Water depth (m)	14.8
$A_C.A_L$	65

"$A_C.A_L$" refers to catchment-to-lake area ratio

Data are reported from Tartari et al. (1998b)

Carbon stable isotope analysis of chironomid remains

The rest of sediment samples were pooled to reach a sample thickness of 2 cm ($n = 34$), and reconstruction of carbon sources sustaining chironomid biomass was performed using carbon stable isotope analysis of chironomid remains archived in lake sediments. We selected *Pseudodiamesa nivosa*-type morphotype (based on classification provided by Ilyashuk et al., 2010) because this morphotype belongs to the deposit-feeder trophic guild (feeding on phytoplankton deposits, terrestrial detritus and associated bacteria; Pegast, 1947). Thus, it is an excellent indicator of recycling of organic matter in lake sediments. Moreover, *Pseudodiamesa nepalensis* (belonging to *Pseudodiamesa nivosa*-type morphotype; Appendix 2) has a widespread occurrence in Nepalese lakes (Manca

et al., 1998), and *Pseudodiamesa nepalensis* has been found at high density in the profundal zone of Lake Pyramid Inferior (Manca et al., 1998). Sediment samples were pretreated using successive washings with NaOH (10%) and HCl (10%) solutions and be sieved through a 100-µm mesh following Schimmelmann & DeNiro (1986) and van Hardenbroek et al. (2009). Following recommendations from Frossard et al. (2013), carbon stable isotope composition of chironomid remains ($\delta^{13}C_{HC}$) belonging to the 4th larval instar of *Pseudodiamesa nivosa*-type morphotype was then analysed using an isotope ratio mass spectrometer interfaced with an elemental analyser at INRA Nancy (Champenoux; UMR INRA 1137). Approximately 15 entire HC were loaded into tin capsules to reach a sample weight always higher than 20 µg. Results were expressed according to the delta notation with Vienna Pee Dee Belemnite as the standard: $\delta^{13}C$ (‰) $= ([R_{sample}/R_{standard}]-1) \times 1,000$, where $R = {^{13}C}/{^{12}C}$. Replication of sample measurements from internal laboratory standards (with a target weight of 20 µg) produced analytical errors (1σ) of $\pm 0.2‰$ ($n = 8$).

Data analysis

Zonation in sedimentological data (OM, TC and CD) was determined by constrained hierarchical cluster analysis using a Bray–Curtis distance and CONISS linkage method (*Rioja* package for R, Juggins, 2017), and significance of zones was assessed using the broken-stick model (Bennett, 1996). For graphical displays, main temporal trends were visually tracked by smoothing the time-series using a LOESS

Table 2 Radiocarbon dates for cores collected in Lake Pyramid inferior

| Depth (cm)	Lab code	Material	Coring	Year BP	Age–depth model (1/2)
20.5	Poz-2317	Bulk	2002	1510 ± 30	I/R
23.1	OZD-210	Bulk	1993	1070 ± 80	R/I
25.5	Poz-2318	Bulk	2002	1690 ± 30	I/R
29.6	GrA-7043	Bulk	1993	1420 ± 110	R/I
33.2	OZD-212	Bulk	1993	1670 ± 80	R/I
43	OZD-215	Bulk	1993	2170 ± 70	I
65.5	Poz-2587	Bulk	2002	3050 ± 30	I

In column Age–depth model, "R" refers to rejected dates and "I" to included dates. Core depths are given for core collected in 2002 (Pinf-02-03) according to cross-correlation shown in Fig. 1B

regression (LOESS parameters: span = 0.2; polynomials degree = 2; *analogue* package for R, Simpson and Oksanen, 2016). Pigment concentrations in lake sediments were expected to have a significant, and potentially non-linear, influence on carbon sources fuelling chironomid biomass. Statistical relationships between $\delta^{13}C_{HC}$ values and sedimentary pigment variables (TC and CD) were then examined using a generalized additive model (GAM; fitted using the *mgcv* package for R; Wood, 2011) approach, with a continuous-time first-order autoregressive process to account for temporal autocorrelation (Simpson & Anderson, 2009). Logarithmic transformations [log(x + 1)] were applied to sedimentary pigment data (i.e. explanatory variables) before statistical analysis in order to approximate a normal distribution. Significance of fitted trends was checked using the standard statistical inference for GAM. All statistical tests and graphical displays were performed using R 3.4.0 statistical software (R Core Team, 2017).

