Changes in carbon sources fueling benthic secondary production over depth and time: coupling Chironomidae stable carbon isotopes to larval abundance

Victor Frossard · Valérie Verneaux · Laurent Millet · Michel Magny · Marie-Elodie Perga

Abstract Stable C isotope ratio (δ^{13}C) values of chironomid remains (head capsules; HC) were used to infer changes in benthic C sources over the last 150 years for two French sub-Alpine lakes. The HCs were retrieved from a series of sediment cores from different depths. The HC δ^{13}C values started to decrease with the onset of eutrophication. The HC δ^{13}C temporal patterns varied among depths, which revealed spatial differences in the contribution of methanotrophic bacteria to the benthic secondary production. The estimates of the methane (CH_4)-derived C contribution to chironomid biomass ranged from a few percent prior to the 1930s to up to 30 % in recent times. The chironomid fluxes increased concomitantly with changes in HC δ^{13}C values before a drastic decrease due to the development of hypoxic conditions. The hypoxia reinforced the implication for CH_4-derived C transfer to chironomid production. In Lake Annecy, the HC δ^{13}C values were negatively correlated to total organic C (TOC) content in the sediment (C_{org}), whereas no relationship was found in Lake Bourget. In Lake Bourget, chironomid abundances reached their maximum with TOC contents between 1 and 1.5 % C_{org}, which could constitute a threshold for change in chironomid abundance and consequently for the integration of CH_4-derived C into the lake food webs. Our results indicated that the CH_4-derived C contribution to the benthic food webs occurred at different depths in these two large, deep lakes (deep waters and sublittoral zone), and that the trophic transfer of this C was promoted in sublittoral zones where O_2 gradients were dynamic.

Keywords Lakes · Chironomids · Methanotrophy

V. Frossard (✉)
Université Savoie Mont Blanc, UMR42 CARRTEL, 73376, Le-Bourget-du-lac, France
e-mail: victor.frossard@univ-savoie.fr

V. Frossard · V. Verneaux · L. Millet · M. Magny
Laboratoire Chrono-Environnement, UMR6249, 16 route de Gray, 25030 Besançon Cedex, France

M.-E. Perga
INRA, UMR42 CARRTEL, 75 avenue de Corzent, 74203 Thonon les Bains Cedex, France

Introduction

Over the last decade, benthic habitats have been reintegrated into the picture of lake food webs (Vadeboncoeur et al. 2002; Reynolds 2008; Rooney and McCann 2012). The energy sources that fuel benthic secondary production originate from both terrestrial and aquatic habitats (including benthic, littoral and pelagic production) and are derived from different C sources [chemo- or photosynthesis (Grey and Deines 2005; Solomon et al. 2011; Jones and Grey 2011; Devlin et al. 2013)]. For a decade, CH_4 has been recognized as an energy source that supports benthic production via methanotrophy (Kiyashko et al. 2001; Jones et al. 2008; Jones and Grey 2011). Different from photosynthesis, methanotrophy is a chemosynthetic metabolic pathway supported by methanotrophic bacteria that oxidize most of the CH_4 produced in freshwater lakes (Bastviken et al. 2008) under both aerobic and anaerobic conditions (Hanson and Hanson 1996; Borrel et al. 2011; Blees et al. 2014). Methanotrophic bacteria constitute a significant food source for benthic (Deines et al. 2009; van

Hardenbroek et al. 2010; Wooller et al. 2012; Belle et al. 2014) and pelagic (Kankaala et al. 2006; Jones and Lennon 2009) invertebrates. As a consequence, CH_4-derived C can support part of a lake's total productivity, and even that of the surrounding terrestrial environments (Jones and Grey 2011).

C stable isotope ratios ($\delta^{13}C$) are traditionally used to track CH_4-derived C assimilated by aquatic consumers because methanotrophic bacteria are typically strongly ^{13}C depleted. The singularly low $\delta^{13}C$ values of methanotrophic bacteria are due to the very low $\delta^{13}C$ values of the CH_4 used as their metabolic substrate [i.e., -90 ‰ (Eller et al. 2005)] and because of variable but significant trophic fractionation of methanotrophic bacteria (~ -5 to -30 ‰; Summons 1994). Chironomids (Diptera) are a suitable candidate to track the assimilation of CH_4-derived C because the $\delta^{13}C$ values of the larvae and their remains are closely related to those of their food sources [i.e., ^{13}C depletion of ~1 ‰ (Heiri et al. 2012; Frossard et al. 2013a)]. Furthermore, they are typically highly abundant and grow at both the water–sediment interface and within the sediment (Armitage et al. 1994; Moller Pillot 2009) where strong O_2 gradients promote development of methanotrophic bacteria. Among chironomids, the tribe of Chironomini and, more broadly, benthic tube-dwelling chironomid species efficiently consume methanotrophic bacteria, therefore relying on CH_4-derived C to support their growth (Eller et al. 2007; Deines et al. 2009; Jones and Grey 2011). For example, van Hardenbroek et al. (2013) recently found a strong correlation between CH_4 fluxes and the $\delta^{13}C$ values of different chironomid taxa, particularly Chironomini and Tanytarsini. Diverse patterns were detected concerning methanotrophic bacteria as food sources for chironomids. Kiyashko et al. (2001), Jones et al. (2008) and Ravinet et al. (2010) all observed an increase of the methanotrophic bacteria contribution to chironomid biomass with depth. However, Agasild et al. (2014) identified shallow habitats with macrophytes in Lake Võrtsjär (Estonia) also as suitable habitat for CH_4-derived C assimilation by chironomids. Blees et al. (2014) recently showed that CH_4 oxidation occurred efficiently even at very low O_2 concentrations (a few nanomoles per liter). This suggested that a periodically anoxic hypolimnion, such as found in deep lakes, could also host methanotrophic bacteria production and subsequent assimilation by the benthic fauna. Therefore, in large, deep lakes with a wide variety of habitats, localizing preferential habitats for the assimilation of CH_4-derived C by benthic fauna remains challenging, but it is crucial to understanding how C and energy flow in benthic food webs of lakes.

