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Influence of thermal regime, oxygen conditions and land use on source and pathways of carbon in lake pelagic food webs

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ABSTRACT

Intensification of anthropogenic activities in many lake catchments during the twentieth century led to increased autochthonous organic matter sedimentation and degradation of hypolimnetic oxygen conditions due to the intensification of heterotrophic processes. These processes can be amplified by the effect of climate warming on thermal stratification in lakes. This study aimed to assess how metabolic disruptions affect carbon sources and pathways in lake pelagic food webs, focusing on methanogenic carbon. The studied lakes showed strong seasonal variations of carbon source availability and transfers to pelagic food webs, characterized by increased methanogenic carbon transfers to *Daphnia* populations in winter. The magnitude of these winter transfers seems to largely depend on the amount of methane stored in the hypolimnion during the stratification period, and thus on the amount of methane released with autumnal turnover. Methane production, storage and transfer mechanisms partly depend on thermal stratification intensity, but also on external factors such as land use. This study provides new insights into the impacts of global changes on the sources and pathways of carbon in pelagic food webs through their influence on lake metabolism and thermal regimes. These functional changes may lead to greater production and release of greenhouse gases into the atmosphere.

R  SUM  

L'intensification des activit  s anthropiques sur de nombreux bassins versants lacustres durant le XX^e si  cle a conduit    une augmentation de la s  dimentation organique autochtone et    la d  gradation des conditions d'oxyg  nation hypolimniques en raison de l'intensification des processus h  t  rotrophes. Ces processus peuvent   tre amplifi  s par l'effet du r  chauffement climatique sur la stratification thermique des lacs. Cette   tude visait      valuer comment ces perturbations m  taboliques affectent les sources et les voies de transfert du carbone dans les r  seaux trophiques p  lagiques lacustres, en se focalisant sur le carbone d'origine m  thanog  ne. Les lacs   tudi  s ont r  v  l   de fortes variations saisonni  res des sources de carbone disponibles et transf  r  es aux r  seaux trophiques p  lagiques, caract  ris  es par l'intensification des transferts de carbone d'origine m  thanog  ne aux populations de *Daphnia* en hiver. L'intensit   de ces transferts hivernaux semble grandement d  pendre de la quantit   de m  thane stock  e dans l'hypolimnion durant la p  riode de stratification, et donc de la quantit   de m  thane lib  r  e lors du brassage automnal. Les m  canismes de production, stockage et transfert de m  thane semblent en partie d  pendre de l'intensit   de la stratification thermique, mais aussi de facteurs externes comme l'utilisation des sols. Cette   tude fournit de nouvelles connaissances sur les cons  quences potentielles des changements plan  taires sur les sources et les voies de transfert du carbone dans les r  seaux trophiques p  lagiques via leur influence sur le m  tabolisme et le r  gime thermique des lacs. Ces changements fonctionnels pourraient mener    une augmentation de la production et des   missions atmosph  riques de gaz    effet de serre.

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Introduction

Lakes are important ecosystems for the transport, emission and storage of considerable quantities of carbon. Although they cover only 0.9% of the earth's surface (Downing et al. 2006), they exert a disproportionate effect on carbon cycle (Tranvik et al. 2009). However, the carbon balance of lakes is still under-documented

(Tranvik et al. 2009) due to the complexity of the carbon cycle in lakes. Indeed, lakes play a dual role as ecosystems that, on the one hand, fix inorganic carbon (atmospheric CO₂) through photosynthesis and, on the other hand, mineralize organic carbon from autochthonous and allochthonous sources (production of CO₂ and CH₄). Understanding the trophic functioning of lakes

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and in particular the mechanisms of carbon production and transfer in food webs is therefore a major challenge in the context of global change.

One of the main carbon pathways in lakes is the fixation by primary producers of dissolved carbon which can be of atmospheric origin ($\text{CO}_{2\text{atm}}$) or released by heterotrophic activities ($\text{CO}_{2\text{resp}}$). Fixation of methanogenic carbon (C-CH_4) by methane oxidising bacteria (MOBs) is another carbon pathway linked to a different form of heterotrophic activity, the methanogenesis, performed by methanogenic archaea that degrade organic matter under anoxic conditions (Rudd and Hamilton 1978).

Global changes are likely to cause disruptions in the trophic functioning of lakes with profound cascading consequences on the associated ecosystem goods and services. On one hand, it is now well established that global warming will have serious impact on the thermal cycle of lakes (Butcher et al. 2015; Woolway and Merchant 2019). An increase in the strength and duration of summer stratification, and less frequent mixing for mid-altitude lakes, will have deep consequences on hypolimnetic oxygen conditions (Golosov et al. 2012; Butcher et al. 2015; Pilla and Williamson 2021). Production, storage and transfers process of C-CH_4 to pelagic food webs are partly controlled by lake thermal regimes (Tranvik et al. 2009; Duc et al. 2010; Yvon-Durocher et al. 2014). In stratified lakes, a large part of methane produced in sediment can indeed accumulate in hypolimnion during summer stratification period, and be released in large quantity into water column in oxic conditions during autumnal turnover (Utsumi et al. 1998). CH_4 can then be oxidised by MOBs and support higher trophic level (Rudd and Taylor 1980; Utsumi et al. 1998). However, knowledge remains limited on the influence of stratification and turnover mechanisms on the intensity of gas production and storage process in deep layers of lakes, and subsequently on the extent of CH_4 transfers in pelagic food webs.

On the other hand, the accelerated eutrophication of waters linked to intensification of anthropogenic activities on the watershed is one of the main forcing factors that may influence the carbon cycle and the trophic functioning of lakes. Over the last decades, the intensification of agropastoral practices and associated disturbances (e.g., increase in organic matter and nutrients inputs in lakes) have led to a strong increase in autochthonous organic sedimentation rates (Anderson et al. 2014), and to the development of unprecedented anoxic conditions in hypolimnion of many lakes due to increasing heterotrophic activities (Frossard et al. 2014; Jane et al. 2021). Numerous studies assessing past trophic

functioning of lakes highlighted that these trophic state changes, marked by increasing heterotrophic activities, are associated to an increase in $\text{CO}_{2\text{resp}}$ and CH_4 transfers to consumers in lakes food webs (Frossard et al. 2014; Belle et al., 2016a,2016b; Schilder et al. 2017). Indeed, intensity of CH_4 production and oxidation is strongly dependent on oxygen conditions (Rudd and Hamilton 1978), which could be strongly affected by eutrophication. Moreover, several experiments have highlighted that the methanogenic activities can be strongly promoted by the increase in autochthonous organic matter inputs (Duc et al. 2010; West et al. 2012; Praetzel et al. 2020), the phytoplankton-derived organic matter being rich in nitrogen and then easily degradable for methanogenic Archaea. Belle et al. (2016a) suggested that agropastoral practice intensity on watershed may partly drive increased methane transfers to benthic consumers in 14 French lakes. Nonetheless, few studies attempted to assess the implication of watershed coverage, or sediment and thermal stratification characteristics on the production and seasonal transfer processes of these heterotrophic derived carbon sources in lake pelagic food webs.

Stable isotope analysis (SIA) of carbon is a tool that has been widely used over the last decades for studying carbon flows in aquatic ecosystems, especially in lakes (Grey et al. 2001; Fry 2006; Taipale et al. 2007; Frossard et al. 2014). Isotopic carbon signature ($\delta^{13}\text{C}$) of organisms closely reflects the isotopic signature of their diet (DeNiro and Epstein 1978) and are therefore commonly used to track and highlight the importance of heterotrophic process in lakes (Del Giorgio and France 1996). Indeed, $\text{CO}_{2\text{resp}}$ usually exhibits $\delta^{13}\text{C}$ values that are consistently lower than $\text{CO}_{2\text{atm}}$ as a result of photosynthetic and metabolic fractionation process occurring during carbon recycling (Degens 1969; Keough et al. 1996; Raven 1996; Fry 2006). Biogenic methane is moreover highly depleted in ^{13}C (Jedrysek 2005), and isotopic fractionation by MOBs during CH_4 oxidation leads to a further ^{13}C depletion (Templeton et al. 2006). ^{13}C -depleted carbon transfers to consumers are thus easily recognizable and result in $\delta^{13}\text{C}$ values highly distinct from $\text{CO}_{2\text{atm}}$ transfers. This approach involving SIA to track these carbon transfers has been used successfully in pelagic (e.g., *Daphnia*, Lennon et al. 2006; Wooller et al. 2012; Rinta et al. 2016b; Morlock et al. 2017) and benthic (e.g., chironomids, Frossard et al. 2014; Belle et al., 2016b) consumers.

Daphnia is a ubiquitous consumer whose isotopic signature can be monitored to assess the origin and transfer of carbon in pelagic food webs. *Daphnia* diet in lake is mainly composed of phytoplankton and detritus (Perga et al. 2008; Lampert and Kinne 2011; Hiltunen

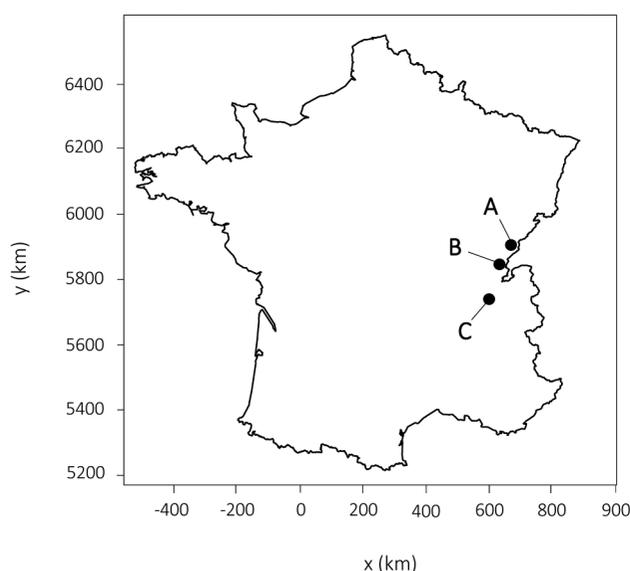


Figure 1. Location of Lake Remoray (A), lakes Abbaye, Chalain, Chambly, Grand Etival, Petit Etival, Maclu, and Ilay (B) and lakes Ambleon and Barterand (C) in France. Projection = WGS84/Pseudo-Mercator system.

et al. 2019; Grosbois et al. 2020). Its $\delta^{13}\text{C}$ values are therefore often considered to be dependent on phytoplankton $\delta^{13}\text{C}$ (Matthews and Mazumder 2003; Lennon et al. 2006; van Hardenbroek et al. 2014), and reflects the fixation of the different forms of available dissolved inorganic carbon for photosynthesis (e.g., $\text{CO}_{2\text{resp}}$ and/or $\text{CO}_{2\text{atm}}$). However, some studies highlighted that a significant part of the carbon consumed by *Daphnia*, and zooplankton in general, may originate from methane (CH_4) via MOB's consumption (Bastviken et al. 2003; Kankaala et al. 2006b, Lennon et al. 2006; Wooller et al. 2012; Rinta et al. 2016b; Morlock et al. 2017). The transfer of C- CH_4 in pelagic food webs seems nevertheless to be seasonal and depends on lakes thermal regime (Taipale et al. 2007, 2008, 2009).

The aim of this study was to assess the influence of thermal regime, oxygen conditions, land use and sediment characteristics on source and pathways of carbon transfer in lake pelagic food web with a particular attention paid on methane. For this purpose, we implemented a physico-chemical and biological survey on 11 lakes located in the French Jura, characterized by different land use, hypolimnetic hypoxia and thermal regime. Our objectives were to assess (1) the seasonal dynamics of dissolved gas in the water column ($[\text{O}_2]$, $[\text{CO}_2]$ and $[\text{CH}_4]$) from direct measurements, (2) the seasonal changes in the carbon sources transferred in pelagic food web using carbon isotopic signature of zooplankton (*Daphnia*), and (3) the main controlling factors of CH_4 storage and transfer in pelagic food webs.

Material and methods

Site description

The 11 studied lakes are located in Jura Mountains (eastern France) (Figure 1). Due to the calcareous nature of the bedrock in the area, the lake water is highly mineralised with high carbonate concentrations and high conductivity. The 11 lakes differ in elevation, geomorphological characteristics (water depth, lake area) and watershed surface and land use (Table 1). The catchment area of these lakes is typically mainly composed of forest (mid-mountain beech-fir forest) and agricultural areas (mainly fertilized grassland for milk production) in variable proportions. All are medium to small size lakes with area ranging from 5 to 232 ha and maximum depth ranging from 7 to 30.5 m. The lakes studied are located in a temperate climate context, with annual air temperatures ranging from 6 to 11°C depending on the lake. Therefore, the period of ice cover is limited in time for these systems, ranging from a few days to a few weeks.

