

1 **Methanotrophy within the water column of a large** 2 **meromictic tropical lake (Lake Kivu, East Africa)**

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10 **Abstract**

11 The permanently stratified Lake Kivu is one of the largest freshwater reservoirs of
12 dissolved methane (CH₄) on Earth. Yet CH₄ emissions from its surface to the atmosphere
13 have been estimated to be 2 orders of magnitude lower than the CH₄ upward flux to the mixed
14 layer, suggesting that microbial CH₄ oxidation is an important process within the water
15 column. A combination of natural abundance stable carbon isotope analysis ($\delta^{13}\text{C}$) of several
16 carbon pools and ¹³CH₄-labelling experiments was carried out during the rainy and dry season
17 to quantify (i) the contribution of CH₄-derived carbon to the biomass, (ii) methanotrophic
18 bacterial production (MBP), and (iii) methanotrophic bacterial growth efficiency (MBGE),
19 defined as the ratio between MBP and gross CH₄ oxidation. We also investigated the
20 distribution and the $\delta^{13}\text{C}$ of specific phospholipid fatty acids (PLFA), used as biomarkers for
21 aerobic methanotrophs. Maximal MBP rates were measured in the oxycline, suggesting that
22 CH₄ oxidation was mainly driven by oxic processes. Moreover, our data revealed that
23 methanotrophic organisms in the water column oxidized most of the upward flux of CH₄, and
24 that a significant amount of CH₄-derived carbon was incorporated into the microbial biomass
25 in the oxycline. The MBGE was variable (2-50%) and negatively related to CH₄:O₂ molar
26 ratios. Thus, a comparatively smaller fraction of CH₄-derived carbon was incorporated into
27 the cellular biomass in deeper waters, at the bottom of the oxycline where oxygen was scarce.
28 The aerobic methanotrophic community was clearly dominated by type I methanotrophs and
29 no evidence was found for an active involvement of type II methanotrophs in CH₄ oxidation

1 in Lake Kivu, based on fatty acids analyses. Vertically integrated over the water column, the
2 MBP was equivalent to 16-60% of the average phytoplankton particulate primary production.
3 This relatively high magnitude of MBP, and the substantial contribution of CH₄-derived
4 carbon to the overall biomass in the oxycline, suggest that methanotrophic bacteria could
5 potentially sustain a significant fraction of the pelagic food-web in the deep, meromictic Lake
6 Kivu.

8 **1 Introduction**

9 Although the atmospheric methane (CH₄) concentration is low compared to carbon dioxide
10 (CO₂), CH₄ contributes significantly to the anthropogenic radiative forcing (18%) because of
11 its 25 times higher global warming potential than CO₂ (Forster et al. 2007). CH₄ has several
12 natural and anthropogenic sources and sinks, whereby natural and artificial wetlands are
13 recognized as major CH₄ sources to the atmosphere (e.g. Kirschke et al. 2012). Bastviken et
14 al. (2011) estimated that CH₄ emissions to the atmosphere from freshwater ecosystems (0.65
15 Pg C yr⁻¹ as CO₂ equivalent) would correspond to 25% of the global land carbon (C) sink (2.6
16 ± 1.7 Pg C yr⁻¹, Denman et al. 2007). Tropical regions are responsible for approximately half
17 of the estimated CH₄ emissions from freshwater ecosystems to the atmosphere, although they
18 have been consistently undersampled (Bastviken et al. 2011). Thus, more information on both
19 the magnitude and controlling factors of CH₄ emissions from tropical inland waters are
20 warranted. CH₄ is produced mainly in anoxic sediments by methanogenic archaea following
21 two different pathways: acetoclastic methanogenesis, using acetate produced from organic
22 matter degradation, or CO₂ reduction. Although both methanogenic pathways may co-occur,
23 CO₂ reduction is dominant in marine sediments, while acetate fermentation is the major
24 pathway in freshwater sediments (Whiticar et al. 1986).

25 CH₄ production rates are typically higher than CH₄ emission fluxes to the atmosphere, since
26 aerobic and anaerobic microbial CH₄ oxidation within lacustrine sediments or in water
27 columns are effective processes that limit the amount of CH₄ reaching the atmosphere, in
28 particular when vertical CH₄ transport occurs mainly through diffusive transport, rather than
29 through ebullition. A wide variety of electron acceptors can be used during microbial CH₄
30 oxidation, including but not limited to oxygen (O₂, Rudd et al. 1974). Micro-organisms using
31 O₂ as electron acceptors belong to the Proteobacteria phylum. The use of an enzyme known as
32 CH₄ monooxygenase (either under a soluble or membrane-bound form) to catalyze the

1 oxidation of CH₄ to methanol is a defining characteristic of aerobic methanotrophs (Hanson &
2 Hanson 1996). Methanol is then oxidized to formaldehyde, which is assimilated to form
3 intermediates of central metabolic routes that are subsequently used for biosynthesis of cell
4 material (Hanson & Hanson 1996 and references therein). Hence, aerobic methanotrophs use
5 CH₄ not only as an energy source, but also as a C source. Aerobic methanotrophs are typically
6 classified into two phylogenetically distinct groups that use different pathways for the
7 formaldehyde assimilation : the type I methanotrophs belong to the *Gammaproteobacteria*
8 and use the ribulose monophosphate pathway while the type II methanotrophs belong to the
9 *Alphaproteobacteria* and use the serine pathway.