The objectives of this study were to track the changes in the CH_4-derived C contribution to chironomid production in two large, deep sub-Alpine French lakes over time

(~150 years) and space (different depths). For this, we combined previously published (Frossard et al. 2014b) and original $\delta^{13}C$ values of chironomid remains (head capsules; HC) sampled from sediment cores retrieved at different depths. To reinforce the interpretations based on HC $\delta^{13}C$ values, original reconstructions of chironomid fluxes associated with previous results (Frossard et al. 2013a) were used to detect changes in O_2 conditions related to CH_4 production and oxidation.

Sediments with high amount of organic matter influence benthic food webs in two ways. First, increased availability of this food source may stimulate benthic secondary production (Armitage et al. 1994; Moller Pillot 2009). Second, sediment organic matter promotes benthic microbial respiration in benthic habitats, which leads to O_2 depletion (Verneaux et al. 2004). In the hypolimnion and sublittoral zones of deep lakes, high organic matter contents are typically associated with strong O_2 depletion because of microbial respiration (Verneaux et al. 2004; Jyväsjärvi et al. 2013). As a consequence, over a gradient of organic matter content, chironomid abundances could be negatively affected by the increase in organic matter, whereas methanotroph production could be increased. This contradictory pattern has direct implications for the transfer of CH_4-derived C within lake food webs because the environmental windows suitable for both chironomid development and methanotrophy likely differ over space and time. To account for this local environmental driver, we assessed the relationships between TOC and both chironomid $\delta^{13}C$ values and fluxes via modeling.

Materials and methods

Study sites

Lake Annecy and Lake Bourget are two large, deep sub-Alpine lakes located on the southwestern edge of the French Alps (Table 1). The nutrient history of these two lakes over the last 150 years was documented by paleolimnological assessments (Millet et al. 2010; Perga

Table 1 Characteristics of Lake Annecy and Lake Bourget

	Lake Annecy	Lake Bourget
Depth (maximum) (m)	67	145
Area (km^2)	27.4	45
Watershed area (km^2)	251	580
Altitude (m a.s.l.)	446	232
Mixing behavior	Monomictic/ holomictic	Monomictic/ meromictic
Current trophic state	Oligotrophic	Oligo/mesotrophic

et al. 2010; Frossard et al. 2013b; Berthon et al. 2014). These two lakes were initially oligotrophic [total P (TP) concentrations ~5–10 µg l^{-1} (Berthon et al. 2014)]. Increased urban and agricultural activity in the watershed led to eutrophication following World War II. From the second to the third quarter of the twentieth century, the increase in nutrient concentrations in the open water led to a mesotrophic level in Lake Annecy (TP concentrations ~15 µg l^{-1}) and to a eutrophic level in Lake Bourget (TP concentrations ~120 µg l^{-1}). Remediation efforts decreased nutrient concentrations over the last quarter of the twentieth century, and these two lakes have almost recovered to a pre-industrial state with respect to their nutrient concentrations in the pelagial, since the 1990s for Lake Annecy and in current times for Lake Bourget (Berthon et al. 2014). Despite the reductions in nutrients, the ecological state of these lakes remains different from that preceding the industrial revolution, with for example, the maintenance of a hypolimnetic hypoxic zone (Perga et al. 2010; Jenny et al. 2013), which prevents abundant benthic fauna from settling (Frossard et al. 2013b). The lack of benthic recovery despite efficient nutrient abatements has been attributed to anthropogenic forcing other than eutrophication since the 1990s, i.e., fish management practices in Lake Annecy (Perga et al. 2010) and climate warming in the two lakes (Frossard et al. 2014a; Jenny et al. 2013). For Lake Bourget, recent changes in the benthic/littoral habitats also include the development and further extension of macrophyte beds toward deeper waters as water transparency has increased. The most recent inventory (2011) of macrophytes in Lake Bourget identified 22 species, including *Nitellopsis obtuse*, *Chara contraria* and *Chara globularis* in association with *Potamogeton* spp., *Myriophyllum* spp. and *Ceratophyllum* spp. that colonized more than 300 ha of the lake. The mats of *Ceratophyllum* spp. were sampled down to a depth of 12 m, whereas those of *C. globularis* were present down to a depth of 14 m (data from Conservatoire des Espaces Naturels de Savoie).

Sampling

The sediment cores (diameter = 90 mm) were retrieved at 30 and 60 m in Lake Annecy in 2009 and at 30, 60 and 145 m in Lake Bourget in 2010 using a gravity corer (Uwitec, Austria). Additionally, two cores were collected in the deepest part of each lake in 2009 and 2010, respectively, in Lake Annecy and Lake Bourget, to provide reference chronologies.

Dating

In each lake, high-resolution chronologies of the last 150 years were obtained from reference cores sampled in the deepest part of the lakes. The age/depth models for the reference cores were obtained using radiometric methods (^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am activities), counting of annual laminations, lithostratigraphic analysis and magnetic susceptibility. Details of the age/depth models of the reference cores are extensively described in Frossard et al. (2013b) for Lake Annecy and in Jenny et al. (2013) for Lake Bourget. The chronologies of the cores used in this study were obtained by correlations with (1) lithological tie points, (2) lamina counting, and (3) high-resolution spectrophotometry as specified in Frossard et al. (2013b). The mean sedimentation rates were ~2 mm $year^{-1}$ in Lake Annecy and ~4 mm $year^{-1}$ in Lake Bourget.

Laboratory analyses

The cores were cut transversally, and each half core was assigned either for HC carbon isotope measurements or for HC flux reconstructions and measurements of TOC content in the sediment. The half cores were then subsampled at 5-mm intervals.