Table 1. Characteristics of the studied lakes. Forest cover = percentage of watershed covered by forest; Agricultural cover = percentage of watershed covered by agricultural land, according to CORINE Land Cover data.

Lake	Longitude	Latitude	Altitude (m a.s.l.)	Maximal water depth (m)	Lake area (ha)	Watershed area (ha)	Forest cover (%)	Agricultural cover (%)
Abbaye	5.91 E	46.52 N	910	19	82	245.7	2.1	76.2
Ambleon	5.58 E	45.75 N	712	11	5.58	548.3	90.5	9.4
Barterand	5.74 E	45.79 N	295	14	21	793.1	51.9	48
Chalain	5.79 E	46.67 N	490	30.5	232	3468.3	61.9	32.5
Chambly	5.80 E	46.64 N	520	10	35	3438.6	62	30.9
Grand Etival	5.80 E	46.50 N	795	8	15	391.4	87.1	10.6
Maclu	5.90 E	46.62 N	779	25	22	190.1	81.6	0
Ilay	5.89 E	46.62 N	778	30	72	165.6	44.7	9.9
Petit Etival	5.80 E	46.50 N	795	7	4.7	71.1	93.7	5.5
Remoray	6.26 E	46.77 N	850	28	85	2486.3	47.3	44.4
Val	5.81 E	46.62 N	520	24	64	2038.6	75.6	15.6

Sampling strategy

A continuous monitoring of oxygen concentrations and temperatures in the water column during a whole annual cycle was implemented in the 11 lakes by the deployment of a sensor mooring line at the point of maximal depth. Six to 15 temperature loggers (TidbiT MX400, HOBO, Bourne, USA) were installed depending on the depth of the lake. These thermistors were equally distributed on the sensor line from the lake surface to the lake bottom. Additionally, two or three O₂ sensors (Minidot, PME, Vista, USA) were positioned in the hypolimnion of the 11 lakes. Oxygen and temperature loggers performed automatic measurements every 4 hours. The deepest sensor is installed at 1 meter from the bottom. The other oxygen sensors were equally vertically distributed in the hypolimnion of the lakes.

In addition, three field campaigns were carried out from September 2020 to March 2021, one at the end of summer stratification period (September 2020), and two after the autumnal turnover, in early (between the end of November 2020 and the beginning of January 2021 depending on lakes) and late winter (in March 2021). During each field campaign, vertical profiles of temperature and dissolved oxygen concentrations ([O₂]) were performed along the whole water column using a multiparameter probe (EXO1, YSI, Yellow Springs, USA) at the point of maximum depth. Membrane covered optical sensors (Mini CO₂/CH₄, Pro-Oceanus, Bridgewater, Canada) were also used to record CO₂ and CH₄ partial pressure at 5 to 6 points along the water column of each lake. Deepest point (Z_{max}) measures were taken at 30 cm above sediments. Vertical profiles of dissolved methane ([CH₄]) and carbon dioxide ([CO₂]) concentrations were determined by using CO₂ and CH₄ solubility and taking into account water temperature and air pressure, according to Weiss (1974) and Wiesenburg and Guinasso (1979).

The lake transparency was measured for each field campaign using a Secchi disk, and the depth of the euphotic zone was determined as 2.5 x the Secchi depth. Two integrated zooplankton samples were made with a 200-µm mesh size plankton net: one in the euphotic zone, and one in the whole water column of the 11 lakes. Zooplankton samples were frozen after each field campaign and stored at -20°C.

Surface sediment samples (the first cm) were collected during the early winter campaign at the deepest point using a 90-mm-diameter gravity corer (UWITECH, Mondsee, Austria).

Laboratory analyses

δ¹³C analysis of zooplankton and surface sediment

In laboratory, the zooplankton samples were thawed, and the *Daphnia* sp. were hand sorted using fine forceps under a binocular microscope without species

differentiation. For each sample, a minimum of 20 *Daphnia* were picked up to obtain the minimum dry weight of 0.07 mg required for isotope analysis. All the samples were previously exposed to 3.7% HCl solution for carbonate removal, rinsed 3 times with demineralized water, and put into ultra-clean tin capsules. The samples were then dried at 60°C in an oven for 2 days. Surface sediment samples were also exposed to 3.7% HCl solution for carbonate removal, then dried at 60°C before being put into ultra-clean tin capsules for carbon isotope analysis.

The carbon stable isotope analyses were performed using an isotope ratio mass spectrometer interfaced with an elemental analyser (EA-IRMS) at INRA Nancy (Champenoux, France). The isotope ratios were expressed in the delta notation with Vienna Pee Dee Belemnite as the standard: $\delta^{13}\text{C} (\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$, where $\text{R} = {}^{13}\text{C}/{}^{12}\text{C}$. The replication of samples measured on an internal standard gave analytical errors of ±0.3‰ for δ¹³C measurements.

Nitrogen, organic carbon and pigment analyses in surface sediment samples

The surface sediment samples of the 11 lakes were dried and acidified with a solution of HCl (3.7%) to remove carbonate. Total organic carbon content and total nitrogen content were measured using a VARIO TOC CUBE analyser (Elementar) and a VARIO MAX CNS, respectively. The results are expressed as mass percentage of organic carbon (C_{org}, %) and C_{org}/N ratio.

Photosynthetic pigments were extracted overnight from fresh sediments with a solution of acetone and water (9:1). Supernatant was then removed and used to quantify the total carotenoids (TC) via spectrophotometry following the formula provided by Züllig (1989) and the recommendations of Guilizzoni et al. (2011). Carotenoid concentrations were expressed in micrograms per gram of sediment dry weight (µg/g dw). TC concentrations can be used as a proxy of autotrophic organic matter sedimentation and may reflect primary production intensity (Guilizzoni et al. 1983; Adams et al. 1978).

Data analyses

The estimation of the watershed surface area used for agropastoral practices relative to lake surface area (relative agricultural area, ha/ha) was derived from Corine Land Cover data. Stratification characteristics were derived from the continuous temperature and oxygen concentration monitoring performed on the 11 lakes during a whole annual cycle (Supplementary material). The hypolimnetic hypoxia duration was determined by the number of days with [O₂] <2 mg/L at the relative

depth of 80% of the maximum depth. For each lake, the strength of the thermal stratification was assessed by the mean temperature differences between surface and bottom layers during stratification period ($\Delta^{\circ}\text{C}_{\text{stratification period}}$). Summer stratification period was defined as the period when the temperature difference between surface and bottom layer exceed 2°C .

Due to the limited number of observations for each variable ($n = 11$ lakes), as well as heteroscedasticity and non-normality of the residuals, non-parametric Spearman's rank-order correlation test was used to assess the relationships between gas concentration profiles, carbon isotopic signature of *Daphnia* and lake properties. Spearman's correlation coefficient (ρ) as well as p -value were calculated. Differences in $\delta^{13}\text{C}$ or gas concentrations between different sampling periods and sampling zones (photic zone vs whole water column) in the 11 studied lakes were determined using Wilcoxon signed-rank test.

All statistical analyses and figure creations were performed using R 3.5.1 statistical software (R Core Team, 2019). The significance level was set at $\alpha = 0.05$ for all statistical tests.

Results

Dissolved gas measurements ($[\text{O}_2]$, $[\text{CH}_4]_{\text{aq}}$ and $[\text{CO}_2]_{\text{aq}}$)

Summer campaign

The studied lakes were strongly thermally stratified during the late summer campaign (Supplementary material), except for the two Etival lakes which present a very weak thermal stratification at this period (Figure 2; Supplementary material). All stratified lakes had clinograde oxygen concentration profiles, with high $[\text{O}_2]$ in the epilimnion ranging from 8.1 to 11.4 mg/L, and low $[\text{O}_2]$ in the hypolimnion ranging from 0 to 0.87 mg/L. The Etival lakes had a less pronounced hypoxia in deep water than the other studied lakes with $[\text{O}_2]$ around 4 mg/L. The CO_2 and CH_4 concentration profiles of the 9 stratified lakes were also quite similar between lakes, with increasing concentrations with depth (Figure 2). In the epilimnion of stratified lakes, $[\text{CO}_2]_{\text{aq}}$ ranged from 13.4 to 31.6 $\mu\text{mol/L}$ and $[\text{CH}_4]_{\text{aq}}$ from 3.5 to 8.7 $\mu\text{mol/L}$, except for the Lake Barterand which showed particularly high epilimnetic $[\text{CO}_2]_{\text{aq}}$ at 46.8 $\mu\text{mol/L}$ and $[\text{CH}_4]_{\text{aq}}$ at 26.2 $\mu\text{mol/L}$. In the hypolimnion, $[\text{CH}_4]_{\text{aq}}$ ranged from 6.2 to 49.2 $\mu\text{mol/L}$ whereas $[\text{CO}_2]_{\text{aq}}$ were comprised between 122.7 and 454.9 $\mu\text{mol/L}$ depending on the lakes. The two shallow Etival lakes featured a relatively homogenous CO_2 and CH_4 concentrations profiles. In Grand and Petit Etival, $[\text{CO}_2]_{\text{aq}}$ varied from 20.4 to

21.1 $\mu\text{mol/L}$ and from 16.8 to 41.12 $\mu\text{mol/L}$ respectively, and $[\text{CH}_4]_{\text{aq}}$ ranged from 4.7 to 6.5 $\mu\text{mol/L}$ and from 3 to 3.4 $\mu\text{mol/L}$ respectively. When considering the whole dataset (i.e. including all lakes and depths), Spearman's rank correlation test indicates a significant positive relationship between $[\text{CO}_2]_{\text{aq}}$ and $[\text{CH}_4]_{\text{aq}}$ concentrations ($\rho = 0.53$, $p = 4.04e^{-5}$), and significant negative relationships between $[\text{O}_2]$ and $[\text{CH}_4]_{\text{aq}}$ ($\rho = -0.41$, $p = 0.001$) and between $[\text{O}_2]$ and $[\text{CO}_2]_{\text{aq}}$ ($\rho = -0.79$, $p = 2.2e^{-16}$). Furthermore, considering only the deep water concentrations in the 11 studied lakes, $[\text{CH}_4]_{\text{aq}}$ were strongly correlated to $[\text{CO}_2]_{\text{aq}}$ according to a Spearman's rank correlation test ($\rho = 0.85$, $p = 0.001$) (Figure 3).

Winter campaigns

In early and late winter, the gas concentration profiles were orthograde with relatively low variability along depth for the 11 lakes. $[\text{CH}_4]_{\text{aq}}$ ranged from 2.84 to 8.70 $\mu\text{mol/L}$ in early winter and from 3.14 to 9.44 $\mu\text{mol/L}$ in late winter, while $[\text{CO}_2]_{\text{aq}}$ ranged from 28.5 to 105.7 $\mu\text{mol/L}$ in early winter and from 21.8 to 133.3 $\mu\text{mol/L}$ in late winter for the 11 lakes (Figure 3). $[\text{CO}_2]_{\text{aq}}$ and $[\text{CH}_4]_{\text{aq}}$ in the deep water were significantly lower during the early and late winter campaigns than during the summer period according to the Wilcoxon paired sample test ($[\text{CH}_4]_{\text{aq}}$ early winter: $p = 0.001$; $[\text{CH}_4]_{\text{aq}}$ late winter: $p = 0.006$; $[\text{CO}_2]_{\text{aq}}$ early winter: $p = 0.006$; $[\text{CO}_2]_{\text{aq}}$ late winter: $p = 0.002$) (Figure 3). O_2 concentration profiles measured in winter were also orthograde, indicating a complete re-oxygenation of the water columns in the 11 lakes. $[\text{O}_2]$ in the deep water were significantly higher during the early and late winter campaigns (from 3 to 12.4 mg/L) than during the summer period according to the Wilcoxon paired sample test ($[\text{O}_2]$ early winter: $p = 9.7e^{-4}$; $[\text{O}_2]$ late winter: $p = 9.7e^{-4}$).

$\delta^{13}\text{C}$ analysis

Two *Daphnia* samples were collected for each studied lake and sampling campaign, one in the euphotic zone and one in the whole water column. According to Wilcoxon rank test for paired samples, there was no significant difference in *Daphnia* carbon isotopic signatures between the euphotic zone and the whole water column for the three sampling campaigns (stratification period: $p = 0.83$, early winter: $p = 0.11$, late winter: $p = 0.27$) (Figure 4). Therefore, $\delta^{13}\text{C}_{\text{Daphnia}}$ values are further discussed in the following sections are the average of euphotic and total column $\delta^{13}\text{C}_{\text{Daphnia}}$.