Results

Organic matter content values (OM) in lake sediments ranged from 2.9 to 30.1% (Fig. 2A), whereas total carotenoids (TC) and chlorophyll derivatives (CD) ranged from 0 to 5.4 mg g^{-1} of OM, and from 0.5 to 245.8 U g^{-1} of OM, respectively (Fig. 2A). Dendrogram based on sedimentological data (Bray–Curtis distance and CONISS linkage method) showed 5 significant zones defined by their major temporal patterns (Fig. 2A). The succession of zones along the sediment core seemed to correspond to a regular temporal pattern (Fig. 2A). "Black" zones (1, 3 and 5) referred to the periods with the high OM contents, and TC and CD values (Fig. 2A). On the opposite side, the lowest OM contents and TC and CD values were observed in "white" zones (2 and 4; Fig. 2A).

Subfossil chironomid $\delta^{13}C$ values ($\delta^{13}C_{HC}$) were relatively high and ranged from − 16.9 to − 8.1‰ (Fig. 2B). The temporal trend of $\delta^{13}C_{HC}$ values
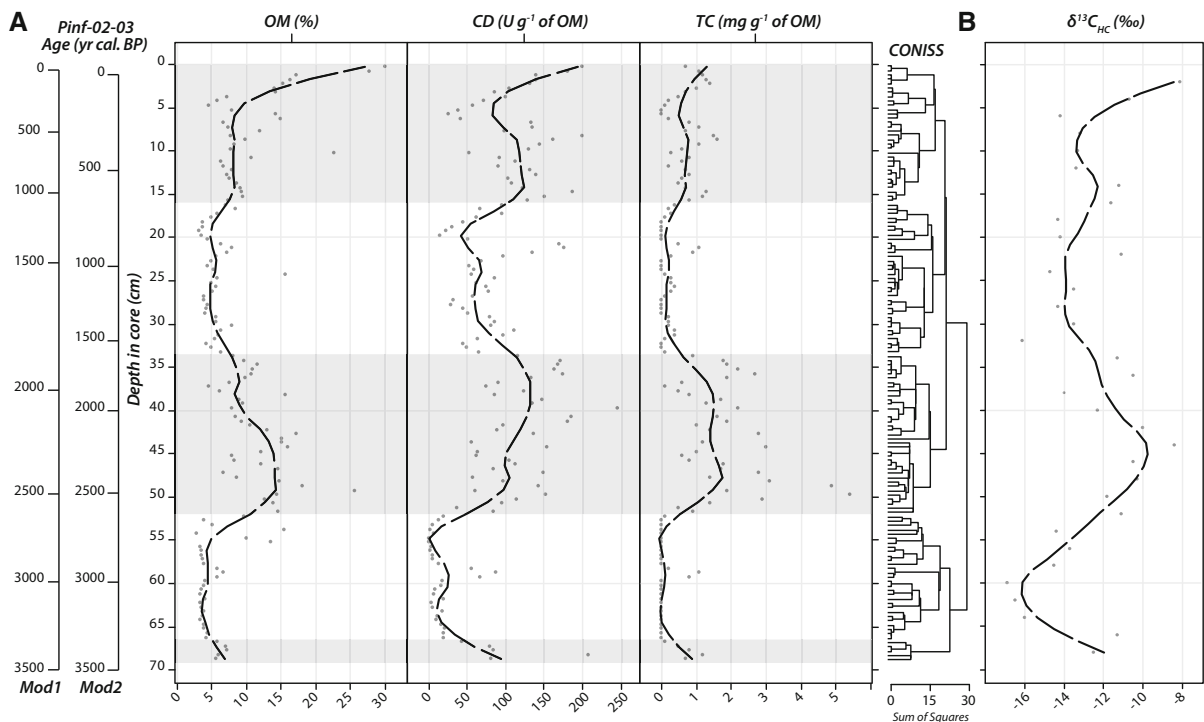
Fig. 2 A Temporal dynamics of organic matter content (OM; %), sedimentary pigments (total carotenoids (TC) and chlorophyll derivatives (CD) expressed in mg g^{-1} OM and U g^{-1} OM, respectively). The dendrogram was constructed by hierarchical clustering analysis (Bray–Curtis distance, CONISS linkage method). The shaded areas in stratigraphic diagram indicate significant zones according to the broken-stick model. The sample ages were also reported on the y-axis according to the two possible age–depth models (Fig. 1B). **B** Temporal trend in carbon isotopic composition of *Pseudodiamesa nivosa* type ($\delta^{13}C_{HC}$; ‰)