10 Besides aerobic processes, anaerobic CH₄ oxidation coupled with SO₄²⁻ reduction has been
11 found to be carried out by a syntrophic consortium of CH₄-oxidizing archaea and sulphate-
12 reducing bacteria. The association between the archaea and bacteria is commonly interpreted
13 as an obligate syntrophic interaction in which the archaeal member metabolizes CH₄ leading
14 to the production of an intermediate, which in turn is scavenged as an electron donor by its
15 SO₄²⁻ reducing partner (Knitell and Boetius 2009). The identity of the intermediates
16 transferred between the CH₄ oxidizers and the SO₄²⁻ reducers is still uncertain. In contrast to
17 aerobic CH₄ oxidation, the contribution of CH₄ as a C source is minimal as only ~ 1% of the
18 oxidized CH₄ is channelled to biosynthesis pathway and the growth of the partners of the
19 consortium is slow, with generation times of months to years (Knitell and Boetius 2009). Both
20 partners of the consortium are strictly intolerant to O₂ (Knitell and Boetius 2009). Initially
21 reported in marine sediments (Boetius et al. 2000), this consortium was later identified in the
22 water column of marine euxinic basins, such as the Black Sea (Schubert et al. 2006), but
23 rarely in lacustrine systems probably because fresh waters are usually depleted in SO₄²⁻ in
24 comparison with other electron acceptors (NO₃⁻, Fe³⁺, Mn⁴⁺) in contrast with the oceans.
25 Nevertheless, it appeared during the last decade that anaerobic CH₄ oxidation could be
26 coupled to a wider variety of electron acceptors that previously thought, including nitrite
27 (NO₂⁻), nitrate (NO₃⁻), manganese (Mn), and iron (Fe) (Raghoebarsing et al. 2006 ; Beal et al.
28 2009).

29 Aerobic methanotrophic organisms not only use CH₄ as electron donors but they are also able
30 to incorporate a substantial fraction of the CH₄-derived C into their biomass, and could
31 therefore contribute to fuel the pelagic food web (Bastviken et al. 2003; Jones and Grey 2011;
32 Sanseverino et al. 2012). A recent study carried out in small boreal lakes (surface area < 0.01

1 km²) demonstrated that methanotrophic bacterial production (MBP, i.e. incorporation rates of
2 CH₄-derived carbon into the biomass) contributed to 13-52% of the autochthonous primary
3 production in the water column (Kankaala et al. 2013). However, in spite of the potential
4 importance of this alternative C source in aquatic ecosystems, most of the studies carried out
5 in aquatic environments reported gross CH₄ oxidation, while direct measurements of MBP in
6 lakes are still scarce. Also, the methanotrophic bacterial growth efficiency (MBGE), defined
7 as the amount of biomass synthesized from CH₄ per unit of CH₄ oxidized, was found to vary
8 widely in aquatic environments (15-80% according to King 1992 ; 6-77% according to
9 Bastviken et al. 2003), but little is known about the factors driving its variability so that it is
10 currently not possible to derive accurate estimations of the MBP based solely on gross CH₄
11 oxidation rates. A better understanding of the environmental control of MBGE would help to
12 assess more accurately the importance of methanotrophic organisms as carbon sources for
13 higher trophic level of the food-web.

14 Lake Kivu, located in a volcanic area, is one of the largest freshwater CH₄ reservoirs, with
15 approximately 60 km³ (at standard temperature and pressure) dissolved in its permanently
16 stratified water (Schmid et al. 2005). One third of the CH₄ accumulated in its deep waters is
17 estimated to be produced via the acetoclastic pathway and two thirds by reduction of geogenic
18 CO₂ (Schoell et al. 1988). Based on a modelling approach, Schmid et al. (2005) estimated that
19 CH₄ production recently increased by threefold since the 1970s for a still unknown reason.
20 Although the deep layers of the lake contain a huge amount of dissolved CH₄, Lake Kivu
21 ranks globally among the lakes with the lowest CH₄ emissions to the atmosphere (Borges et
22 al. 2011). Moreover, the emission of CH₄ from surface waters to the atmosphere (0.038 mmol
23 m⁻² d⁻¹, Borges et al. 2011) is several orders of magnitude lower than the upward flux of CH₄
24 to the mixed layer (9.38 mmol m⁻² d⁻¹, Pasche et al. 2009), suggesting that CH₄ oxidation
25 prevents most of CH₄ to reach the surface of the lake. Our knowledge on bacterial CH₄
26 oxidation in Lake Kivu has so far been based on circumstantial evidence such as mass balance
27 considerations (Borges et al. 2011; Pasche et al. 2011), identification of
28 *Gammaproteobacteria* aerobic CH₄ oxidizers in the oxycline using molecular tools (Pasche et
29 al. 2011), and a few incubations carried out almost 40 years ago (Jannasch 1975).