$\delta^{13}\text{C}_{\text{Daphnia}}$ showed a seasonal variability between summer and winter with a varying magnitude depending on the lake. The summer $\delta^{13}\text{C}_{\text{Daphnia}}$ ranged from

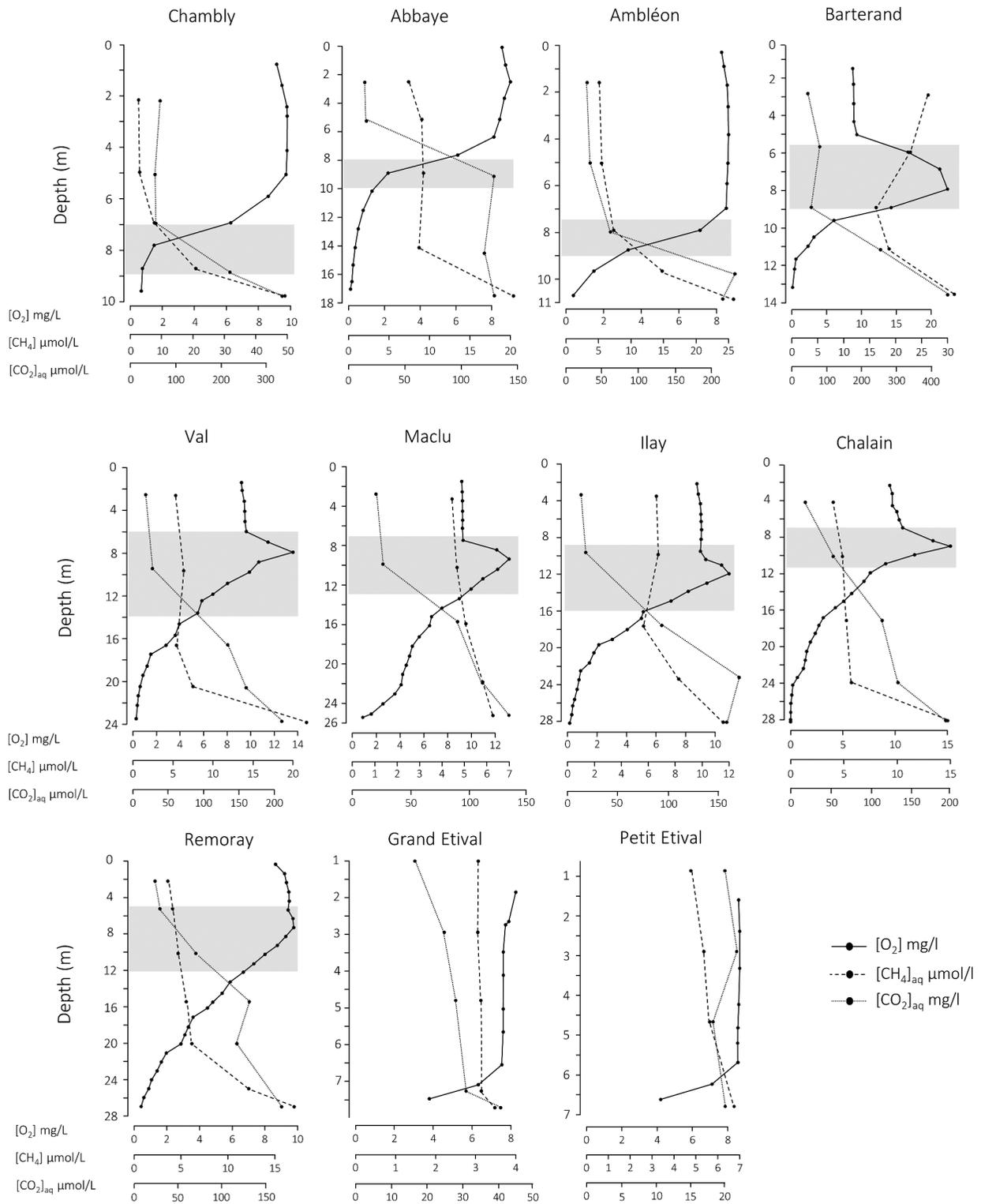


Figure 2. Vertical profiles of dissolved oxygen ($[O_2]$ mg/L), dissolved methane ($[CH_4]_{aq}$ $\mu\text{mol/L}$) and dissolved carbon dioxide ($[CO_2]_{aq}$ $\mu\text{mol/L}$) in the 11 sampled lakes during the summer stratification sampling campaign. The grey zone indicates the thermocline delimiting the mixed surface layer from the hypolimnion. Solid line = $[O_2]$ profile, dashed line = $[CH_4]_{aq}$ profile, and dotted line = $[CO_2]_{aq}$ profile.

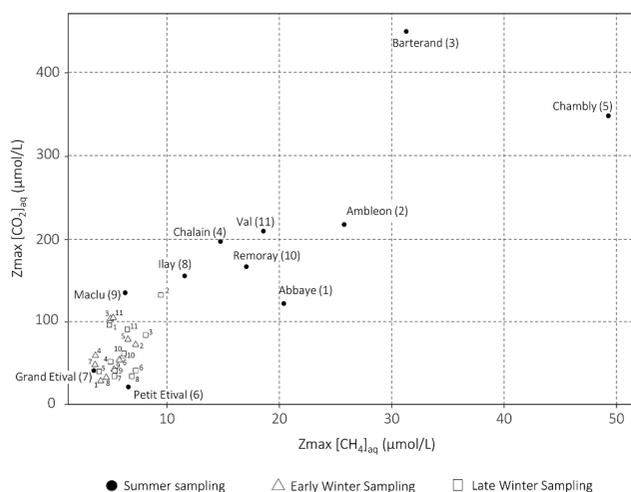


Figure 3. Dissolved CO_2 and CH_4 concentrations at Z_{max} (deepest point) for the 11 sampled lakes in summer and winter. Each point is labeled with a number corresponding to the 11 sampled lakes in alphabetical order. A significant positive relationship was observed between $Z_{\text{max}} [\text{CH}_4]_{\text{aq}}$ and $Z_{\text{max}} [\text{CO}_2]_{\text{aq}}$ according to the Spearman rank correlation test ($\rho = 0.85$, $p = 0.001$).

–40.9 to –33.9 ‰ for the 11 studied lakes (Table 2). During the winter sampling campaigns, $\delta^{13}\text{C}_{\text{Daphnia}}$ range from –54.9 to –37 ‰ and were thus lower than in summer for all lakes except for Petit and Grand Etival (Table 2; Figure 5). According to a Wilcoxon rank test for paired samples, early and late winter $\delta^{13}\text{C}_{\text{Daphnia}}$ were significantly lower than $\delta^{13}\text{C}_{\text{Daphnia}}$ during the stratification period (early winter: $p = 0.004$, late winter: $p = 0.009$, Figure 4). Furthermore, $\delta^{13}\text{C}_{\text{Daphnia}}$ during late winter were significantly higher than $\delta^{13}\text{C}_{\text{Daphnia}}$ during early winter ($p = 0.006$) (Figure 4).

There was a positive correlation of $\delta^{13}\text{C}_{\text{Daphnia}}$ between summer and winter (spearman rank correlation

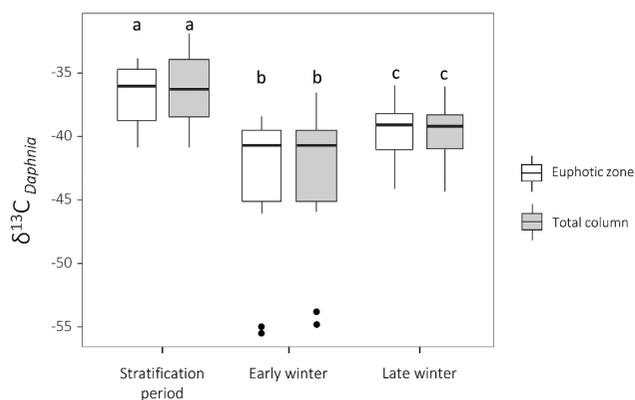


Figure 4. Distribution of $\delta^{13}\text{C}_{\text{Daphnia}}$ values for the 11 sampled lakes according to sample period and sampling zone. Means sharing the same letter are not significantly different according to Wilcoxon rank tests for paired samples ($\alpha = 0.05$).

Table 2. Isotopic carbon signatures ($\delta^{13}\text{C}_{\text{Daphnia}}$, ‰) of *Daphnia* sampled during three sampling campaigns, $\delta^{13}\text{C}_{\text{Daphnia}}$ difference between the summer and early winter campaigns (seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$), and $\delta^{13}\text{C}_{\text{Daphnia}}$ difference between the summer and mean winter values (mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$).

Lake	Summer $\delta^{13}\text{C}_{\text{Daphnia}}$	Early winter $\delta^{13}\text{C}_{\text{Daphnia}}$	Late winter $\delta^{13}\text{C}_{\text{Daphnia}}$	Seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$	Mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$
Abbaye	–33.88	–37.48	–38.28	3.60	4.00
Ambleon	–33.98	–39.60	–38.20	5.61	4.91
Barterand	–39.96	–54.91	–44.22	14.94	9.60
Chalain	–35.51	–44.22	–42.38	8.71	7.79
Chambly	–33.89	–42.02	–41.31	8.12	7.77
Grand Etival	–40.74	–39.41	–38.79	–1.32	–1.63
Etival					
Maclu	–36.01	–39.25	–37.25	3.23	2.23
Ilay	–34.47	–40.17	–36.02	5.69	3.62
Petit Etival	–40.86	–40.68	–40.01	–0.17	–0.51
Etival					
Remoray	–37.23	–45.99	–40.69	8.75	6.10
Val	–36.00	–54.59	–39.13	18.58	10.85

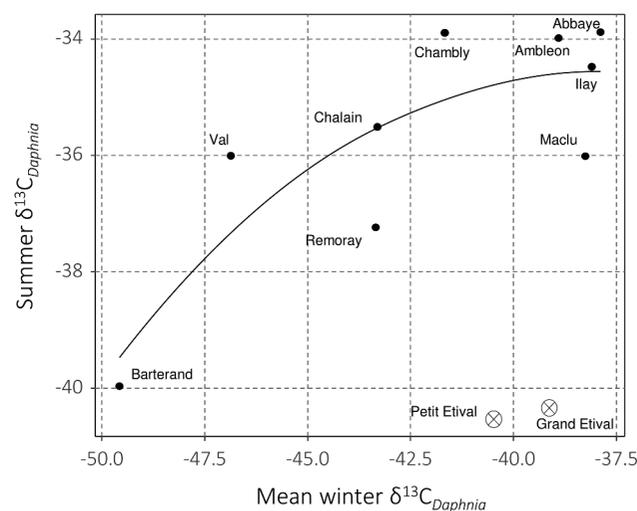


Figure 5. Mean winter $\delta^{13}\text{C}_{\text{Daphnia}}$ values plotted as a function of summer $\delta^{13}\text{C}_{\text{Daphnia}}$ values for the 11 sampled lakes. Spearman rank correlation test: $\rho = 0.7$, $p = 0.04$. Both lakes at Etival, which are weakly stratified in summer compared to the other studied lakes, were not considered in this analysis.

test, $\rho = 0.7$, $p = 0.04$), when excluding the two Etival lakes characterized by quite similar $\delta^{13}\text{C}_{\text{Daphnia}}$ between the sampling seasons (Figure 5). Nevertheless, the differences in $\delta^{13}\text{C}_{\text{Daphnia}}$ between summer and early winter (Seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$) or between summer and mean winter (Mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$) were variable among the studied lakes (Table 2). For the nine stratified lakes, the seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ranged from 3.23 to 18.58 ‰, while mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ranged from 2.23 to 10.85 ‰ (Table 2). In comparison, the Etival lakes showed smaller differences in $\delta^{13}\text{C}_{\text{Daphnia}}$ between summer and winter (Seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}} = -0.1$ and -1.3 ; Mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}} = -0.5$ and -1.6).

Relation between $\delta^{13}\text{C}_{Daphnia}$ dissolved gas measurements and lake and watershed properties

Effects of lake and watershed properties on dissolved gas concentrations

Three types of explanatory variables were considered in order to assess the influence of lake and watershed properties on dissolved gas concentrations in the water column, especially gas storage in the hypolimnion during summer stratification: (1) sediment organic matter features, (2) thermal stratification and oxygen conditions and (3) land use on the watershed (Table 3). The results of Spearman rank correlation test performed on these datasets are detailed in supplementary material.