seemed also to follow a regular pattern (Fig. 2B), matching very well with those observed in sedimentological data (Fig. 2A). The highest $\delta^{13}C_{HC}$ values (i.e. less ^{13}C-depleted) were reported during the highest sedimentary pigment concentrations (Fig. 2A). A GAM approach was applied to $\delta^{13}C_{HC}$ values to understand how aquatic primary production (TC and CD) dynamics affected carbon sources sustaining chironomid biomass. After logarithmic transformation of explanatory variables, model residues followed a normal distribution and the use of the Gaussian family was then relevant. Both GAMs showed strong significant positive effects of TC ($F = 14.8$; e$df = 2.42$) and CD concentrations ($F = 7.5$; $edf = 2.44$) on $\delta^{13}C_{HC}$ values, explaining 57 and 34% of $\delta^{13}C_{HC}$ variability, respectively (Fig. 3B and Table 3).

Discussion

Climate change, E–m flux and benthic secondary production

Reconstructions of past climate changes in the Himalayan Mountains are scarce, but they revealed marked alternations of warm and cold periods over the last 3,500 years (Röthlisberger & Geyh, 1985; Owen et al., 2009; Holmes et al., 2009; Henderson & Holmes, 2009; Yang et al., 2009). Since current glacier dynamics and associated in-lake solute concentrations are strongly regulated by climate variability (Salerno et al., 2008; Salerno et al., 2016; Chudley et al., 2017), past warming periods should also correspond to periods of glacial retreats. Clustering analysis of the sedimentary pigment stratigraphy of Lake Pyramid Inferior revealed a strong concordance between primary producer's dynamics and main known climatic phases in the Eastern part of the Himalayan Mountains (Lami et al., 2010). During warm phases ("dark" zones in Fig. 2A), autochthonous primary productivity was higher due to increase in in-lake solute concentrations, whereas in contrast the lowest aquatic primary productivity was reported during cold events ("white" zones in Fig. 2A). According to the conceptual E–m flux framework (Leavitt et al., 2009), phytoplankton community seemed then to be indirectly controlled by climate change through temporal changes in in-lake solute concentrations (m-fluxes; Fig. 3A; Lami et al., 2010). Effects of climate change were then mediated by the surrounding catchment and the glacier dynamics, playing the role of environmental filters.
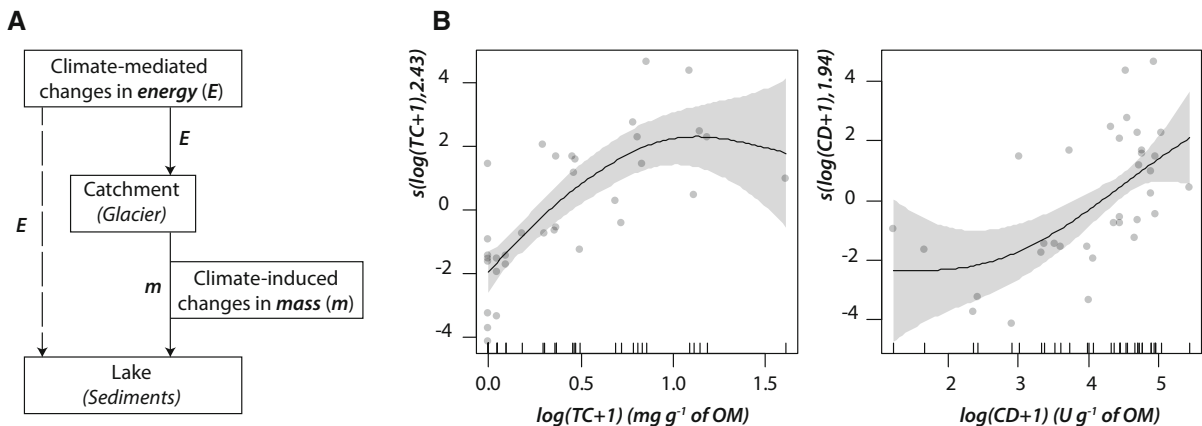
Fig. 3 A Conceptual overview showing the impact of climate change on the energy–mass framework in Lake Pyramid Inferior. Energy (E) refers to irradiance and atmospheric heat, while mass (m) transfers correspond to nutrients and solute dynamics. The dashed arrow represents a minor process, whereas the full arrows correspond to main processes that affected the Lake Pyramid Inferior dynamic. In the case of Lake Pyramid Inferior, a warmer climate induces a decrease in the glacial area in the catchment, leading to increase in solute concentrations in lake. B