Micropsectra HC preparations

Micropsectra contracta-type (hereafter "*Micropsectra*") was used in this study because it was the most abundant taxon in the two lakes at all depths. Furthermore, the use of a single taxon, and therefore, a single feeding behavior [detritivore (Moog 2002)] limited the complications in interpretation of HC δ^{13}C values (Reuss et al. 2013). Moreover, because the *Micropsectra* larvae were tube-dwellers and typically completed their larval development within a year (Brooks et al. 2007), the depth-specific δ^{13}C signals were not likely attenuated by vertical migrations, as occasionally observed for some other chironomid species [e.g., *Chironomus acerbiphilus* (Takagi et al. 2005)]. The subsamples were treated for 2 h with HCl (10 %) and then with KOH (10 %) and were then sieved using a net with a 100-µm mesh. The chironomid remains were removed from the sieved residues under ×40–70 magnification and were mounted on microscope slides using Aquatex mounting agent. The HCs of *Micropsectra* were identified according to Brooks et al. (2007) with a microscope at ×100–1,000 magnification. The *Micropsectra* counts were then converted to HC fluxes (HC $year^{-1}$ 10 cm^{-2}) according to accumulation rates from the age/depth models and the sediment surface for each sample.

C isotope analyses

The samples for HC carbon isotope measurements were treated using the same procedure as that used for *Micropsectra* HC identifications. This procedure did not

significantly affect the $\delta^{13}C$ values of the HCs (van Hardenbroek et al. 2010). For Lake Annecy, the chironomid HC isotopic analyses were conducted at the CNRS laboratory of Nancy (France) using an Isoprime mass spectrometer coupled with a Eurovector EA3000 elemental analyzer. The internal precision was tested using sugar (sucrose) with a known $\delta^{13}C$ value (i.e., -26.1 ‰) and a SD of 0.2 ‰ ($n = 67$). For Lake Bourget, the chironomid HC isotopic analyses were conducted using a Finnigan Delta S mass spectrometer coupled with an elemental analyzer and a Gas Bench II (Finnigan) at the INRA laboratory of Champenoux (France). The internal precision was tested using beet sugar (sucrose) with a known $\delta^{13}C$ value (i.e., -26.7 ‰) and a SD of 0.1 ‰ ($n = 9$). The stable C isotopic ratios were expressed as $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1,000$, where $R = {}^{13}C/{}^{12}C$ and Vienna Pee Dee belemnite is the standard.

Total organic C and hydrogen index analyses

Total organic C (TOC) content in the sediment (% C_{org}) of the two lakes was assessed following two distinct methods. In Lake Annecy at 30- and 60-m depths as well as in Lake Bourget at 145-m depth, the TOC was determined using Rock–Eval pyrolysis (Espitalié et al. 1985a, b) with a Model 6 device (Vinci Technologies). This pyrolysis induced a progressive release of hydrocarbon products (HP) contained in sediments. This allowed for calculation of the hydrogen index (HI), which represented the hydrogen to C ratio of sediment organic matter (in mg HP g^{-1} TOC). Allochthonous organic matter typically exhibits a lower HI index than autochtonous organic matter due to higher lignin content. Therefore, the HI was used to assess temporal variability in the origin of sediment organic matter (Meyers and Ishiwatari 1993). Analyses were performed on 50–100 mg of dried, crushed sediment. For Lake Bourget, at 30- and 60-m depths, the TOC was determined using a vario MAX CNS analyzer (Elementar, Germany) from ~170 mg of dried sediment. The samples were dried for 4 h at 105 °C prior to analysis.

Statistical analyses

The temporal trends for chironomid fluxes and $\delta^{13}C$ values as well as TOC and HI were described using local polynomial regressions (loess; order = 2; window size = 50 % of the data).

The chironomid responses (abundance or $\delta^{13}C$) to sediment organic matter and subsequent O_2 conditions were expected to be nonlinear (Jones et al. 2008; Brodersen et al. 2008). Additionally, the data set exhibited a nested structure that might have biased the statistical independence of individual values and consequently statistical inferences

(Zuür et al. 2007). Therefore, generalized additive mixed models (GAMMs) were used to analyze the relationships between TOC and both HC $\delta^{13}C$ values and *Micropsectra* fluxes. The GAMMs fit smooth additive functions and allow nonlinear relationships to be considered (Hastie and Tibshirani 1990). Selected by generalized cross-validation, the extent of the smoothing was expressed as estimated *df* (e*df*), where e*df* = 1 related to a linear effect and values greater than 1 indicated a progressively stronger nonlinear effect (Wood 2006). To account for the nested structure of the data set, the random elements of the GAMMs for *Micropsectra* flux modeling were either lakes or depths as categorical random variables. The categories "lakes" and "depths" were not introduced together in the random part of the models because the size of the data set did not accommodate that many *df*. The relationships between TOC and HC $\delta^{13}C$ were analyzed independently for each lake because $\delta^{13}C$ values of primary producers were not available to assess lake-specific $\delta^{13}C$ baselines. In Lake Annecy, the relationship between HC $\delta^{13}C$ values and TOC was examined using a GAMM that included depth as a random variable. In Lake Bourget, a generalized additive model (GAM) was used because of the low number of samples per depth. The Akaike information criterion (AIC) was used as the basis for model selection. Prior to modeling, the *Micropsectra* fluxes were log transformed using $\log_{10}(x + 1)$ to achieve normality. One was added to HC fluxes because of the occurrence of zero flux for some samples. Mann–Whitney and Kruskal–Wallis tests were used to determine significant differences between TOC and HI and among lakes and depths, respectively. Statistical analyses and graphical displays were produced using R.3.0.2 (R Core Team Development 2012), as well as the packages mgcv (Wood 2011) and nlme (Pinheiro et al. 2012). The R-codes for the GAMMs are provided in the electronic supplementary material.