Total carotenoids (TC) was strongly correlated to gas concentrations in the deep water (Figure 6) ($[\text{CH}_4]_{\text{aq}} \sim \text{TC}$: $p = 2.2e^{-16}$, $\rho = 0.96$; $[\text{CO}_2]_{\text{aq}} \sim \text{TC}$: $p = 4.2e^{-4}$, $\rho = 0.89$). However, there was no relationship between $[\text{CH}_4]_{\text{aq}}$ or $[\text{CO}_2]_{\text{aq}}$ in the deep water and the other organic matter descriptors during summer stratification. The relative agricultural area was also positively correlated to $[\text{CH}_4]_{\text{aq}}$ and $[\text{CO}_2]_{\text{aq}}$ in deep water during summer (spearman rank correlation test, $\rho = 0.8$, $p = 0.004$, and $\rho = 0.79$, $p = 0.006$, respectively, Figure 7). There were no significant relationships between dissolved gas concentrations in deep water and hypoxia duration or the proxy used for the thermal stratification intensity.

Relationship between dissolved gas concentrations and $\delta^{13}\text{C}_{Daphnia}$

During the summer stratification, no significant relationship could be evidenced between mean $\delta^{13}\text{C}_{Daphnia}$ and $[\text{CH}_4]_{\text{aq}}$ or $[\text{CO}_2]_{\text{aq}}$ in epilimnion or in deep water. Nevertheless, there was a significant negative relationship between epilimnetic $[\text{CO}_2]_{\text{aq}}$ and the $\delta^{13}\text{C}$ of *Daphnia* sampled only in euphotic zone ($p = 0.02$, $\rho = -0.69$) (Figure 8). The two Etival lakes, which were only weakly thermally stratified during summer

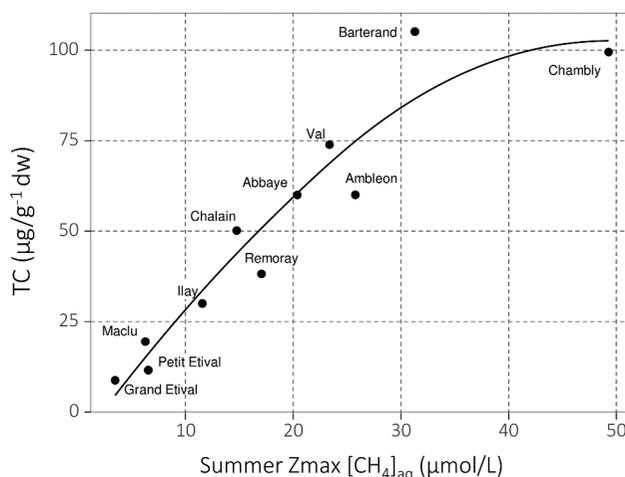


Figure 6. Summer $Z_{\text{max}} [\text{CH}_4]_{\text{aq}}$ plotted as a function of TC (total carotenoid concentration) in sediments. Spearman rank correlation test: $\rho = 0.96$, $p = 2.2e^{-16}$.

period (Figure 2), did not follow this relationship. Indeed, in these lakes, the highly negative summer $\delta^{13}\text{C}_{Daphnia}$ corresponded to relatively low epilimnetic $[\text{CO}_2]_{\text{aq}}$ (Figure 8).

For both winter campaigns, no relationship was evidenced between $\delta^{13}\text{C}_{Daphnia}$ and gas concentrations in the water columns in the 11 lakes. Furthermore, there was not any significant relationship between the amount of CH_4 or CO_2 stored in deep water during summer period and the $\delta^{13}\text{C}$ of *Daphnia* of the two winter campaigns. However, the decrease in the $\delta^{13}\text{C}_{Daphnia}$ between summer and winter campaigns (seasonal $\Delta\delta^{13}\text{C}_{Daphnia}$) seemed proportional to the amount of gas stored in the hypolimnion during summer stratification. Indeed, according to Spearman correlation rank test, summer hypolimnetic $[\text{CH}_4]_{\text{aq}}$ was positively correlated with seasonal $\Delta\delta^{13}\text{C}_{Daphnia}$ ($p = 0.03$, $\rho = 0.66$) and mean seasonal $\Delta\delta^{13}\text{C}_{Daphnia}$ ($p = 0.008$, $\rho = 0.77$) (Figure 9a). Summer hypolimnetic $[\text{CO}_2]_{\text{aq}}$ was also

Table 3. Sediment, stratification and watershed characteristics of the 11 studied lakes. C_{org} is expressed in %, $\delta^{13}\text{C}_{\text{OM}}$ is expressed in ‰ and TC (total carotenoids) is expressed in $\mu\text{g/g}$ of dry sediment. Hypoxia duration = duration of $[\text{O}_2] < 2 \text{ mg/l}$ at $0.8 \times$ maximal depth in days, and $\Delta \text{ }^\circ\text{C}_{\text{stratification period}}$ = mean temperature difference in $^\circ\text{C}$ between the surface and bottom layers during the summer stratification period. Relative agricultural area and relative forest area represent the watershed area used for forest and agricultural activities relative to lake area (in ha), according to CORINE Land Cover data.

Lake	C_{org}/N	C_{org}	$\delta^{13}\text{C}_{\text{OM}}$	TC	Hypoxia duration	$\Delta \text{ }^\circ\text{C}_{\text{stratification period}}$	Relative agricultural area	Relative forest area
Abbaye	6.4	14.1	-33.6	59.9	121	11.5	2.28	0.06
Ambleon	8.8	6.8	-36.5	60	23	8.1	9.24	89.03
Barterand	9.2	2.9	-36.3	105.1	138	12.5	18.15	19.60
Chalain	9.8	2.3	-33.9	50.1	175	11.5	4.86	9.26
Chambly	9.0	5.8	-35.6	99.4	51	8.7	30.41	60.92
Grand Etival	8.8	6.4	-33.3	8.7	5	4.7	2.78	22.74
Maclu	9.4	7.4	-34.2	19.4	60	10.6	0.0	7.06
Ilay	9.1	12.3	-33.1	29.9	140	10.9	0.22	1.02
Petit Etival	6.4	11.5	-36.7	11.5	14	3.12	0.85	14.25
Remoray	9.2	5.7	-34.7	38.1	127	10.5	13.28	13.83
Val	12.6	6.3	-28.8	73.8	153	13.3	4.97	24.10

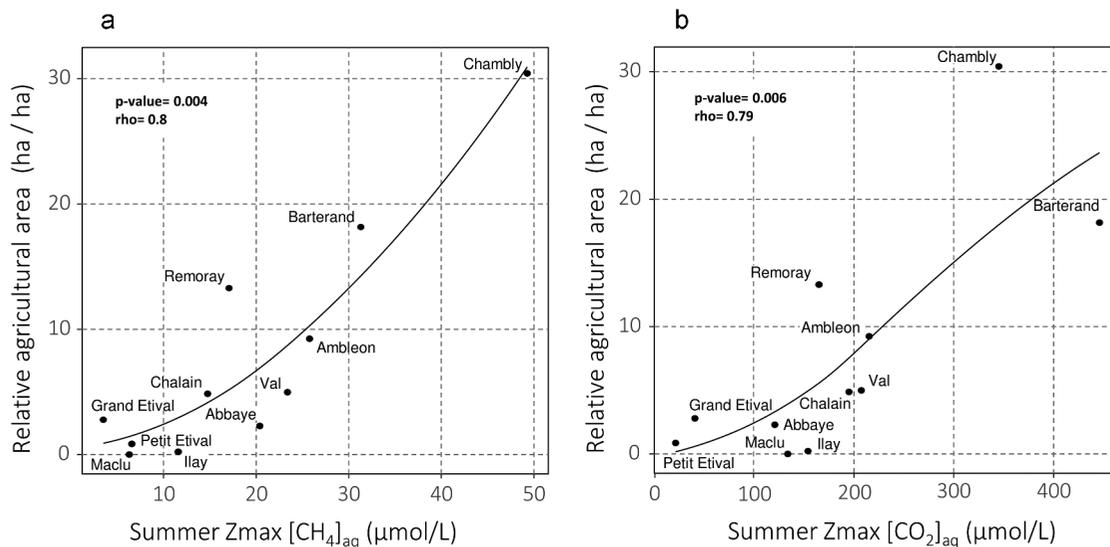


Figure 7. Relative agricultural area as a function of summer $[\text{CH}_4]_{\text{aq}}$ at Z_{max} (A) and summer $[\text{CO}_2]_{\text{aq}}$ at Z_{max} (B) and corresponding Pearson rank correlation test.

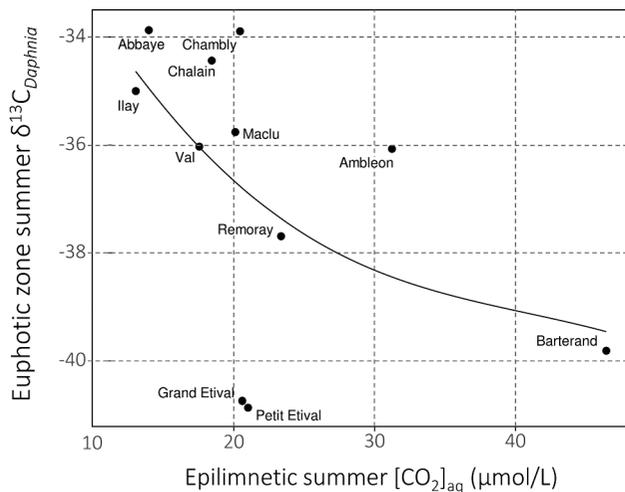


Figure 8. Mean epilimnetic summer $[\text{CO}_2]_{\text{aq}}$ as a function of $\delta^{13}\text{C}$ of *Daphnia* sampled in the euphotic zone in the summer stratification period. Spearman rank correlation test: $\rho = -0.69$, $p = 0.02$.

positively correlated with seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.008$, $\rho = 0.77$) and mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.002$, $\rho = 0.83$) (Figure 9b).

Relationships between $\delta^{13}\text{C}_{\text{Daphnia}}$ sediment, watershed and stratification characteristics

The mean $\Delta^\circ\text{C}_{\text{stratification period}}$ was significantly positively correlated with seasonal and mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.003$, $\rho = 0.81$, $p = 0.005$, $\rho = 0.8$, respectively, Figure 10a). The lakes with the strongest thermal stratification in summer thus showed the highest decrease in $\delta^{13}\text{C}_{\text{Daphnia}}$ between summer and winter

period. According to the Spearman rank test, there was no significant relationship between mean $\Delta^\circ\text{C}_{\text{stratification period}}$ and summer $\delta^{13}\text{C}_{\text{Daphnia}}$, early winter $\delta^{13}\text{C}_{\text{Daphnia}}$, or late winter $\delta^{13}\text{C}_{\text{Daphnia}}$.

Although no significant relationship could be evidenced between the hypoxia duration in the 11 lakes and the summer or winter $\delta^{13}\text{C}_{\text{Daphnia}}$, there was a significant relationship between the hypoxia duration and seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.007$, $\rho = 0.78$) and mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.01$, $\rho = 0.70$) according to Spearman correlation tests (Figure 10b).

A significant positive relationship was observed between pigment concentrations (TC) and seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ (spearman rank test $p = 0.01$, $\rho = 0.74$) or mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.001$, $\rho = 0.86$) (Figure 11). No significant relationship was found between $\% C_{\text{org}}$, C_{org}/N or $\delta^{13}\text{C}_{\text{OM}}$ and seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ or $\delta^{13}\text{C}_{\text{Daphnia}}$ for all sampling periods.