30 Because of the permanently stratified nature of its water column and the large amount of CH₄
31 dissolved in its deep water, the meromictic Lake Kivu offers an ideal natural laboratory to
32 investigate the role of methanotrophy in large tropical lakes. In this study, we used the

1 difference in C stable isotope abundance ($\delta^{13}\text{C}$) of different C sources to estimate the fraction
2 of CH_4 inputs to the mixed layer from deep waters that is microbially oxidized within the
3 water column, and to quantify the relative contribution of CH_4 -derived C to the particulate
4 biomass. Additionally, phospholipid fatty acids (PLFA) and their $\delta^{13}\text{C}$ signatures were
5 analyzed to characterize the populations of methanotrophic bacteria present in the water
6 column. We also carried out ^{13}C -labelling experiments to trace the incorporation of CH_4 -
7 derived C into the biomass, to quantify methanotrophic bacterial production, and its
8 conversion to CO_2 , to quantify methanotrophic bacterial growth efficiency). Finally, stable
9 isotope probing (SIP) of specific PLFA (SIP-PLFA) after ^{13}C - CH_4 labelling allowed to
10 characterize the bacterial populations active in methanotrophy.

11

12 **2 Material and methods**

13 **2.1. Study site description and sampling**

14 Lake Kivu (East Africa) is a large (2370 km²) and deep (maximum depth of 485 m)
15 meromictic lake. Its vertical structure consists of an oxic and nutrient-poor mixed layer
16 (seasonally variable depth, up to 70 m), and a permanently anoxic monimolimnion rich in
17 dissolved gases (CH_4 , CO_2) and inorganic nutrients (Damas, 1937; Degens et al., 1973;
18 Schmid et al., 2005). Seasonal variations of the vertical position of the oxycline are driven by
19 contrasting hygrometry and long wave radiation between rainy (October-May) and dry (June-
20 September) seasons (Thiery et al. 2014), the latter being characterized by a deepening of the
21 oxic zone, and an increased input of dissolved gases and inorganic nutrients into the mixed
22 layer (Sarmiento et al. 2006, Borges et al. 2011). Sampling was carried out in the Northern
23 Basin (1.72°S, 29.23°E) in February 2012 (rainy season), and in the Northern Basin and
24 Southern Basin (2.34°S, 28.98°E) in September 2012 (dry season) (Figure 1).

25 O_2 concentration was measured with a YSI-proODO probe with a optical O_2 sensor (detection
26 limit is 3 $\mu\text{mol L}^{-1}$), calibrated using air saturated water. Hereafter, “ O_2 -depleted waters”
27 stands for waters with concentration < 3 $\mu\text{mol L}^{-1}$. Lake water was collected with a 7 L Niskin
28 bottle (Hydro-Bios) at a depth interval of 5 m from the lake surface to the top of the
29 monimolimnion, at 80 m.

30 **2.2. Chemical analyses**

1 Samples for CH₄ concentrations were collected in 50 ml glass serum bottles from the Niskin
2 bottle with a silicone tubing, left to overflow, poisoned with 100 µl of saturated HgCl₂ and
3 sealed with butyl stoppers and aluminium caps. Concentrations of CH₄ were measured by
4 headspace technique (Weiss 1981) using gas chromatography with flame ionization detection
5 (GC-FID, SRI 8610C), after creating a 20 ml headspace with N₂ in the glass serum bottles,
6 and then analyzed following the method described by Borges et al. (2011). Samples for the
7 determination of the δ¹³C signature of CH₄ (δ¹³C-CH₄) were collected in 250 ml glass serum
8 bottles similarly to CH₄ concentration samples. δ¹³C-CH₄ was determined by a custom
9 developed technique, whereby a 20 ml helium headspace was first created, and CH₄ was
10 flushed out through a double-hole needle, CO₂ was removed with a CO₂ trap (soda lime), and
11 the CH₄ was converted to CO₂ in an online combustion column similar to that in an Elemental
12 Analyzer (EA). The resulting CO₂ was subsequently preconcentrated by immersion of a
13 stainless steel loop in liquid nitrogen in a custom-built cryofocussing device, passed through a
14 micropacked GC column (HayeSep Q 2m, 0.75mm ID ; Restek), and finally measured on a
15 Thermo DeltaV Advantage isotope ratio mass spectrometer (IRMS). Certified reference
16 standards (IAEA-CO1 and LSVEC) were used to calibrate δ¹³C-CH₄ data.

17 Samples for the determination of δ¹³C signatures of dissolved inorganic carbon (DIC) were
18 collected by gently overfilling 12 ml glass vial (Labco Exetainer), preserved with 20 µl of
19 saturated HgCl₂. For the analysis of δ¹³C-DIC, a 2 ml helium headspace was created and 100
20 µl of H₃PO₄ (99 %) was added into each vial to convert all DIC species into CO₂. After
21 overnight equilibration, a variable volume of the headspace was injected into an EA coupled
22 to an isotope ratio mass spectrometer (EA-IRMS; Thermo FlashHT with Thermo DeltaV
23 Advantage). Calibration of δ¹³C-DIC measurements was performed with certified reference
24 materials (LSVEC and either NBS-19 or IAEA-CO-1).

25 Samples for particulate organic carbon (POC) concentrations and its stable C isotope
26 signature (δ¹³C-POC) were filtered on pre-combusted (overnight at 450°C) 25 mm glass fiber
27 filters (Advantec GF-75; 0.3 µm), and dried. These filters were later decarbonated with HCl
28 fumes for 4 h, dried and packed in silver cups. POC and δ¹³C-POC were determined on an
29 EA-IRMS (Thermo FlashHT with Thermo DeltaV Advantage). Calibration of POC and δ¹³C-
30 POC was performed with IAEA-C6 and acetanilide, and reproducibility of δ¹³C-POC
31 measurements, estimated based on triplicate measurements, was typically better than 0.2 %.