Fitted smooth function between explanatory variables (TC and CD) and $\delta^{13}C_{HC}$ values from generalized additive models (GAMs), with a continuous-time first-order autoregressive process to account for temporal autocorrelation. Grey surface marks the 95% uncertainty interval of the fitted function. On the x-axis, black ticks show the distribution of the observed values for variables. Numbers in brackets on the y-axis are the effective degrees of freedom (edf) of the smooth function

Table 3 Summary of statistics for generalized additive models (GAMs) fitted with a Gaussian distribution to carbon stable isotopic composition of chironomid remains ($\delta^{13}C_{HC}$; ‰)

	R^2	Intercept	t value	P	edf	F	P
$\delta^{13}C_{HC} \sim s(TC)$	0.57	− 12.7	− 51.6	< 0.001	2.42	14.8	< 0.001
$\delta^{13}C_{HC} \sim s(CD)$	0.34	− 12.7	− 41.6	< 0.001	2.44	7.5	< 0.001

The explanatory variables were sedimentary pigments (total carotenoids (TC) and chlorophyll derivatives (CD)) expressed in mg g^{-1} of OM. "*edf*" refers to the effective degrees of freedom

The surrounding catchment of Lake Pyramid Inferior is mainly constituted by bare rocks (ca. 90%; Tartari et al., 1998b), and inputs of terrestrial organic matter to lakes can then assume to be negligible. In this context, the availability of food sources at the base of aquatic food webs was only controlled by the intensity of aquatic primary productivity, and it was likely the limiting factor of benthic secondary production (as suggested by Morgan et al., 1980), inducing a bottom-up control on benthic consumers. Indeed, benthic secondary production of lakes can be strongly reliant on phytoplankton dynamics in a phenomenon also described as the pelagic–benthic coupling (Goedkoop & Johnson, 1996). In this study, we demonstrated the positive relationship between photosynthetic pigments' concentrations in sediments and carbon sources incorporated in chironomid biomass (illustrated by $\delta^{13}C$ values of *Pseudodiamesa nivosa* type; Fig. 3B). The target morphotype used in this study belonged to *Pseudodiamesa nivosa*-type morphotype which is known to feed on phytoplankton deposits and associated bacteria (and terrestrial detritus if any; Pegast, 1947). The deposit-feeder trophic guild is then particularly able to feed on phytoplankton deposits on lake sediments especially during phytoplankton blooms (e.g. Johnson, 1987). Warm periods and associated rise in aquatic primary production (Fig. 2A; Lami et al., 2010) induced changes in food source availabilities at the base of aquatic food webs (i.e. phytoplankton), impacting trophic interactions in the benthic food web. Finally, the present study also confirmed the strong sensitivity of carbon processing in the benthic food web to climate change. Indeed, previous studies revealed that temperature was the principal driver of the food availability at the base of the benthic food web in small lakes (van Hardenbroek et al., 2013; Belle et al., 2017a, c).