Finally, the relative agricultural area was negatively correlated to winter $\delta^{13}\text{C}_{\text{Daphnia}}$ (relative agricultural area \sim early winter $\delta^{13}\text{C}_{\text{Daphnia}}$: $p = 0.03$, $\rho = -0.64$; \sim late winter $\delta^{13}\text{C}_{\text{Daphnia}}$: $p = 0.02$, $\rho = -0.7$; \sim mean winter $\delta^{13}\text{C}_{\text{Daphnia}}$: $p = 0.02$, $\rho = -0.69$). The relative agricultural area was also significantly positively correlated with the seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.04$, $\rho = 0.62$) and the mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ ($p = 0.02$, $\rho = 0.69$). Moreover, relative agricultural area was significantly positively correlated with TC ($p = 0.01$, $\rho = 0.74$). Lakes with the largest area of agricultural parcels relative to their surface area and the highest pigment concentrations in sediments showed the lowest winter $\delta^{13}\text{C}_{\text{Daphnia}}$ and the largest seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$. No

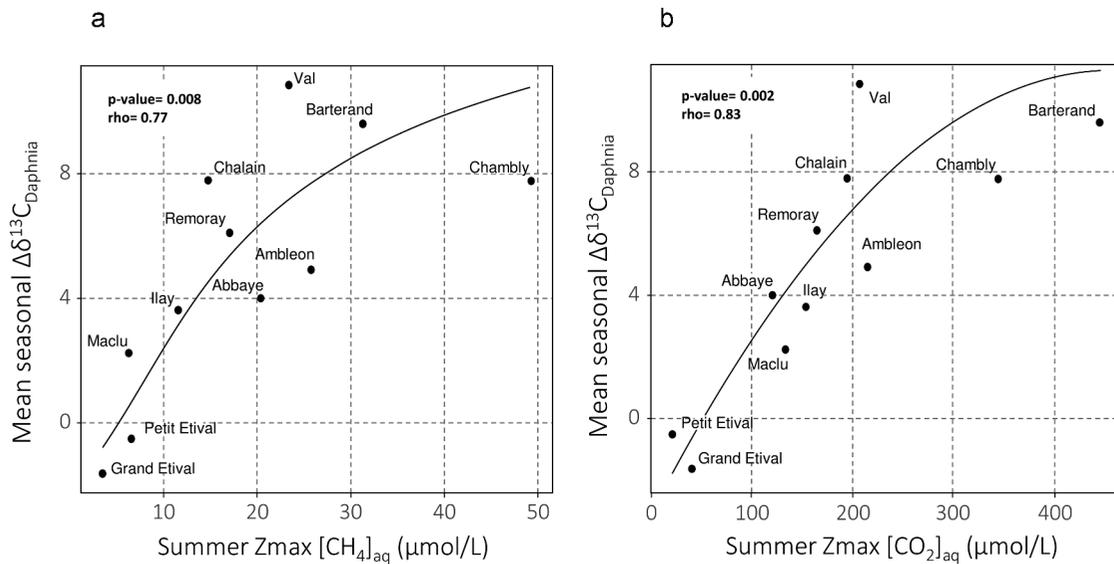


Figure 9. Mean seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ as a function of summer $[\text{CH}_4]_{\text{aq}}$ at Z_{max} (A) and summer $[\text{CO}_2]_{\text{aq}}$ at Z_{max} (B) and corresponding Pearson rank correlation test.

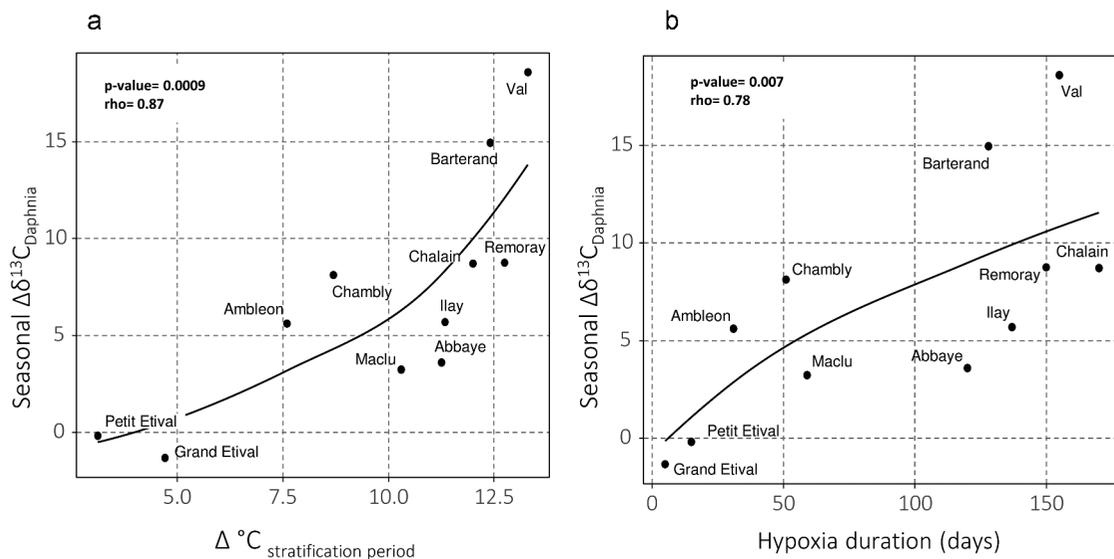


Figure 10. Seasonal $\Delta\delta^{13}\text{C}_{\text{Daphnia}}$ as a function of summer $\Delta^\circ\text{C}_{\text{stratification period}}$ (A) and hypoxia duration during the summer period (B) and corresponding Pearson rank correlation test.

relationship was evidenced between the relative agricultural area and the summer $\delta^{13}\text{C}_{\text{Daphnia}}$ according to a Spearman correlation rank test.

Discussion

Seasonal dynamics of dissolved gas concentrations derived from heterotrophic processes

Similar summer clinograde profiles of gas concentrations were observed for the stratified lakes of our study. They were all characterised by an increase in $[\text{CH}_4]_{\text{aq}}$ and $[\text{CO}_2]_{\text{aq}}$

in the hypolimnion which was comparable to that reported in many studies (Rudd et al. 1976; Bastviken et al. 2003; Kortelainen et al. 2006; Miettinen et al. 2015; Rinta et al. 2015; Laas et al. 2016; Thalasso et al. 2020). In this water layer, gas concentrations ranged from 6.2 to 49.2 $\mu\text{mol/L}$ for CH_4 and from 122.7 to 454.9 $\mu\text{mol/L}$ for CO_2 , depending on the lake. These values, comparable to those measured in several other lakes (Bastviken et al. 2003; Kortelainen et al. 2006; Rinta et al. 2015; Thalasso et al. 2020), are highly supersaturated according to the dissolved concentration at air-water equilibrium. Indeed, air water equilibrium concentration range between 0.002 to 0.004 $\mu\text{mol/L}$ for CH_4

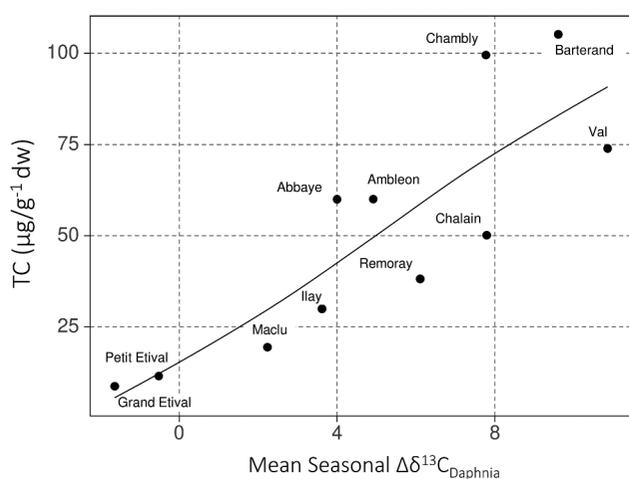


Figure 11. Mean Seasonal $\Delta\delta^{13}C_{Daphnia}$ as a function of carotenoid concentration in sediments (TC, $\mu\text{g/g}^{-1} \text{ dw}$). Spearman rank correlation test: $\rho = 0.86$, $p = 0.001$.

and 13 to 17 $\mu\text{mol/L}$ for CO_2 depending on water temperature (according to solubility equations by Weiss (1974) and Wiesenburg and Guinasso (1979) and by considering atmospheric concentrations of 407 ppm of CO_2 and 1850 ppb of CH_4 (www.eea.europa.eu)). The strong summer hypolimnetic anoxia and the negative relationship between O_2 and CO_2 concentrations observed during the stratification period indicated sustained heterotrophic activities (Rantakari and Kortelainen 2005; Laas et al. 2016). The high concentration of CH_4 found in the hypolimnion of our lakes at the same time can be explained by the degradation of organic matter carried out by methanogenic Archaea under such anoxic conditions (Rudd and Hamilton 1975; Rudd et al. 1976; Utsumi et al. 1998).

In the 9 stratified lakes, although lower than in hypolimnion, epilimnetic $[\text{CO}_2]_{\text{aq}}$ were close or higher than air-water equilibrium. These results are in accordance with previous studies that evidenced supersaturation of CO_2 in the surface layer of stratified lakes (Laas et al. 2016; Pighini et al. 2018), reflecting high heterotrophic activities in the epilimnion during summer stratification. However, our results about epilimnetic CO_2 concentrations should be considered with caution, as they result from a balance between respiration and CO_2 fixation by primary producers, and are consequently possibly subject to large temporal variability (Peeters et al. 2016). Alternatively, several previous studies suggested that, in calcareous lakes analogous to our study sites, CO_2 concentration may be rather linked to the production of CO_2 by calcite precipitation in alkalinitrophic context (Marcé et al. 2015; Khan et al. 2020). CO_2 supersaturation in our study may then be explained by these processes, the Jura lakes being known to have a significant calcic precipitation by littoral macrophytes (Magny 1992).

These epilimnetic CO_2 concentrations could be sustained by some inputs from respiration in the sedimentary compartment, as shown by Kortelainen et al. (2006). Epilimnetic $[\text{CH}_4]_{\text{aq}}$ were always highly supersaturated for all the studied lakes, especially for Lake Barterand which had considerable surface $[\text{CH}_4]_{\text{aq}}$ in summer. Similar epilimnetic CH_4 supersaturation has been evidenced in many lakes during thermal stratification (Rudd and Hamilton 1975; Rudd et al. 1976; Pighini et al. 2018). This may be the result of vertical diffusive gas transport from bottom sediment to the surface water. Nevertheless, this process seems limited in thermally stratified lakes (Bastviken et al. 2008). Other mechanisms may also explain the presence of CH_4 in surface layers. Some studies suggest indeed that methane-rich air bubbles rising from the lake bottom may contribute to the supersaturation of surface waters (McGinnis et al. 2006). The methane supersaturation in oxic conditions may also be the result of CH_4 production by phototroph organisms together with oxygen tolerant methanogens (Bogard et al. 2014; Tang et al. 2014; Wang et al. 2017). Finally, the horizontal transfers of CH_4 produced in the littoral zones of the lakes could explain these epilimnetic concentrations (Fernández et al. 2016).

In winter, CO_2 and CH_4 concentrations decreased significantly in the hypolimnion compared to summer measurements, and concentrations profiles became orthograde with low variability along water columns. A large part of methane produced in sediment and accumulated in the hypolimnion during summer stratification was released in the whole water column with autumnal turnover (Rudd and Hamilton 1975; Utsumi et al. 1998). During the turnover and throughout winter, favourable oxygen conditions promote oxidation by MOB (methane oxidizing bacteria) of a large part of the released CH_4 (Rudd and Hamilton 1975; Rudd and Taylor 1980; Utsumi et al. 1998; Kankaala et al. 2007).

In contrast to the stratified lakes of our study, the two weakly stratified Etival lakes showed homogeneous and similar gas concentration profiles in summer and in winter. Both lakes of Etival are small polymictic lakes. Their water columns were thus permanently oxygenated and no gas was stored in deep water layer. CH_4 produced in sedimentary compartment of these lakes was then directly available for emission or oxidation by MOB and the CO_2 produced can be directly used for photosynthesis (Laas et al. 2016) or released to the atmosphere.

Seasonal dynamics of carbon transfers to pelagic food web

During summer stratification, the $\delta^{13}C_{Daphnia}$ values ranged from -33.8‰ to -40‰ in the stratified lakes. *Daphnia* diet is usually mainly composed of phytoplankton and

terrestrial detritus during summer (Perga et al. 2008; Lampert and Kinne 2011). Nevertheless, in our study, allochthonous detritus did not appear to contribute significantly to *Daphnia* diet. Indeed, a study about the $\delta^{13}\text{C}$ of the vegetation in the buffer zones of 4 Jura mountains lakes (50 m all around the lake shore) reported values from -24.2‰ (for *Phragmitia*) to -30.3‰ (for grassland) (Borderelle 2006). The $\delta^{13}\text{C}$ of *Daphnia* during summer stratification are however in the range of the carbon isotopic signature usually found for phytoplankton (-25‰ to -40‰ , Peterson and Fry 1987; France 1995; Vuorio et al. 2006; Masclaux et al. 2013; Wang et al. 2013; de Kluijver et al. 2014).