1 Samples (~ 2 L) for measurements of phospholipid fatty acid concentrations (PLFA) and their
2 $\delta^{13}\text{C}$ signature were filtered on pre-combusted 47 mm glass fiber filters (Advantec GF-75; 0.3
3 μm), and kept frozen until further processing. Extraction and derivatisation of PLFA was
4 performed following a modified Bligh and Dyer extraction, silica column partitioning, and
5 mild alkaline transmethylation as described by Boschker et al. (2004). Analyses were made
6 on a Isolink GC-c-IRMS coupled to a Thermo DeltaV Advantage. All samples were analyzed
7 in splitless mode, using an apolar GC column (Agilent DB-5) with a flow rate of 2 ml min^{-1} of
8 helium as carrier gas. Initial oven temperature was set at 60°C for 1 min, then increased to
9 130°C at $40^\circ\text{C min}^{-1}$, and subsequently reached 250°C at a rate of 3°C min^{-1} . $\delta^{13}\text{C}$ -PLFA were
10 corrected for the addition of the methyl group by a simple mass balance calculation, and were
11 calibrated using internal (C19:0) and external (mixture of C14:0, C16:0, C18:0, C20:0, C22:0)
12 fatty acid methyl ester (FAME) standards. Reproducibility estimated based on replicates
13 measurement was $\pm 0.6\%$ or better for natural abundance samples.

14 **2.3. Determination of the isotope fractionation factor**

15 In September 2012 (Southern Basin), the isotope fractionation factor (ϵ) was estimated by
16 monitoring the changes in CH_4 concentration and $\delta^{13}\text{C}$ - CH_4 over time in microcosms at
17 several depths (60 m, 62.5 m, 65 m, 67.5 m) across the oxycline. Six glass serum bottles (60
18 ml) were gently overfilled at each depth and tightly capped with a butyl rubber stopper and an
19 aluminium cap. They were then incubated in the dark at the lake temperature during 0, 24, 48,
20 72, 96 or 120 h. The incubation was stopped by poisoning the bottles with $100\ \mu\text{l}$ of saturated
21 HgCl_2 . The measurement of the concentration of CH_4 and the $\delta^{13}\text{C}$ - CH_4 in every bottle was
22 performed as described before. The isotope fractionation factor was calculated according to
23 Coleman et al. (1981).

24 **2.4. Methanotrophic bacterial production and growth efficiency measurement**

25 At several depths throughout the water column, the methanotrophic bacterial production and
26 methanotrophic bacterial growth efficiency were estimated by quantifying the incorporation
27 of ^{13}C -labelled CH_4 (^{13}C - CH_4 , 99.9%, Eurisotop) into the POC and DIC pool. Water from
28 each sampling depth was transferred with a silicone tubing into 12 serum bottles (60 ml),
29 capped with butyl stoppers and sealed with aluminium caps. Thereafter, 4 different volumes
30 ($50\ \mu\text{l}$, $100\ \mu\text{l}$, $150\ \mu\text{l}$, or $200\ \mu\text{l}$) of a ^{13}C - CH_4 gas mixture (1:10 in He) were injected in
31 triplicate and $100\ \mu\text{l}$ of saturated HgCl_2 was immediately added to one bottle per gas
32 concentration treatment, serving as control bottle without biological activity. After vigorous

1 shaking, the bottles were incubated in the dark during 24 h at the lake temperature. The
 2 incubation was stopped by filtration of a 40 ml subsample on 25 mm glass fiber filters
 3 (Advantec GF-75; 0.3 μm) to measure the ^{13}C -POC enrichment, and a 12 ml Exetainer was
 4 filled and poisoned with the addition of HgCl_2 in order to measure the ^{13}C -DIC enrichment.
 5 The exact amount of ^{13}C - CH_4 added in the bottles was determined from the bottles poisoned
 6 at the beginning of the experiment. The measurements of the concentration of POC, the $\delta^{13}\text{C}$ -
 7 POC, the $\delta^{13}\text{C}$ -DIC and the $\delta^{13}\text{C}$ - CH_4 were performed as described above. Methanotrophic
 8 bacterial production (MBP, $\mu\text{mol L}^{-1} \text{d}^{-1}$) rates were calculated according to Hama et al.
 9 (1983) :

$$10 \quad \text{MBP} = \text{POC}_f * (\%^{13}\text{C-POC}_f - \%^{13}\text{C-POC}_i) / (t * (\%^{13}\text{C-CH}_4 - \%^{13}\text{C-POC}_i)) \quad (1)$$

11 where POC_f is the concentration of POC at the end of incubation ($\mu\text{mol L}^{-1}$), $\%^{13}\text{C-POC}_f$ and
 12 $\%^{13}\text{C-POC}_i$ are the percentage of ^{13}C in the POC and the end and the beginning of incubation,
 13 t is the incubation time (d^{-1}) and $\%^{13}\text{C-CH}_4$ is the percentage of ^{13}C in CH_4 directly after the
 14 inoculation of the bottles with the ^{13}C tracer. The methanotrophic bacterial respiration rates
 15 (MBR, $\mu\text{mol L}^{-1} \text{d}^{-1}$) were calculated according to :

$$16 \quad \text{MBR} = \text{DIC}_f * (\%^{13}\text{C-DIC}_f - \%^{13}\text{C-DIC}_i) / (t * (\%^{13}\text{C-CH}_4 - \%^{13}\text{C-DIC}_i)) \quad (2)$$