Results

TOC temporal trends

The two lakes exhibited different TOC contents at different depths. The TOC contents were higher in the sediments of Lake Bourget than in those of Lake Annecy (Mann–Whitney test, $p < 0.01$). In both lakes, the TOC content tended to increase over time (Fig. 1). Over the entire study period, the Lake Annecy TOC content did not differ significantly among sample depths (Mann–Whitney test, $p = 0.51$), although recently, the TOC content was higher at 60 than at 30 m (Fig. 1). By contrast, in Lake Bourget, the TOC content differed among sample depths (Kruskal–Wallis test, $p < 0.01$), with lower TOC content at 145 than at 60 m from
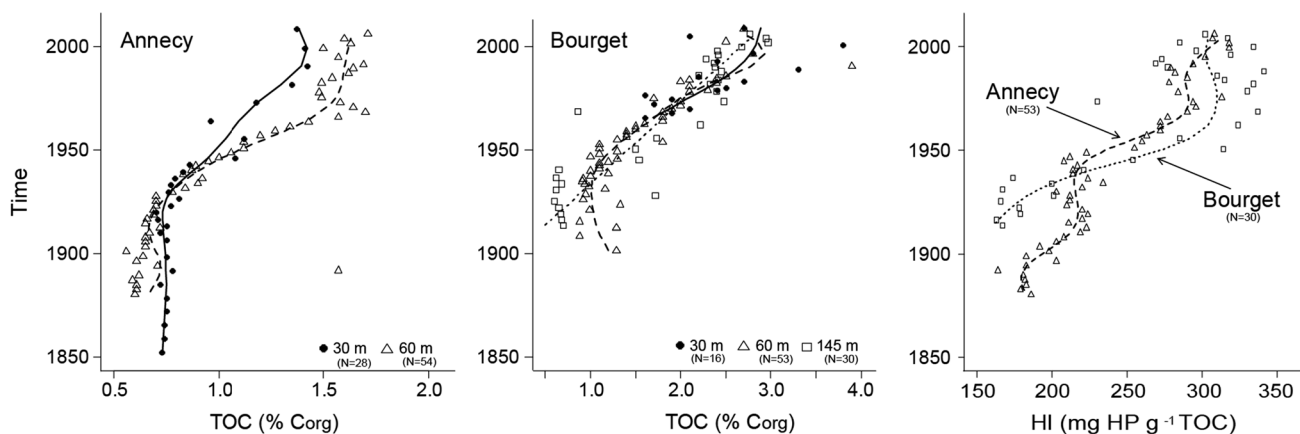
Fig. 1 Temporal changes in the organic matter content [total organic C (*TOC*); TOC content in the sediment (*C*org)] and the hydrogen index (*HI*) of the sediments in Lake Annecy and Lake Bourget at the different depths. *Black circles*, *solid line* 30-m core; *open triangles*, *dashed line* 60-m core; *open squares*, *dotted line* 145-m core. *Smooth lines* were produced using local polynomial regressions (loess)
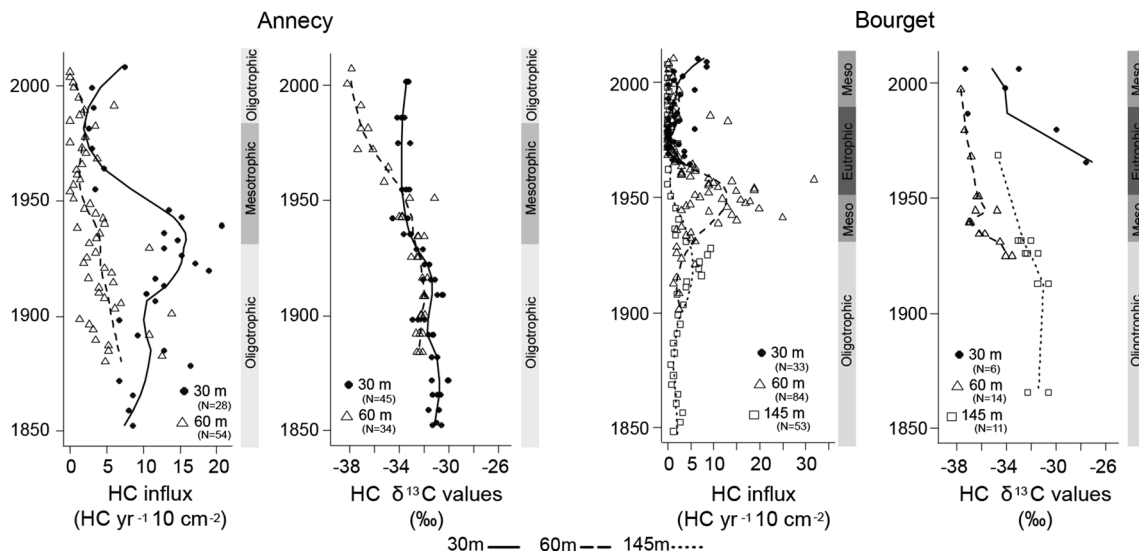

Fig. 2 Patterns for *Micropsectra* HC fluxes and head capsule (*HC*) stable C isotope ratio (δ^{13}C) values in Lake Annecy and Lake Bourget at the different depths. *Black circles*, *solid line* 30-m core; *open triangles*, *dashed line* 60-m core; *open squares*, *dotted line* 145-m core. The *smooth lines* were produced using loess regressions. *Vertical bars* Temporal changes of the trophic status of the lakes according to Wetzel's classification (2001) based on total P concentrations

the 1900s to the 1950s. From the 1950s, the TOC content drastically increased, particularly at 145 m, which led to an overlap between 60 and 145 m, whereas at 30 m the TOC content was even higher in recent times. The TOC content in the two lakes also changed qualitatively over time according to the HI. Whereas the HI from sediments of deeper cores did not differ among the two lakes (Mann–Whitney test, $p = 0.08$), the HI increased in both cores over time from approximately 150–200 between the 1900s and the 1920s to 300–350 between the 1970s and the 2000s (Fig. 1), which indicated increased organic matter sedimentation that originated from autochthonous primary production.