In the 9 stratified lakes of our study, we evidenced a negative correlation between $\delta^{13}\text{C}$ of *Daphnia* from the euphotic zone and the epilimnetic CO_2 concentrations. Some previous studies showed analogous relationships between phytoplankton $\delta^{13}\text{C}$ (and by extension zooplankton $\delta^{13}\text{C}$) and $[\text{CO}_2]_{\text{aq}}$ in lakes (Mohamed and Taylor 2009; Smyntek et al. 2012; de Kluijver et al. 2014). The phytoplankton $\delta^{13}\text{C}$ is determined by a combination of (i) the degree of isotopic fractionation performed during photosynthesis and (ii) the $\delta^{13}\text{C}$ value of the dissolved inorganic carbon used (de Kluijver et al. 2014). The isotope fractionation by phytoplankton increases with increasing dissolved CO_2 concentration. Indeed, the increase in dissolved inorganic carbon availability leads to an increase in ^{13}C discrimination, and therefore a ^{13}C depletion of phytoplankton (Peterson and Fry 1987; Laws et al. 1995; Smyntek et al. 2012; de Kluijver et al. 2014). In our study, high CO_2 concentrations in the epilimnion may be due to intense respiration processes (de Kluijver et al. 2014). Respiration-derived CO_2 exhibits ^{13}C -depleted signatures compared to other dissolved carbon sources available for photosynthesis (Fry 2006; Parker et al. 2010). As $\delta^{13}\text{C}_{Daphnia}$ were in the lower range of phytoplankton $\delta^{13}\text{C}$ according to literature (Peterson and Fry 1987; France 1995; Vuorio et al. 2006; Masclaux et al. 2013; Wang et al. 2013) and according to the relationship between epilimnetic $[\text{CO}_2]_{\text{aq}}$ and $\delta^{13}\text{C}_{Daphnia}$, the $\delta^{13}\text{C}_{Daphnia}$ could reflect the intensity of respired carbon transfers to phytoplankton and then to primary consumers. Furthermore, other DIC sources can also sustained the ^{13}C depletion of phytoplankton, such as the CO_2 derived from the oxidation of CH_4 (Wachniew and Róžański 1997). Finally, even if the *Daphnia* diet seems to be mainly based on phytoplankton, other sources may contribute to the observed ^{13}C -depletion of their signatures, such as MOBs (Templeton et al. 2006), considering the availability of CH_4 in epilimnion during summer stratification.

In winter, the $\delta^{13}\text{C}_{Daphnia}$ values were significantly lower than in summer in the stratified lakes of our study, with values ranging from -54.9 to -37 ‰. Moreover, these winter $\delta^{13}\text{C}_{Daphnia}$ were substantially lower than the $\delta^{13}\text{C}$ of allochthonous detritus and phytoplankton usually reported in the literature for most of the lakes (-25‰ to -40‰ , Peterson and Fry 1987; O'Leary 1988; France 1995; Grey et al. 2001; Vuorio et al. 2006; Masclaux et al. 2013; Wang et al. 2013; de Kluijver et al. 2014). The transfer of methanogenic carbon (C-CH_4) in food web via MOBs consumption most likely explains these low carbon isotopic signatures of pelagic consumers. Indeed, C-CH_4 is highly ^{13}C -depleted, with $\delta^{13}\text{C}$ values ranging between -40 and -80 ‰ in European calcareous hypoxic lakes (Rinta et al. 2015). Moreover, the $\delta^{13}\text{C}$ values of MOBs are further reduced due to isotopic fractionation during methanotrophy, and range between -90‰ and -60‰ , making them highly distinct from the carbon isotopic signature of phytoplankton and allochthonous detritus (Jedrysek 2005; Templeton et al. 2006). Similar highly ^{13}C -depleted signatures of pelagic consumers have already been reported in several stratified lakes and indicated the transfers of C-CH_4 to pelagic food webs in winter (Harrod and Grey 2006; Taipale et al. 2008; Morlock et al. 2017). In such lakes, CH_4 accumulated in the hypolimnion during the summer stratification. During the autumnal turnover, the methane accumulated in the hypolimnion is released into the entire water column, which is simultaneously reoxygenated (Utsumi et al. 1998). When CH_4 become available in oxic conditions, it can be oxidized by MOBs (Rudd and Hamilton 1978; Bastviken et al. 2002; Kankaala et al. 2006a). The release of methane thus results in a high production of MOBs after the autumnal turn-over (Utsumi et al. 1998; Kankaala et al. 2007; Schubert et al. 2012), which then become an abundant carbon source for *Daphnia* (Taipale et al. 2008, 2009). For the studied lakes, this hypothesis is further supported by the significant negative relationship observed between bottom CH_4 concentration measured in summer and the $\delta^{13}\text{C}_{Daphnia}$ decrease between the summer and winter campaigns. This relationship suggests that the intensity of C-CH_4 transfer in pelagic food webs after the autumnal turnover is proportional to the amount of methane stored in the hypolimnion during summer stratification. This hypothesis agrees with literature that indicates that a large part of the methane is oxidised by the MOBs after autumnal mixing regardless of the magnitude of the summer storage in the hypolimnion (Kankaala et al. 2006a, 2007). Zooplankton $\delta^{13}\text{C}$ can also decrease in winter due to the increased storage of lipid in their tissues (Syväranta and Rautio 2010). Indeed, lipids are known to be depleted in ^{13}C , leading to lower $\delta^{13}\text{C}$ values for tissues with a higher lipid content (DeNiro and Epstein 1977).

However, winter lipid storage by *Daphnia* appears to be very limited compared to other zooplanktonic taxa, especially copepods (Grosbois et al. 2017). Furthermore, these lipid storage mechanisms can lead to maximum variations in cladoceran signatures of only 3 to 4.5 ‰ (Smyntek et al. 2007; Syväranta and Rautio 2010). The seasonal variations of $\delta^{13}\text{C}_{Daphnia}$ observed in our study are larger than these values for the majority of the stratified lakes, thus suggesting that these variations are mainly due to changes in the carbon sources consumed by the daphnids rather than winter storage of lipid.

Both lakes of Etival, which were weakly stratified during summer, had the most negative summer $\delta^{13}\text{C}_{Daphnia}$ of the 11 studied lakes with $\delta^{13}\text{C}$ values slightly below -40‰ . These signatures are lower than those reported for phytoplankton (Peterson and Fry 1987; France 1995; Vuorio et al. 2006; Masclaux et al. 2013; Wang et al. 2013), and could be explained by the use of a substantial amount of C-CH₄ by *Daphnia* during summer. Indeed, in unstratified or polymictic lakes, CH₄ is available all year round under oxic conditions and can thus be transferred to food webs at all seasons (Laas et al. 2016; Vachon et al. 2019). Due to the weak thermal stratification, and the absence of methane storage in deep water layer in summer, no significant difference in $\delta^{13}\text{C}_{Daphnia}$ was then observed between summer and winter for these two lakes. Moreover, the lowest values were observed in summer rather than in winter for these two lakes (even if these differences are not significant). Despite winter lipid storage, the $\delta^{13}\text{C}_{Daphnia}$ probably still increased in winter due to less methanogenic activities under lower water temperature.

Influence of thermal stratification, hypoxia duration and land-use on CH₄ storage and transfer in food webs

Influence of thermal stratification and hypoxia duration

Unexpectedly, our results did not reveal a clear impact of thermal stratification and hypoxia duration on gas concentrations in the hypolimnion during summer stratification. Indeed, lakes with strong thermal stratification and prolonged hypolimnetic hypoxia are usually reported to store large quantity of CH₄ in summer due to the high production rates of CH₄ and CO₂ and the limitation of vertical gas exchanges between the hypolimnion and the epilimnion (Juutinen et al. 2009; Rinta et al. 2016a; Vachon et al. 2019). These unexpected results may be partly linked to our study design which involved only point-in-time physico-chemical measurements. The gas concentration measurements may correspond to different moments of the thermal cycle, which is specific to

each studied lake. These differences may explain the lack of significant relationship between the gas concentrations in deep water and the thermal stratification strength or the hypolimnetic anoxia duration. Nevertheless, these factors were significantly correlated with the seasonal decrease in $\delta^{13}\text{C}$ of pelagic consumers (*Daphnia*). Indeed, $\delta^{13}\text{C}$ signature of organisms are probably more time integrative (Tieszen et al. 1983) and are therefore less affected by the one-off nature of the sampling. Our results suggest therefore that the thermal stratification strength ($\Delta^{\circ}\text{C}_{\text{stratification period}}$), as well as hypoxia duration, at least partly explained the amplitude of $\delta^{13}\text{C}_{Daphnia}$ decrease between summer and winter. These relationships suggest that lakes with stronger stratification and prolonged hypolimnetic hypoxia are likely to store a greater quantity of CH₄ in summer and are characterized by an enhanced transfer of C-CH₄ to the pelagic food web in winter. For the lakes with weaker stratification, a greater proportion of the methane produced in the hypolimnion may be released in summer to pelagic consumers, resulting in a smaller $\delta^{13}\text{C}_{Daphnia}$ decrease between summer and winter.

Finally, our study shows that the storage of the methanogenic carbon and its transfer dynamic in pelagic food webs are highly dependent on the physical conditions prevailing in lakes. This finding implies that the methane cycle in particular, and the carbon cycle more generally, may be impacted by global warming. Indeed, the duration and strength of lake stratification will increase with global warming (Butcher et al. 2015; Winslow et al. 2015; Pilla and Williamson 2021). This will be accompanied by a decrease in hypolimnetic oxygen and an increase in anoxia/hypoxia duration (Fang and Stefan 2009; Foley et al. 2012) which could lead to an increase in CH₄ production during the summer stratification (Rudd and Hamilton 1978). As evidenced by our results, a part of this CH₄ could finally be transferred to the pelagic food web after the autumnal turnover. In addition, many temperate lakes are expected to show a shift of their thermal regime under climate change, from dimictic to either monomictic, polymictic and even permanently stratified in some cases (Woolway and Merchant 2019). These shifts would then induce strong changes in the methanogenic carbon seasonal transfer in pelagic food webs.

Influence of land-use

In the 11 lakes involved in the present study, the relative area of the watershed used for agriculture is positively correlated with the amount of CO₂ and CH₄ stored in the hypolimnion during the summer stratification. The influence of agricultural land-use on the CH₄ fluxes to the

atmosphere was already suggested by Huttunen et al. (2003). Moreover, Kortelainen et al. (2006) also evidenced relationships between agricultural catchment area and increase in concentrations in CO₂ derived from heterotrophic activities in lake water columns, but to our knowledge, our study is the first to report a significant relationship between agricultural land on watershed and hypolimnetic CH₄ concentrations. Furthermore, we observed a positive relationship between agricultural catchment area and TC (proxy of primary production), as well as between TC and hypolimnetic CH₄ concentrations. These results are consistent with other studies showing that methane production is positively linked to primary productivity variables such as nutrients or *chlorophyll a* concentration (Kortelainen et al. 2000; Beaulieu et al. 2019). Indeed, agriculture is already identified as a major disturbance factor for the ecological status and functioning of lakes (Carpenter et al. 1998; Knoll et al. 2003), leading to their accelerated eutrophication. The increase in primary production induced by water eutrophication and thus in sedimentation rates of easily degradable organic matter induces oxygen depletion in the hypolimnion (Foley et al. 2012) and promote methanogenesis (Beaulieu et al. 2019). In addition, several studies report that CH₄ fluxes to the atmosphere are greater in lakes where sedimentary organic matter is mainly autochthonous (Huttunen et al. 2003; West et al. 2012). Moreover, a strong organic sedimentation rate combined to low C/N ratio of lake sediments are particularly suitable for methanogenesis (Duc et al. 2010; Praetzel et al. 2020). Unexpectedly, in our dataset, we did not observe any relationship between C_{org} or C/N ratio in sediment organic matter and hypolimnetic gas concentrations in this study. A possible explanation is that we only measured the proportion of organic carbon in sediments and not considered the rate of organic sedimentation, which would be a more direct measurement of the amount of organic matter being sedimented. Furthermore, other factors such as temperature, O₂ concentration in the sediments, or competition with other bacteria for substrate acquisition can influence the methanogenic activity in sediments (Borrel et al. 2011).