17 where DIC_f is the concentration of DIC after the incubation ($\mu\text{mol L}^{-1}$), $\%^{13}\text{C-DIC}_f$ and $\%^{13}\text{C-}$
 18 DIC_i are the final and initial percentage of ^{13}C in DIC. Finally, the methanotrophic bacterial
 19 growth efficiency (MBGE, %) was calculated according to :

$$20 \quad \text{MBGE} = \text{MBP} / (\text{MBP} + \text{MBR}) * 100 \quad (3)$$

21 The CH_4 concentration in the bottles sometimes increased drastically because of the $^{13}\text{C-CH}_4$
 22 addition, which could have induced a bias in the estimation of MBP and MBR in case of CH_4 -
 23 limitation of the methanotrophic bacteria community. However, performing incubation along
 24 a gradient of CH_4 concentrations allowed us to assess if the measured MBP and MBR were
 25 positively related to the amount of tracer inoculated in the bottles. In case of such an effect
 26 (only at 50 m in the Northern Basin in February 2012 and at 60 m in the Southern Basin in
 27 September 2012) we applied a linear regression model (r^2 always better than 0.90) to estimate
 28 the intercept with the y-axis, which was assumed to correspond to the MBP or MBR rates at
 29 in-situ CH_4 concentration.

30 **2.5. Stable isotope probing of PLFA (SIP-PLFA) with $^{13}\text{C-CH}_4$**

1 At each sampling depth and in parallel with the MBP measurement, 4 serum bottles (250 ml)
2 were filled with water, overflowed and sealed with butyl stopper and aluminium caps. Bottles
3 were spiked with 500 μl of $^{13}\text{C}\text{-CH}_4$ (99.9%). After 24 h of incubation in the dark at lake
4 temperature, the water from the 4 bottles was combined and filtered on a single pre-
5 combusted 47 mm glass fiber filter (Advantec GF-75; 0.3 μm) to quantify the incorporation of
6 the tracer in bacterial PLFA. The filters were kept frozen until further processing. The
7 extraction, derivatisation and analysis by GC-c-IRMS were carried out as described above.

8

9 **3 Results**

10 **3.1. Physico-chemical parameters**

11 In September 2012, the water column in the Southern Basin was oxic ($> 3 \mu\text{mol L}^{-1}$) from the
12 surface to 65 m (Figure 2a). CH_4 was abundant in deep waters, with a maximum
13 concentration of $899 \mu\text{mol L}^{-1}$ at 80 m, however CH_4 decreased abruptly at the bottom of the
14 oxycline, being 4 orders of magnitude lower in surface waters (Figure 2a). Consistent with its
15 biogenic origin, CH_4 was depleted in ^{13}C in deep waters ($\delta^{13}\text{C}\text{-CH}_4$: -55.0 ‰) but became
16 abruptly enriched in ^{13}C at the transition between oxic and O_2 -depleted waters, where CH_4
17 concentrations sharply decreased, to reach a maximal value of -39.0 ‰ at 62.5 m depth
18 (Figure 2a). The $\delta^{13}\text{C}\text{-POC}$ values mirrored the pattern of $\delta^{13}\text{C}\text{-CH}_4$: they were almost
19 constant from the surface to 55 m ($-24.4 \pm 0.3 \text{ ‰}$), then showed an abrupt excursion towards
20 more negative values at the bottom of the oxycline, with a minimum value (-42.8 ‰) at 65 m
21 depth (Figure 2a). Similar results were found in September 2012 in the Northern Basin, where
22 the water was oxic ($> 3 \mu\text{mol L}^{-1}$) down to 55 m (Figure 2b). At the transition between oxic
23 and O_2 -depleted waters, an abrupt isotopic enrichment of the CH_4 was also observed and the
24 $\delta^{13}\text{C}\text{-POC}$ was relatively depleted in ^{13}C , similarly as in the Southern Basin (Figure 2b).

25 In February 2012 in the Northern Basin, the water was oxic ($> 3 \mu\text{mol L}^{-1}$) until 45 m depth
26 but the O_2 concentrations were below the limit of detection deeper in the water column
27 (Figure 2c). The gradual decrease in the CH_4 concentration between 60 m and 45 m (from 110
28 $\mu\text{mol L}^{-1}$ to $3 \mu\text{mol L}^{-1}$) was accompanied by a parallel increase of the $\delta^{13}\text{C}\text{-CH}_4$ signature in
29 the same depth interval (from -55.9 ‰ to -41.7 ‰), the residual CH_4 becoming isotopically
30 enriched as CH_4 concentration decreased (Figure 2c). $\delta^{13}\text{C}\text{-POC}$ values were also slightly
31 lower below the oxic zone, with a minimum at 50 m (-26.9 ‰) (Figure 2c).