Temporal changes in *Micropsectra* fluxes

Most *Micropsectra* temporal dynamics were nonlinear except in Lake Annecy at 60 m, where a linear decreasing trend was observed, and at 30 m in Lake Bourget, in which HC fluxes increased in the last decades (Fig. 2). The nonlinear trends were bell-shaped patterns (temporally restricted increase in HC fluxes). At 30 m in Lake Annecy and 60 m in Lake Bourget, the HC fluxes reached approximately 15 HC year^{-1} 10 cm^{-2} during the HC peaks, whereas at 145 m in Lake Bourget, the HC flux peaked at only ~7 HC year^{-1} 10 cm^{-2}. The fluxes then declined greatly, falling to a level

Table 2 Summary statistics of the generalized additive mixed models linking total organic C (*TOC*) and *Micropsectra* fluxes in Lake Bourget and Lake Annecy

		Estimates	*t*	*p*
Parametric part	Intercept	0.6 ± 0.04	17.3	<0.001
		e*df*	*F*	*p*
Nonparametric part	s(TOC): Lake Annecy	2.3	15.3	<0.001
	s(TOC): Lake Bourget	4.9	12.1	<0.001

of quasi-extirpation in Lake Annecy at 30 m (1940s) and in Lake Bourget at 60 m (1950s) and 145 m (1930s). These results allowed for a precise dating of the onset of the summer hypolimnetic hypoxia in the two lakes and indicated that this phenomenon still occurred at the different studied depths.

Trends in δ^{13}C

The HC fluxes varied among depths between the two lakes and had different temporal trends. At 30 m in Lake Bourget, the sediment core was too short to obtain HC fluxes older than 1960, which precluded the assessment of any pre-perturbation HC δ^{13}C baseline. For the other depths, the large amount of HCs resulted in high-resolution and replicated measurements that provided a detailed description of relative changes in the C sources that supported chironomid production. When sufficient HCs were present in a sample, particularly in Lake Annecy, the replicated δ^{13}C values were very similar, which indicated low within-sample variability in HC δ^{13}C values (i.e., the mean SD among replicates within a sample was 0.4 ‰ in Lake Annecy and 0.7 ‰ in Lake Bourget).

The different HC δ^{13}C temporal patterns could be expected because the timing and the extent of changes in C sources for chironomids were unknown a priori. Indeed, the HC δ^{13}C temporal patterns differed depending on sampling depths and were mostly nonlinear in the two lakes, except in Lake Bourget at 30 m. This result suggested that depth-specific variability in C sources contributed to chironomid production over time (Fig. 2). In Lake Annecy, HC δ^{13}C records spanned most of the last 150 years. Until the 1930s, the HC δ^{13}C values were similar between depths (−32.0 ± 0.5 ‰ at 60 m and −31.4 ± 0.6 ‰ at 30 m). This result indicated a similar mixture of C sources fueling chironomids, which was dominated by photosynthetic primary producers (primarily phytoplankton) at different depths. By the 1930s, HC δ^{13}C values started to decrease at both depths, but at 30 m, the ^{13}C depletion stabilized in the 1950s at ~−33 ‰ (depletion ~−2 ‰). By contrast, the HC δ^{13}C values at 60 m continued to decrease from ~−32 down to ~−38 ‰. Hence, deep chironomids were depleted

in current times by approximately 5 ‰ compared with the sublittoral ones.

In Lake Bourget, similar HC δ^{13}C patterns were obtained at different depths with a decrease in HC δ^{13}C values concomitantly with the onset of eutrophication and the increase in autochthonous organic matter sedimentation on the lake bottom, as indicated by the HI values. Nonetheless, the HC δ^{13}C reconstructions did not fully cover the last 150 years because of the lack of HCs (145 m) or the short time period covered by the cores (60 and 30 m). The δ^{13}C values of the oldest HCs from the 1860s at 145 m were similar to those measured in the 1930s (~−32 ‰). From the 1930s to the 2000s, the HC δ^{13}C values decreased both at 60 and at 145 m, from ~−34 to ~−38 ‰.

The HC δ^{13}C values at 60 m in Lake Bourget were slightly (by 2–3 ‰) but significantly (Kruskal–Wallis, $p = 0.02$) more ^{13}C depleted in comparison with those at 145 m. After the 1970s, no more HCs were retrieved from sediment at 145 m. At 30 m, the HC δ^{13}C values were characterized by a sharp decline from ~−28 to ~−38 ‰ from the 1960s to the 2000s (Fig. 2). As a result, the HC δ^{13}C values were almost identical at 30 and 60 m in the 2000s.

Relationships between *Micropsectra* fluxes and TOC

In each lake, the *Micropsectra* fluxes exhibited specific nonlinear relationships with TOC (Table 2). A GAMM that considered lakes a random part of the model (AIC = 128.4) outperformed a GAMM that used depths as a random part (AIC = 134.2) and a GAM without a random part (AIC = 130.3). The model explained 37.4 % of the overall variability in *Micropsectra* fluxes. In Lake Annecy, the HC fluxes decreased almost linearly both at 30 and at 60 m as TOC increased (Fig. 3). By contrast, in Lake Bourget, the nonlinear pattern was an increase in HC fluxes between 0.5 and 1.1 % C_{org} in the sediments. Beyond this threshold, the HC fluxes sharply decreased to stabilize at low values for TOC values in the sediment higher than 2–2.5 % C_{org} (Fig. 3). In Lake Bourget, the initial organic matter enrichment of the sediment benefited the chironomids by supplying greater amounts of food and did not lead to severe hypoxia. The drastic decrease of *Micropsectra* at higher contents of 1.1–1.5 % C_{org} indicated that this was the amount of organic matter necessary to trigger O_2 depletion to lower concentrations than the physiological requirement of this genus.