Finally, in this study, relative area of catchment used for agriculture was clearly correlated with the winter $\delta^{13}\text{C}_{Daphnia}$ and seasonal $\Delta\delta^{13}\text{C}_{Daphnia}$. TC in sediments was also clearly correlated with seasonal $\Delta\delta^{13}\text{C}_{Daphnia}$. These results suggest that agricultural activities in the watershed and primary production of a lake enhance C-CH₄ transfers during winter in the pelagic compartment. Indeed, lakes with large agricultural catchments relative to lake size and intense primary production tend to accumulate more methane in the hypolimnion during the summer period according to our data. These lakes

then release more methane during autumnal turnover, resulting in sustained transfer of C-CH₄ to the pelagic food web. Belle *et al.*, (2016a) also suggested that agro-pastoral practices intensity on catchment may partly drive methane transfers to primary consumers, but to our knowledge, this study is the first to show a direct relationship between catchment occupation, primary production, and transfer of ¹³C-depleted carbon sources to lakes pelagic food webs.

Conclusion

The nine stratified lakes of this study, in contrast to the two weakly stratified lakes, showed marked seasonal dynamics of dissolved gas concentrations. These seasonal dynamics were characterized by an accumulation of CO₂ and CH₄ coupled with a decrease in oxygenation of the hypolimnion during stratification, and a reoxygenation of the deep layers associated with a decrease in CO₂ and CH₄ concentrations after autumnal turnover. *Daphnia* $\delta^{13}\text{C}$ showed strong seasonal variations in the lakes studied, except for the two weakly stratified lakes during summer, marked by a significant decrease of $\delta^{13}\text{C}$ values between summer and winter. These seasonal dynamics suggests the transfer of a non-negligible amount of C-CH₄ into the pelagic food webs after autumnal turnover. According to our results, the intensity of these winters C-CH₄ transfers largely depends on the amount of CH₄ stored in the hypolimnion during the stratification period. These C-CH₄ storage and transfer mechanisms seem partly dependent on the intensity of thermal stratification and the duration of hypoxia periods, but also on external forcing factors such as the land use on watersheds that influence origin and quantity of sedimented organic matter promoting methanogenesis. The ongoing global changes, characterized by global warming and accelerated eutrophication of waters linked to intensification of anthropogenic activities on the watershed, may therefore have a severe impact on the origin and pathways of carbon to pelagic food webs through their influence on thermal regimes and trophic functioning of lakes. More broadly, although some studies suggest that an increase in lake primary production could trap a significant amount of carbon in the sediments (Anderson et al. 2014), our results are consistent with other studies showing on the contrary, that increase in primary production could be at the origin of a greater production and release of greenhouse gases into the atmosphere (Beaulieu et al. 2019). Further studies need to quantify the relative importance of CO₂ and CH₄ production and emission relative to their

incorporation in food webs to improve our understanding of carbon cycle in lakes in the context of global changes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Data are available from the authors upon reasonable request.

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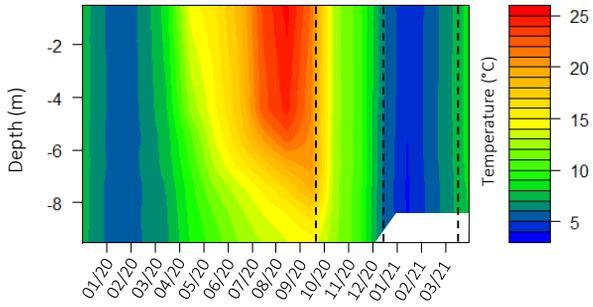
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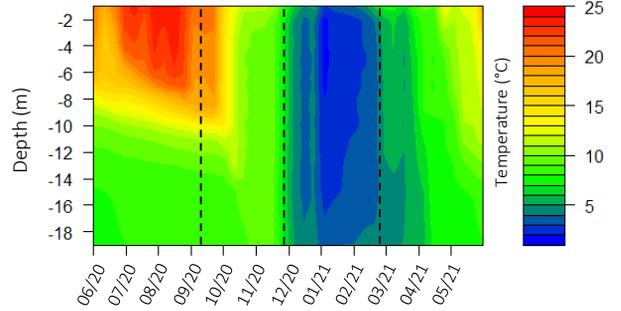
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Supplementary material:

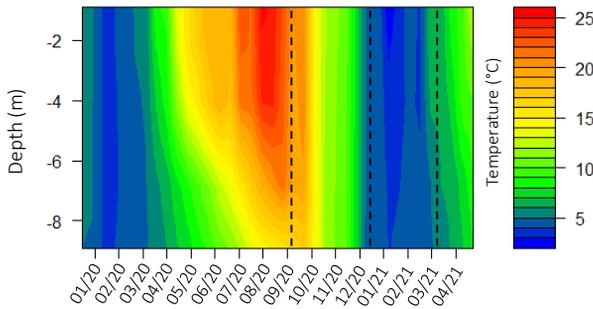
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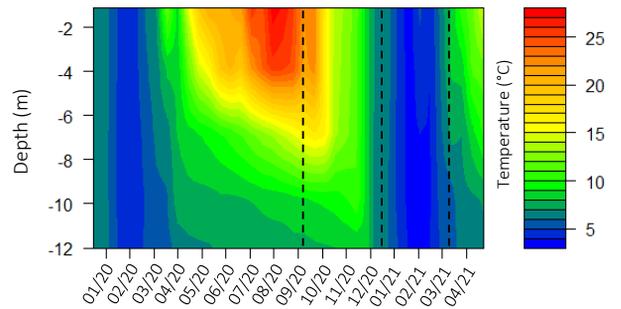
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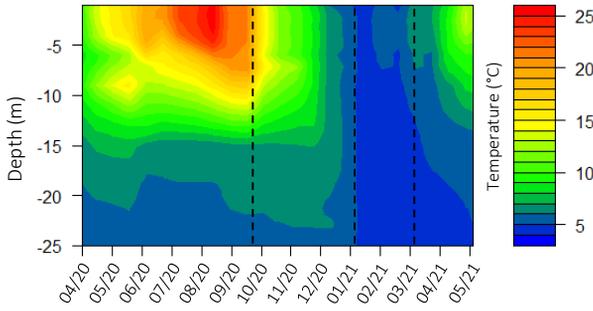
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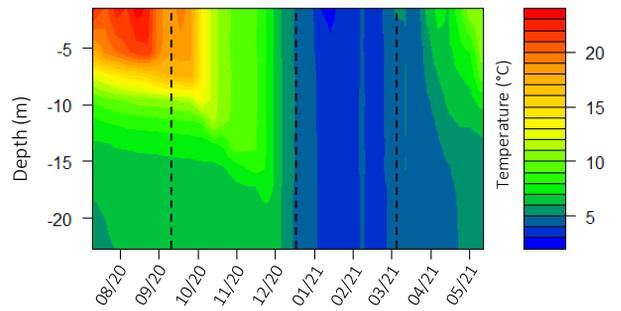
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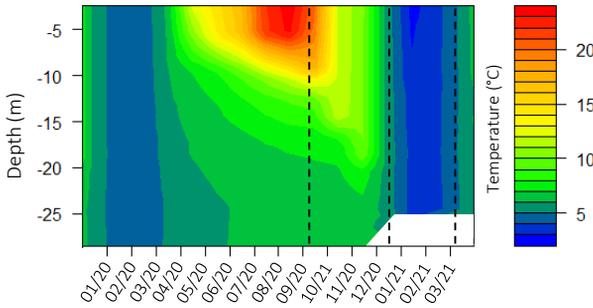
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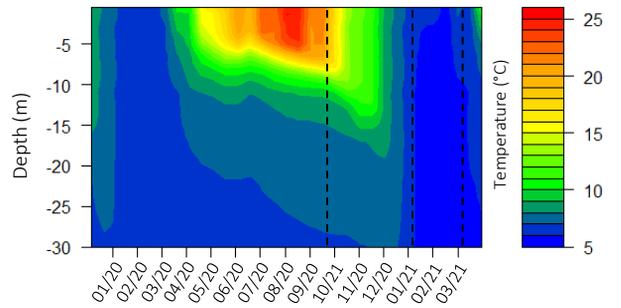
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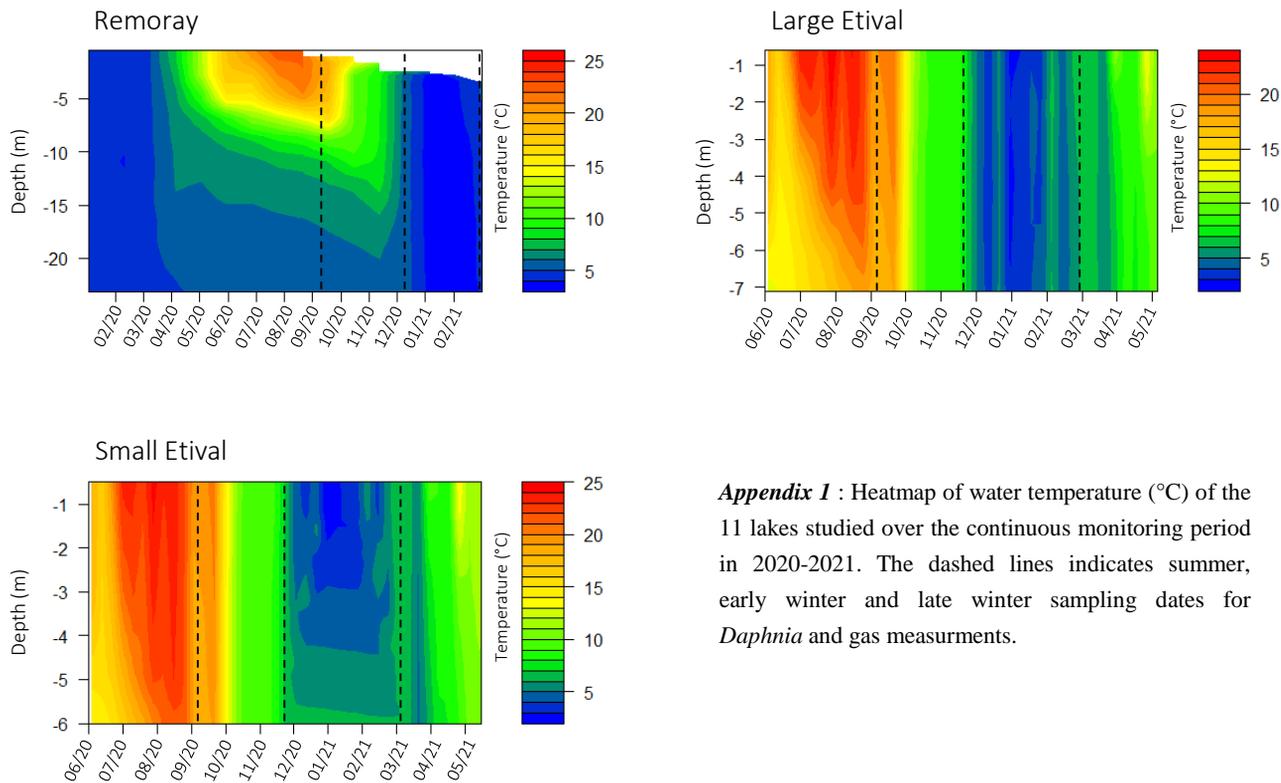


Ilay



Chalain



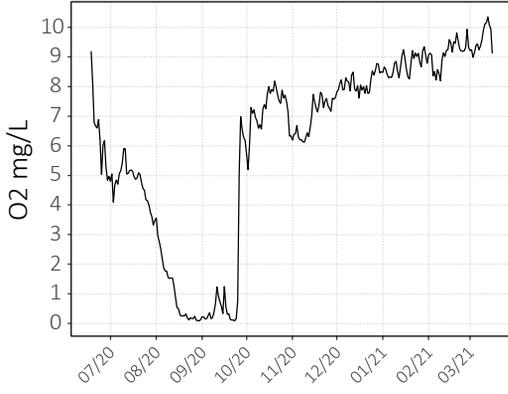


Appendix 1 : Heatmap of water temperature (°C) of the 11 lakes studied over the continuous monitoring period in 2020-2021. The dashed lines indicates summer, early winter and late winter sampling dates for *Daphnia* and gas measurements.

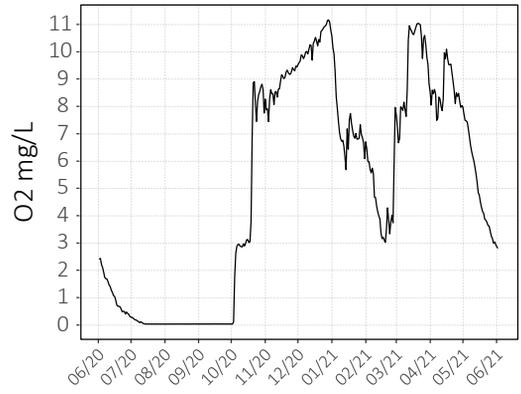
The lakes of Chambly, Abbaye, Ambleon, Barterand, Val, Maclu, Ilay, Chalain and Remoray were strongly thermally stratified during summer period with mean temperature differences between surface and bottom layer ($\Delta^{\circ}\text{C}_{\text{stratification period}}$) ranging from 8.1 to 13.3°C depending on lakes. In contrast, lakes of small and large Etival were weekly stratified during summer period, with $\Delta^{\circ}\text{C}_{\text{stratification period}}$ of respectively 3,1 and 4,7°C, and showed episodes of partial mixing of the water column during summer period.