1 **3.2. Phospholipid fatty acid concentration and stable isotopic composition**

2 Figure 3 show profiles of the relative concentration and the $\delta^{13}\text{C}$ signature of specific PLFA
3 in September 2012 (Figure 3a, 3b ; Southern basin) and February 2012 (Figure 3c, 3d ;
4 Northern Basin). Irrespective of station, season and depth, the C16:0 saturated PLFA was
5 always the most abundant PLFA (18-35% of all PLFA). The relative abundance of the C16
6 monounsaturated fatty acids (C16 MUFA) significantly increased at the bottom of the
7 oxycline in February and September 2012. The $\delta^{13}\text{C}$ signature of the C16 MUFA was
8 comparable to the $\delta^{13}\text{C}$ signature of the C16:0 in oxic waters, oscillating around -27‰ or -
9 29‰ in February and September 2012, respectively. However, C16 MUFA were largely
10 depleted in ^{13}C in the oxycline, with minimal $\delta^{13}\text{C}$ values as low as -55.3‰ at the transition
11 between oxic and O_2 -depleted waters in September 2012, and -49.5‰ in February 2012. This
12 very strong depletion in $\delta^{13}\text{C}$ was only observed for this particular type of PLFA (C16
13 MUFA). The C18 MUFA were slightly more abundant in oxic waters (on average 9%) than in
14 deeper waters (1-4%). Their isotopic composition varied with depth following the same
15 vertical pattern than C16 MUFA, but with a lower amplitude. C18 MUFA minima in $\delta^{13}\text{C}$
16 were observed in O_2 -depleted waters in February 2012 (55 m, -35.1‰) and September 2012
17 (70m, -30.5‰). The relative abundance of iso- and anteiso-branched C15:0 PLFA was
18 systematically low (1-5%) and did not follow any depth pattern. Their isotopic signature was
19 however slightly lower in O_2 -depleted waters than in oxic waters.

20 **3.3 Isotope fractionation factor determination**

21 During the isotope fractionation factor experiment, a significant decrease of the CH_4
22 concentration over time and a parallel enrichment of the residual CH_4 (Figure 4) were
23 monitored in every bottle incubated under oxic conditions. However, no consumption of CH_4
24 was measured in O_2 -depleted waters. The isotope fractionation factor measured at several
25 depths across the oxycline ranged between 1.008 and 1.024, and averaged 1.016 ± 0.007 (n =
26 5).

27 **3.4. Methanotrophic bacterial production**

28 MBP rates within the oxycline were variable (from 0 to $7.0 \mu\text{mol C L}^{-1} \text{d}^{-1}$). Maximum values
29 were always observed at the bottom of the oxycline, near the transition between oxic and O_2 -
30 depleted waters (Figure 2d, 2e, 2f), however substantial MBP (up to $2.2 \mu\text{mol L}^{-1} \text{d}^{-1}$) were
31 also recorded in O_2 -depleted waters in February 2012 (Figure 2f). Vertically integrated over
32 the water column, MBP rates were estimated at $28.6 \text{ mmol m}^{-2} \text{d}^{-1}$ and $8.2 \text{ mmol m}^{-2} \text{d}^{-1}$ in

1 September 2012 in the Southern and Northern Basin, respectively, and 29.5 mmol m⁻² d⁻¹ in
2 February 2012 in the Northern Basin. MBGE was found to be highly variable in the water
3 column ranging between 50% at 52.5 m in the Northern Basin (September 2012) and 2% at
4 67.5 m in the Southern Basin (September 2012). Computed from depth-integrated MBP and
5 MBR rates, the water column mean MBGE were 23% in September 2012 in the Southern and
6 Northern Basins, and 42% in February 2012 in the Northern Basin.

7 Specific CH₄-derived C incorporation rates in PLFA (d⁻¹ ; incorporation rates normalized on
8 PLFA concentration) show that bacteria containing C16 MUFA and C14:0 were particularly
9 active in CH₄-derived C fixation in the oxycline in February and September 2012 (Figure 5a,
10 4b). In contrast, the specific incorporation pattern was dominated by C17 MUFA, and to a
11 lesser extent 10Me16:0 and C16 MUFA in O₂-depleted waters in February 2012 (Figure 5b).

12

13 **4. Discussion**

14 The sharp decrease of CH₄ concentration and the isotopic enrichment of the residual CH₄ in
15 the oxycline, mirrored by the isotopic depletion of the POC pool at these depths indicated that
16 microbial CH₄ oxidation is a strong CH₄ sink within the water column of Lake Kivu. Similar
17 patterns characterized by a strong isotopic depletion of the POC pool in the oxycline were
18 reported in other systems, such as the meromictic Northern Basin of Lake Lugano (Lehmann
19 et al. 2004, Blee et al. 2014). The fraction of the upward CH₄ flux oxidized within a depth
20 interval can be estimated from a closed-system Rayleigh model of isotope fractionation (Blee
21 et al. 2014) described by the following equation (rearranged from Eq. 11 in Coleman et al.
22 1981):

$$23 \ln(1-f) = \ln((\delta^{13}\text{CH}_{4t}+1000)/(\delta^{13}\text{CH}_{4b}+1000))/((1/\alpha)-1) \quad (4)$$

24 where f is the fraction of CH₄ oxidized within the depth interval, $\delta^{13}\text{CH}_{4b}$ and $\delta^{13}\text{CH}_{4t}$ are the
25 $\delta^{13}\text{C}$ values of CH₄ at the bottom and the top of the depth interval, respectively, and α is the
26 isotope fractionation factor for CH₄ oxidation estimated in Lake Kivu in September 2012 ($\alpha =$
27 1.016 ± 0.007). Based on this equation and using a range of isotope fractionation factors
28 (from 1.009 to 1.023), we can estimate that 51-84% of the upward flux of CH₄ was
29 microbially oxidized within a 10 m depth interval in the oxycline (60-70 m) in the Southern
30 Basin during the dry season (September 2012). Similarly, 51-84% of the CH₄ flux was

1 oxidized between 50 m and 55 m in the Northern Basin during the dry season, and 58-89% of
2 the CH₄ flux was oxidized within a wider depth interval (45-70 m) during the rainy season
3 (February 2012). The relatively wide range of the estimated percentage of CH₄ flux oxidized
4 is due to the uncertainty on the isotope fractionation factor. Nevertheless, these calculations
5 illustrate clearly the importance of microbial CH₄ oxidation processes in preventing CH₄ to
6 reach the surface waters of the lake.