Relationships between HC δ^{13}C values of *Micropsectra* and TOC

Among the two lakes, relationships between HC δ^{13}C values and TOC differed and significant relationship could

Fig. 3 The relationships between *Micropsectra* HC fluxes and TOC contents of the sediments in Lake Annecy and Lake Bourget. *Black circles* 30-m core, *open triangles* 60-m core, *open squares* 145-m core, *solid lines* generalized additive mixed model-fitted values showing lake-specific patterns, *dashed lines* 95 % confidence intervals
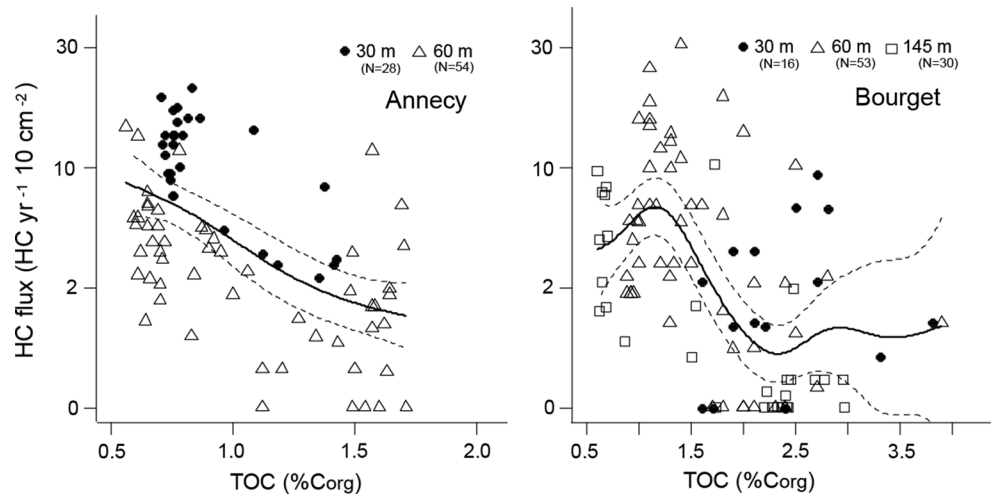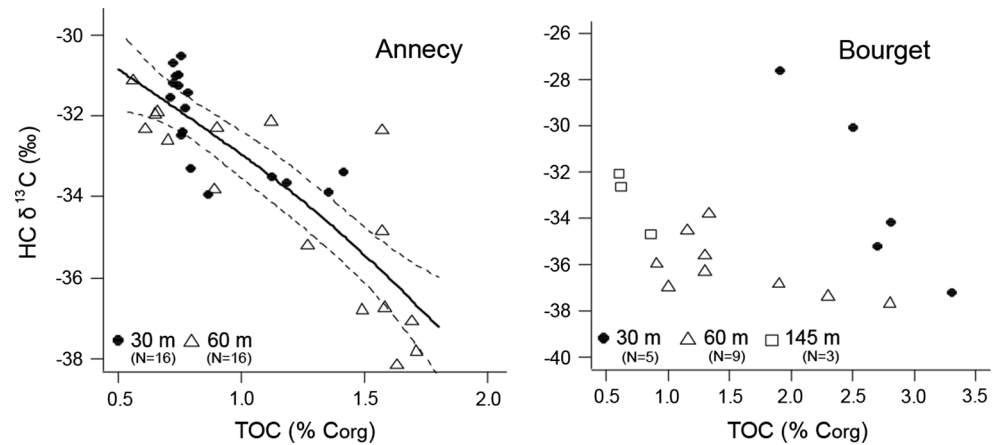
Fig. 4 The relationships between HC δ13C values and TOC contents of the sediments in Lake Annecy and Lake Bourget. *Black circles* 30-m core, *open triangles* 60-m core, *open squares* 145-m core, *solid line* generalized additive model-fitted values for Lake Annecy, *dashed lines* 95 % confidence intervals. In Lake Bourget, no significant relationships were found

only be found for Lake Annecy. For this lake, a negative slightly nonlinear (edf = 1.45) relationship was found between TOC content and the HC δ13C values (GAM, F = 45.44 and $p < 0.001$), which explained 71.7 % of the variability of the HC δ13C values (Fig. 4). The pattern was common for the two depths because the consideration of depth as a random part in the GAMM failed to improve the fit to the model (AIC = 116.7 with depth as a random effect and AIC = 113.1 without a random effect). This result suggested a similar effect of TOC content on change in the C cycle when comparable increases in methanotrophy occurred with increases in TOC. The primary difference was that at 30 m in Lake Annecy the TOC content was lower than at 60 m, and therefore, was too low to support abundant CH_4 production and oxidation to be reflected in the 13C-depleted HC. In Lake Bourget, no significant relationship was found between TOC content and the HC δ13C values (GAM, F = 0.37 and $p = 0.55$; Fig. 4). Nonetheless, two different patterns were identified. In the deep zones, the HC δ13C values slowly decreased with increasing TOC contents. At 30 m, the HC δ13C values exhibited

a sharp decrease as TOC content increased. The increase in CH_4-derived C would have occurred at this particular depth recently.

Discussion

This study reports on the temporal changes of HC δ13C values over the last 150 years to address the spatial and temporal changes in C sources (chemosynthesis vs. photosynthesis) that contributed to chironomid production. The two sub-Alpine lakes were both large, but they exhibited a different nutrient status, and hydrology and thermal regimes (holomixis in Lake Annecy and meromixis in Lake Bourget) that were likely to promote the chemosynthetic pathway of methanotrophy differently. Additionally, the determination of the *Micropsectra* HC fluxes was a first attempt to independently track changes in environmental conditions (i.e., hypoxic conditions) to reinforce interpretations of potential methanotrophy inferred from the HC δ13C values. Conservative patterns for both HC δ13C values and

chironomid fluxes were obtained from the different depths, which suggested similar relationships between changes in environmental conditions (i.e., O_2) and changes C food sources that supported the chironomids.