High elevation, partial pressure and isotopic fractionation

The range of $\delta^{13}C_{HC}$ values reported in this study (ranging from − 16.9 to − 8.1‰, Fig. 2B) was considerably higher than that previously reported from different types of lakes (from − 49.2 to − 22‰; -van Hardenbroek et al., 2013; Frossard et al., 2015; Belle et al., 2016; Belle et al., 2017c). Accurate data interpretation needs a reliable estimation of isotopic baselines (Vander Zanden and Rasmussen, 1999; Post, 2002), and too few stable isotopic studies have been conducted in the Nepalese Himalayas. Whereas $\delta^{13}C$ values of pelagic primary production usually range from − 36 to − 25‰ (Vuorio et al., 2006; Wang et al., 2013), the range of $\delta^{13}C_{HC}$ values could match well with that of the $\delta^{13}C$ values of phytoplankton frequently observed in CO_2-limiting environment (Street-Perrott et al., 1997; Reuss et al., 2013). Lake Pyramid Inferior is located at 5,067 m a.s.l., and atmospheric pressure is consequently about the half of the value reported at the sea level. Based on calculations combining pH and alkalinity values measured in water column of Lake Pyramid Inferior, Tartari et al. (1998a) assumed that dissolved inorganic carbon (DIC) concentrations in the lake may be in equilibrium with ambient atmosphere. The DIC concentrations are then very low and might be a limiting factor for the development of phytoplankton (Hein, 1997). In this CO_2-limiting context, CO_2 invasion from atmospheric origin can be observed during rising phytoplankton productivity (Schindler & Fee, 1974), which could explain the observed high $\delta^{13}C$ values. In addition, a lower fractionation by phytoplankton during periods of high aquatic primary productivity ("dark zones", Fig. 2A) could lead to increase in phytoplankton $\delta^{13}C$ values observed during productive periods. Indeed, it is well

known that a substrate competition (for the light isotope, ^{12}C) can occur when the aquatic primary productivity is high and DIC concentrations are very low (Smyntek et al., 2012), leading to the decrease in the trophic fractionation. Similar observations were previously suggested from sediment records in temperate lakes (Belle et al., 2017b; Schilder et al., 2017).

Future challenges for (paleo)-limnological studies in the Nepalese Himalayas

Further investigations are still needed to better understand the carbon cycle in high-elevation lakes (i.e. from biogeochemical cycles to aquatic food webs), and in particular fractionation by phytoplankton and aquatic primary producers in CO_2-limiting environment (Street-Perrott et al., 1997). The use of compound-specific stable isotopes of phytoplankton markers (e.g. Ninnes et al., 2017) could help us better estimate the temporal changes in isotopic baselines for chironomid larvae. In addition, complementary studies combining stable isotope and chironomid abundance investigations should be conducted to assess the impact on benthic secondary productivity (similarly to Frossard et al., 2015). The reconstruction of carbon processing in aquatic food webs using carbon stable isotopic analysis of biological remains seems to be promising in Nepalese Himalayas lakes and could be coupled with those of cladoceran remains (Rantala et al., 2015), to better assess the complexity of the response of lake carbon cycling to past climate change.

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