7 The theoretical $\delta^{13}\text{C}$ signature of methanotrophs can be estimated at each depth from $\delta^{13}\text{C}$ -
8 CH₄ values and the experimental isotope fractionation factor (α , ranged between 1.009-1023).
9 Applying a simple isotope mixing model with the $\delta^{13}\text{C}$ signature of methanotrophs as an end-
10 member and the $\delta^{13}\text{C}$ -POC in the surface (5 m) as a sedimenting organic matter end-member,
11 it is possible to estimate the contribution of CH₄-derived C to the POC pool. Indeed, the
12 contribution of CH₄-derived C appeared to be substantial at the bottom of the mixolimnion. In
13 September 2012 in the Southern Basin, 32-44% of the depth-integrated POC pool in the
14 oxycline (between 60 m and 70 m) originated from CH₄ incorporation, with a local maximum
15 at the transition between oxic and O₂-depleted waters (65 m, 44-54%). In the Northern Basin,
16 13-16 % of the POC in the oxycline (between 50 m and 60 m) derived from CH₄. However,
17 the contribution of CH₄ to the POC pool was relatively lower during the rainy season, as only
18 4-6% of the POC in the 50-70 m depth interval, below the oxycline, had been fixed by
19 methanotrophic organisms in the Northern Basin in February 2012 (local maximum slightly
20 below the oxycline at 50 m, 8-10%).

21 ¹³CH₄ tracer experiments allowed estimation of the net MBP and the MBGE. Whatever the
22 season, the highest MBP (0.8-7.2 $\mu\text{mol C L}^{-1} \text{d}^{-1}$) rates were found near the transition between
23 oxic and O₂-depleted waters. Hence, CH₄ oxidation in Lake Kivu seems to be mainly driven
24 by oxic processes. Furthermore, maximal MBP rates were observed where the *in situ* CH₄:O₂
25 ratio ranged between 0.1 and 10 (molar units, Figure 6), encompassing the stoichiometric
26 CH₄:O₂ ratio for aerobic microbial CH₄ oxidation (0.5) and the optimal ratio estimated in
27 culture experiment (0.9, Amaral & Knowles 1995). This relationship highlights the
28 importance of the regulation of aerobic methanotrophic production by both CH₄ and O₂
29 availability. Vertically integrated over the water column, the MBP was estimated at 29.5
30 $\text{mmol m}^{-2} \text{d}^{-1}$ during the rainy season in the Northern Basin, and 28.6 $\text{mmol m}^{-2} \text{d}^{-1}$ and 8.2
31 $\text{mmol m}^{-2} \text{d}^{-1}$ during the dry season in the Southern Basin and the Northern Basin,
32 respectively. These rates are comparable to the gross CH₄ oxidation rate reported earlier by

1 Jannasch (1975) in Lake Kivu ($7.2 \text{ mmol m}^{-2} \text{ d}^{-1}$) and the upward CH_4 flux recently estimated
2 ($9.38 \text{ mmol m}^{-2} \text{ d}^{-1}$) by Pasche et al (2009). Areal MBP in Lake Kivu are equivalent to 16-
3 60% of the mean annual phytoplankton primary production ($49 \text{ mmol m}^{-2} \text{ d}^{-1}$, Darchambeau
4 et al. 2014), suggesting that biomass production by methanotrophs has the potential to sustain
5 a significant fraction of the pelagic food-web. For example, it has been shown that cyclopoid
6 copepods (mesozooplankton) of Lake Kivu escape visual predators by migrating below the
7 euphotic zone, sometimes down to O_2 -depleted waters (Isumbiso et al. 2006), where they
8 might feed on CH_4 -derived C sources.