Spatial and temporal change in C sources

In both lakes, the HC $\delta^{13}C$ baselines of ~−32 ‰ indicated similar food sources supported chironomid production at the different depths. Although no $\delta^{13}C$ values of phytoplankton were obtained from the sediment cores, such values suggested that the C sources were largely dominated by phytoplankton (France 1995; Popp et al. 1998), which we expected for these two large and deep lakes with low watershed area to lake area ratios (i.e., 11.8 and 10.5 for Lake Bourget and Lake Annecy, respectively). Therefore, prior to eutrophication, photosynthesis was the main C source at the base of the food webs in these two lakes. Eutrophication occurred concomitantly with the onset of the HC $\delta^{13}C$ decrease and HC fluxes, which indicated that eutrophication acted as the initial forcing factor that triggered changes in the C cycle mediated by changes in O_2 conditions. In most cases, the timing of changes for both HC $\delta^{13}C$ values and chironomid fluxes was time delayed, with the deeper depths being affected first. This upward pattern was likely related to the expansion of the oxycline from deep to shallower depths, which would constrain chironomid settling, whereas methanogenesis and methanotrophy would be enhanced. The influence of respired C (C_{resp}) (Rau 1978), which exhibits significantly ^{13}C-depleted $\delta^{13}C$ values compared with atmospheric C, likely had a minor influence on the HC $\delta^{13}C$ patterns. Indeed, the dissolved organic C pools that promote C_{resp} in the inorganic C pool via microbial respiration (Karlsson 2007) were low, as indicated by the high transparency of the two lakes (minimal transparency = 4.2 m in Lake Annecy and 3.8 m in Lake Bourget). Further, the large size of the two studied lakes suggested that atmospheric CO_2 (CO_{2atm}) was likely to be a major source for aqueous CO_2 (CO_{2aq}) because of gas exchanges (Wanninkhof 1992). The current increase in CO_{2atm} can support the increase in CO_{2aq} (Low-Décarie et al. 2014) influencing the fractionation rate of primary producers (Raven et al. 1994). As a consequence, the increase in CO_{2aq} can lead to low phytoplankton $\delta^{13}C$ baselines due to a high fractionation rate (Smyntek et al. 2012). Nonetheless, according to Frossard et al. (2014b), in Lake Annecy, the C_{resp} could have been responsible for the decrease of ~2 ‰ of HC $\delta^{13}C$ values, and the influence on changes in CO_{2aq} would be of a similar magnitude. Therefore, a major influence of C_{resp} or CO_{2aq} on phytoplankton $\delta^{13}C$ values to explain a stronger $\delta^{13}C$ decrease than ~2 ‰ was unlikely. As a consequence, higher $\delta^{13}C$ depletion in chironomid HCs than 2 ‰ was unlikely to be attributable to changes in the $\delta^{13}C$ values of C sources, but rather to changes in the contribution of C sources supporting chironomids over time.

In Lake Bourget, the ^{13}C depletion of ~2 ‰ of HC $\delta^{13}C$ at 60 m compared with those at 145 m would suggest a more important contribution of CH_4-derived C to chironomid production at 60 than at 145 m. This finding could be partially explained by the higher amounts of organic matter in sediments at 60 m compared with those at 145 m, which would provide more substrate to support methanogenesis and subsequently methanotrophy (Borrel et al. 2011). The sharp ^{13}C depletion at 30 m from the 1960s to the 2000s (~−10 ‰) might indicate that methanotrophy was strongly enhanced over this time period in the sublittoral zone where the oxycline was likely to occur within the superficial sediment rather than within the water column, as suggested by the monitoring data [Comité Intersyndical pour l'Assainissement du Lac du Bourget (CISALB) data]. This result could be associated with an increase in sediment organic matter related to the development of macrophytes (particularly *C. globularis*) downward following the re-oligotrophication. Indeed, lake transparency significantly increased over the last decades (ANOVA, $p < 0.001$; data from the CISALB), and *C. globularis* beds currently flourish up to depths of 14 m (data from Conservatoire des Espaces Naturels de Savoie). Although the sediment core was sampled in deeper water depth (30 m), chironomid assemblages contained a significant fraction of macrophyte dwellers [e.g., *Cricotopus* spp. (Frossard 2013c)], which suggested that the sediment record integrated the macrophyte-associated fauna (van Hardenbroek et al. 2011). Within macrophyte beds, sediment organic matter increases with macrophyte biomass (Squires and Lesack 2003). Therefore, the development of macrophytes in the sublittoral zone could promote, through an increase in organic matter availability, methanogenesis and subsequently methanotrophy (Agasild et al. 2014). Thus, the temporal enhancement of the methanotrophy was comparatively more important in the sublittoral zone that reached similar HC $\delta^{13}C$ values to those found in deeper waters. A simple linear two-sources mixing model [seston = −29 to −32 ‰ (France 1995; Lehmann et al. 2004; Yang et al. 2014) and methanotrophs = −60 to −90 ‰ (Eller et al. 2005)] indicated that a decrease of 1 ‰ in the chironomid diet could be associated with an increase of CH_4-derived C of ~1–4 %. As a consequence, over the time period studied, the CH_4-derived C contribution to chironomids would have increased from less than 10–30 % in different habitats of the two lakes. Using mixing models supported by measured $\delta^{13}C$ values of CH_4, as well as terrestrial and aquatic biomarkers, Wooller et al. (2012) estimated a similar "basal" support of a few percent of CH_4 to chironomid C over the last ~12,000 years in an arctic Alaska wetland. The authors

also reported on marked periods of increasing contributions, with maximal contributions up to 15 %, which was almost half of the maximal contribution estimated at different depths in recent times among the two lakes in our study. Nonetheless, the extent of change in CH_4-derived C contribution to chironomids was in line with Belle et al. (2014), who recently reported on an increase of CH_4-derived C contribution, from ~10 to ~25 %, over the last millennium in Lake Narlay (France).