The 11 lakes studied showed mixing of their water column between the end of September and the end of December depending on lakes. Lakes Etival, Ambleon and Chambly, which are the shallowest lakes of this study, were mixed relatively early compared to the other lakes studied, between late September and early October. The deepest lakes of th study like Chalain, Val, Ilay, or Remoray were mixed later in the season, between early and late December.

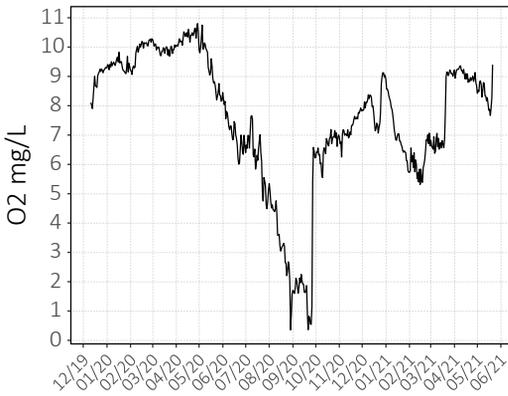
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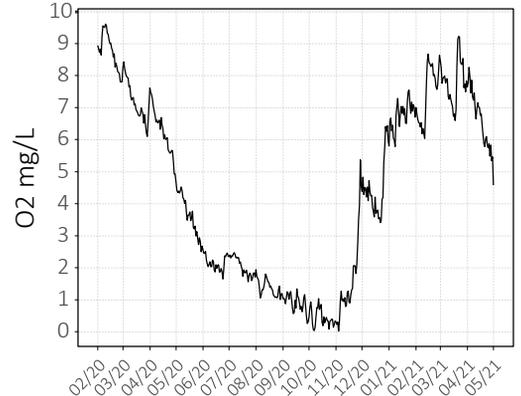
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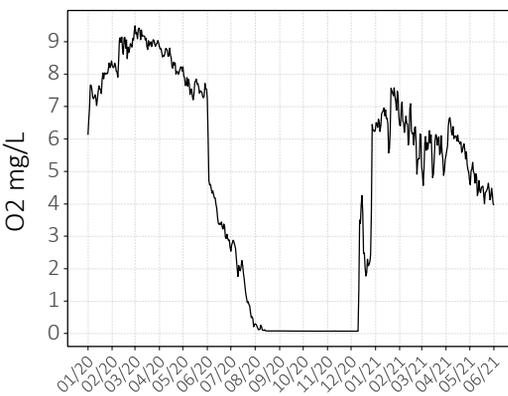
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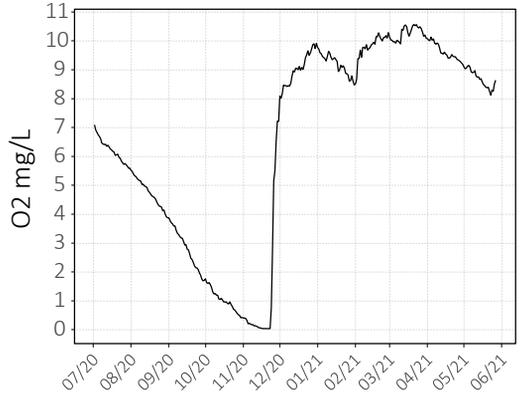
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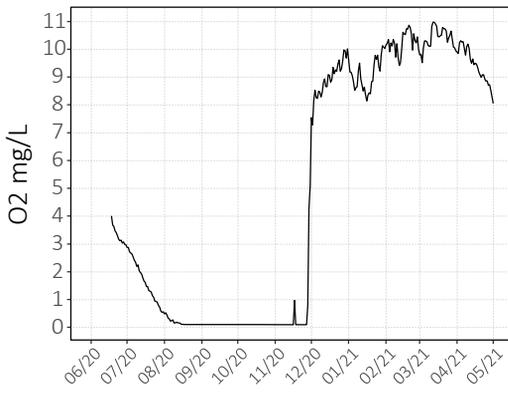
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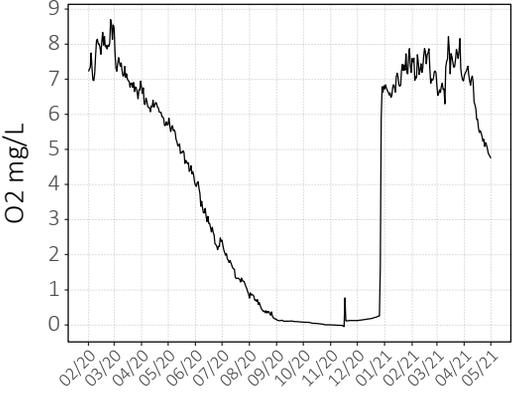
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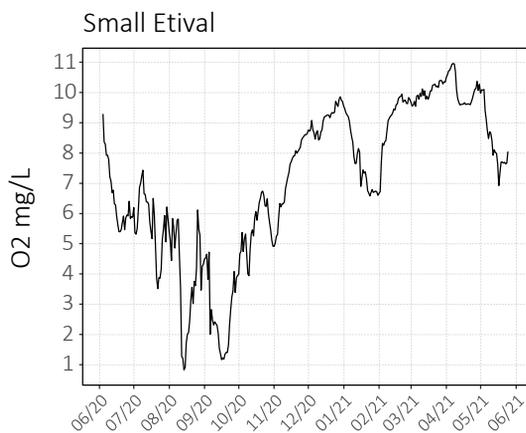
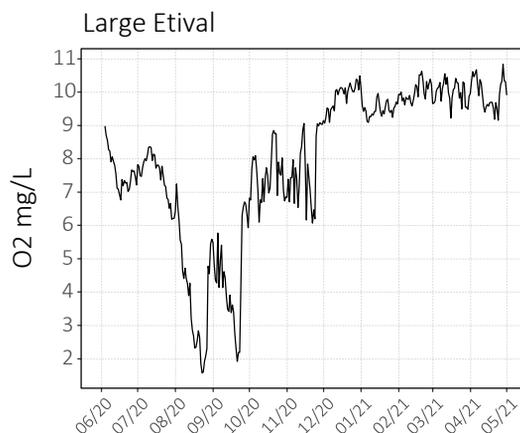
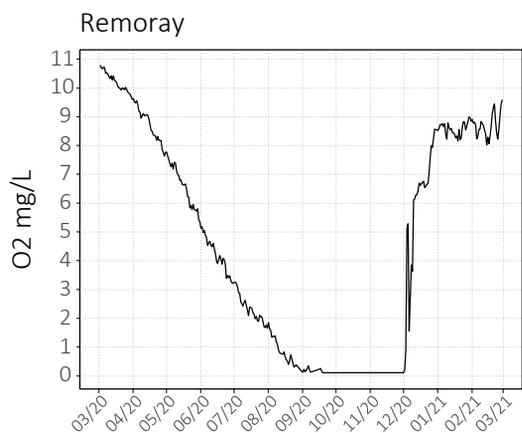


Ilay



Chalain





Appendix 2 : Dissolved oxygen concentration at 0,8*Zmax over the continuous monitoring period in 2020-2021.

The lakes of Chambly, Abbaye, Ambleon, Barterand, Val, Maclu, Ilay, Chalain and Remoray, which were strongly stratified during summer period, showed intense deoxygenation at depth to reach total anoxia at 0,8*Zmax during stratification period. Both lakes of Etival, which were weakly stratified during summer period, showed deoxygenation of lower magnitude than the stratified lakes, with minimum oxygen concentration reaching 1mg/l for small Etival and 2mg/l for large Etival.

Hypoxia duration (days with $[O_2]$ below 2 mg/l at 0,8*Zmax) is variable depending on lakes, ranging from 5 to 175 days. Small and large Etival, which were weakly stratified during summer, showed small hypoxia duration of respectively 14 and 5 days. In strongly stratified lakes, hypoxia duration is nonetheless variable depending on lakes. Lakes Abbaye, Barterand, Chalain, Ilay, Remoray and Val showed prolonged periods of hypoxia ranging from 121 to 175 days. In contrast, the lakes of Ambleon, Chambly and Maclu, which were also strongly stratified during summer, showed less prolonged periods of hypoxia ranging from 23 to 60 days.

	Summer Zmax [CH ₄]aq		Summer Zmax [CO ₂]aq	
	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>
C _{org}	0.65	-0.15	0.23	-0.39
C _{org} /N	0.81	0.08	0.16	0.45
δ ¹³ C _{OM}	0.4	-0.28	0.57	-0.19
TC	2.2 e-16	0.96	4.2 e-4	0.89
Hypoxia duration	0.46	0.24	0.21	0.4
Δ°C _{stratification period}	0.21	0.4	0.13	0.48
Relative agricultural area	0.004	0.8	0.006	0.79
Relative forest area	0.14	0.47	0.11	0.51

Appendix 3 : Spearman rank correlation test performed between lake properties and gas concentrations dataset. *p*-value and *rho* in bold type indicates significant relationship ($\alpha < 0,05$)

	Summer δ ¹³ C _{Daphnia}		Early winter δ ¹³ C _{Daphnia}		Late winter δ ¹³ C _{Daphnia}		seasonal Δδ ¹³ C _{Daphnia}		Mean seasonal Δδ ¹³ C _{Daphnia}	
	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>
Summer Zmax [CH ₄]aq	0,11	0,5	0,13	-0,48	0,18	-0,43	0,03	0,66	0,008	0,77
Summer Zmax [CO ₂]aq	0,31	0,33	0,04	-0,62	0,16	-0,45	0,008	0,77	0,002	0,83
Summer epilimnion [CH ₄]aq	0,28	0,34	0,61	-0,17	0,63	-0,16	0,45	0,25	0,34	0,31
Summer epilimnion [CO ₂]aq	0,12	-0,49	0,32	-0,32	0,17	-0,44	0,92	0,03	0,88	0,05
Early winter mean [CH ₄]aq	0,98	0	0,45	-0,25	0,92	-0,03	0,53	0,2	0,67	0,14
Late winter mean [CH ₄]aq	0,24	-0,38	0,4	-0,28	0,69	0,13	0,71	0,12	0,94	0,02
Early winter mean [CO ₂]aq	0,43	-0,26	0,07	-0,57	0,01	-0,71	0,27	0,36	0,11	0,5
Late winter mean [CO ₂]aq	0,59	0,18	0,35	-0,3	0,32	-0,32	0,15	0,46	0,04	0,61

Appendix 4 : Spearman rank correlation test performed between Daphnia carbon isotopic signatures and gas concentrations dataset. *p*-value and *rho* in bold type indicates significant relationship ($\alpha < 0,05$)

	Summer δ ¹³ C _{Daphnia}		Early winter δ ¹³ C _{Daphnia}		Late winter δ ¹³ C _{Daphnia}		Seasonal Δδ ¹³ C _{Daphnia}		Mean seasonal Δδ ¹³ C _{Daphnia}	
	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>
Δ°C _{stratification period}	0.52	0.21	0.09	-0.53	0.32	-0.32	0.003	0.81	0.005	0.8
Hypoxia duration	0.46	0.24	0.09	-0.53	0.5	-0.22	0.007	0.78	0.01	0.7
C _{org}	0.88	0.05	0.13	0.48	0.1	0.51	0.19	-0.42	0.16	-0.45
C _{org} /N	0.86	-0.06	0.08	-0.54	0.46	-0.24	0.06	0.58	0.07	0.52
δ ¹³ C _{OM}	0.53	0.2	0.73	0.11	0.28	0.35	0.69	0.13	0.88	0.05
TC	0.13	0.48	0.1	-0.52	0.16	-0.45	0.01	0.74	0.001	0.86
Relative agricultural area	0.71	0.12	0.03	-0.64	0.02	-0.7	0.04	0.62	0.02	0.69
Relative forest area	0.79	0.09	0.28	-0.35	0.38	-0.29	0.59	0.18	0.38	0.29

Appendix 5 : Spearman rank correlation test performed between Daphnia carbon isotopic signatures and lake properties. *p*-value and *rho* in bold type indicates significant relationship ($\alpha < 0,05$)