Linking C sources and species abundance

Micropsectra was the dominant genus in the chironomid assemblages of the two lakes prior to the occurrence of modern anthropogenic forcing. Its sensitivity to O_2 conditions (Quinlan and Smol 2001) and ability to tolerate temporally restricted hypoxic conditions (Brodersen et al. 2008) allowed for the identification of severe and prolonged hypoxia following eutrophication through the decrease of *Micropsectra* fluxes. The decrease in *Micropsectra* fluxes supports the hypothesis of methanotrophy to explain the decrease in HC δ^{13}C values because the onset of the decrease was followed by a decrease in HC fluxes at most depths. In the two lakes, the bell-shaped patterns of HC fluxes, likely related to an increase in the amount of organic matter available, indicated that the highest contribution of chironomids to fish production was fueled by C integrated in the food webs via the photosynthetic pathway. Methanotrophy mostly developed after the *Micropsectra* fluxes drastically decreased at the different depths. Therefore, although the proportion of CH_4-derived C was likely to increase in chironomid biomass, the transfer to higher levels of the food webs might be limited. Once the hypoxia became too severe, although methanotrophy could still be efficient, the trophic transfer of CH_4 was strongly limited because of the collapse of the chironomid populations. Interestingly, along with the decrease in HC δ^{13}C values, the *Micropsectra* fluxes increased at 30 m in recent times. As a consequence, CH_4-derived C export to fishes by chironomids could become more important in sublittoral habitats where macrophytes provide suitable conditions for methanotrophy (Agasild et al. 2014) compared with deep zones where hypoxia almost eliminated chironomid larvae.

The lake-specific patterns between *Micropsectra* fluxes and TOC suggested different influences of TOC on *Micropsectra* fluxes between the lakes, likely related to lake morphometry and mixing. In Lake Annecy, the negative relationship between *Micropsectra* HCs and TOC indicated that an increase in TOC content triggered a decrease in O_2 concentration. In Lake Bourget, the TOC threshold value of approximately 1–1.5 % TOC in the sediment was the value at which *Micropsectra* HC fluxes started to collapse, which could define the minimal amount of TOC necessary

to trigger significant changes required in O_2 conditions for C cycle change and an increase in methanotrophy. Therefore, completing biological indicators with δ^{13}C values of organisms provided independent ways to highlight changes in environmental conditions and reinforced our interpretations based on HC δ^{13}C values.

Future changes in C sources

The significant negative relationship between HC δ^{13}C values and TOC, which was almost linear, indicated that TOC might have been a limiting substrate for methanogenesis, methanotrophy and the subsequent integration of CH_4-derived C within the benthic food web in Lake Annecy. The lack of correlation between HC δ^{13}C values and TOC content in the sediments in Lake Bourget might be explained by differences in TOC quality, and other parameters related to O_2 conditions (e.g., mixing efficiency) and availability of C_{resp} (e.g., depth of the primary production in the water column).

Despite reductions of nutrient concentrations in the open water (i.e., re-oligotrophication), no return of HC δ^{13}C values to those of pre-perturbation conditions was observed in the two lakes. This finding suggested that the contribution of the chemosynthetic pathway to chironomid production was still increasing relative to the photosynthetic pathway in recent times. The progressive increases in TOC and HI in all cores suggested that phytoplanktonic organic matter export to the benthos was still increasing. Diagenetic processes could partially be involved in TOC patterns (Meyers and Ishiwatari 1993). Nonetheless, HI is highly conservative over millennia (Talbot and Livingstone 1989; Noël et al. 2001), and therefore, the increase supported an effective increase in sediment organic matter over time. The changes in the transfer of pelagic organic matter in these lakes due to the decrease in size of *Daphnia* because of the top-down effect of planktivorous fishes, as well as climate warming (Perga et al. 2010; Frossard et al. 2014a), are two possible explanations for the increase in TOC content. Changes in *Daphnia* size decrease the C transfer efficiency in the pelagial (Brooks and Dodson 1965), whereas climate warming as well as the increase in CO_{2atm} concentrations, and subsequently in CO_{2aq}, could increase phytoplankton productivity (Schippers et al. 2004; Paerl and Huisman 2008) independently of nutrient enrichment to support the increase of TOC content within the sediments.

In the future, the development of anoxic conditions in the deep waters of these two lakes accompanied by macrophyte development could continue to promote methanogenesis and methanotrophy. Nonetheless, the environmental conditions under which methanotrophy occurs will influence the possibility to integrate CH_4-derived C into the lake food webs because of variable ecological niches for benthic

invertebrates. As a consequence, the consideration of other chironomid genera as well as different taxa exhibiting various ecological traits (Frossard et al. 2014a, b; van Hardenbroek et al. 2014) would help to achieve a better assessment of the extent of the contribution of CH_4-derived C to the entire benthic food web.

Conclusion

The long-term perspectives provided by HC δ^{13}C records at multiple depths revealed the great potential for using biological remains to identify and track changes in the C sources that fuel benthic food webs. Bathymetric variability in the estimated CH_4-derived C contribution to chironomids suggested that in large, deep lakes both deep and sublittoral zones are suitable for chemosynthesis (methanotrophy) as a significant C source to fuel benthic food webs. The sublittoral zones that support active macrophyte growth and provide suitable environmental conditions to shelter abundant chironomid communities are promising habitats to support CH_4-derived C integration and transfer within lake food webs. Because technical limitations are progressively overcome, the application of additional proxies such as stable isotopes [e.g., δ^2H and δ^{34}S (Deines et al. 2009; Grey and Deines 2005)], phospholipid fatty acids and ancient DNA (Belle et al. 2014) are needed to better identify C sources at the base of lake food webs and to track their temporal changes over long time periods.

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