9 The relative contribution of MBP to the autochthonous production in Lake Kivu was distinctly
10 higher than those reported in 3 Swedish lakes during summer, where MBP was equivalent to
11 0.3 and 7.0% of the phytoplankton production (Bastviken et al. 2003). This was unrelated to
12 the phytoplankton production rates in the Swedish lakes that ranged between 7 and 83 mmol
13 $\text{m}^{-2} \text{ d}^{-1}$ and encompassed the average phytoplankton production value in Lake Kivu (49 mmol
14 $\text{m}^{-2} \text{ d}^{-1}$). The MBP rates in the Swedish lakes (based on ^{14}C incubations) were, however,
15 distinctly lower than in Lake Kivu, ranging between 0.3 and $1.8 \text{ mmol m}^{-2} \text{ d}^{-1}$. This difference
16 is probably related to the high CH_4 concentrations at the transition between oxic and O_2 -
17 depleted waters in Lake Kivu, as MBP peaked in the Swedish lakes at CH_4 concentrations $<$
18 $100 \mu\text{mol L}^{-1}$, while MBP peaked in Lake Kivu at CH_4 concentrations one to two orders of
19 magnitude higher. Kankaala et al. (2013) reported seasonally resolved (for the ice-free period)
20 MBP in five small (0.004 to 13.4 km^2) boreal humic lakes (with dissolved organic C
21 concentrations ranging between 7 and 24 mgC L^{-1}) in southern Finland. In these lakes
22 phytoplankton production and MBP were highly variable, ranging between 5 and 50 mmol m^{-2}
23 d^{-1} and $<0.2 \text{ mmol C m}^{-2} \text{ d}^{-1}$ and $41 \text{ mmol m}^{-2} \text{ d}^{-1}$, respectively. MBP was significantly
24 higher in the two smallest lakes (0.004 - 0.008 km^2), characterized by high CH_4 concentrations
25 ($< 750 \mu\text{mol L}^{-1}$) and permanent anoxia throughout the year in bottom waters. Considering a
26 MBGE of 25%, their MBP estimates corresponded to a highly variable percentage of
27 phytoplankton production, between 35% and 100% in the two smallest lakes, and between
28 0.4% and 5.0% in the three larger lakes (0.04 to 13.4 km^2), and therefore they proposed that
29 the relative contribution of methanotrophic bacteria to the total autotrophic production in a
30 lake is related to its size (Kankaala et al. 2013). However, the results reported for the large
31 (2370 km^2) Lake Kivu do not fit with this general pattern, probably because of the permanent
32 and strong stratification of its water column that on one hand promotes a long residence time

1 of deep waters and the accumulation of CH₄, and on the other hand leads to very slow upward
2 diffusion of solutes, promoting the removal of CH₄ by bacterial oxidation as it diffuses to the
3 surface.

4 The MBGE found during this study was variable (2-50%), but within the range of reported
5 values in fresh waters (15-80%, King 1992; 6-72 %, Bastviken et al. 2003). MBGE was
6 negatively related to the CH₄:O₂ ratio (Figure 7), i.e., a smaller fraction of the oxidized CH₄
7 was incorporated into the biomass at the bottom of the oxycline, where O₂ availability was
8 relatively limited compared to CH₄. It has been recently suggested that under O₂-limiting
9 conditions, methanotrophic bacteria are able to generate energy (adenosine triphosphate) by
10 fermentation of formaldehyde (Kalyuzhnaya et al. 2013), the key intermediate in the
11 oxidation of CH₄. This CH₄-based fermentation pathway would lead to the production of
12 excreted organic acids (lactate, formate, ...) from CH₄-derived C instead of converting CH₄
13 into cellular biomass. If the metabolic abilities for this process are ubiquitous in
14 methanotrophic organisms, it may potentially occur within the water column of Lake Kivu, at
15 the bottom of the oxycline or in micro-oxic zone, as suggested by the low MBGE values
16 found at high CH₄:O₂ molar ratio.

17 Almost all known aerobic methanotrophic bacteria are phylogenetically affiliated to
18 Proteobacteria, belonging either to the *Gammaproteobacteria* (also referred to type I
19 methanotrophs) or *Alphaproteobacteria* (type II methanotrophs) classes (Hanson & Hanson
20 1996). The two distinct groups differ in some important physiological characteristics.
21 Notably, they use different C fixation pathway (ribulose monophosphate for type I; the serine
22 pathway for type II) and possess different patterns of PLFA. C16 MUFA are especially
23 abundant in the type I methanotrophs while the type II methanotrophs contain mainly C18
24 MUFA (Le Bodelier et al. 2009). Therefore, the much larger ¹³C depletion of C16 MUFA
25 than C18 MUFA and the strong labelling of C16 MUFA during the incubation with ¹³C-CH₄
26 indicate that the aerobic methanotrophic community was dominated by type I methanotrophs
27 in the water column during this study. In contrast, Type II methanotrophs did not appear to
28 contribute much to the overall CH₄ oxidation in Lake Kivu, in good agreement with the
29 results of Pasche et al. (2011). Nevertheless, in February 2012 the C16 MUFA appeared to be
30 strongly depleted in ¹³C below the transition between oxic and O₂-depleted waters (Figure 3).
31 Strong ¹³C-depletion of bacterial lipid markers for aerobic methanotrophic bacteria in O₂-
32 depleted waters has also been reported in the Black Sea (Schubert et al. 2006) and in Lake

1 Lugano (Blees et al. 2014). The presence of methanotrophic bacterial biomass below the
2 oxycline could simply result from gravity-driven physical particle transport from oxic waters,
3 but it has been also demonstrated that some aerobic methanotrophs are able to persist under
4 low oxygen conditions in a reversible state of reduced metabolic activity (Roslev and King
5 1995). In contrast, the recovery of these aerobic methanotrophs after CH₄ deprivation under
6 oxic conditions is less successful because of a significant degradation of cell proteins (Roslev
7 and King 1995). Blees et al. (2014) suggested that this physiological preference for O₂
8 starvation than CH₄ starvation under oxic conditions would drive aerobic methanotrophs
9 towards the O₂-depleted part of the oxygen continuum. This concept seems particularly
10 important in tropical lakes because the thermal stratification of the water column is usually
11 very dynamic in these systems due to the small temperature gradient, allowing episodic, yet
12 frequent, O₂ intrusion events into deeper waters. Aerobic methanotrophs in dormancy would
13 recover quickly after the episodic O₂ injection, and resume rapidly micro aerobic CH₄
14 oxidation (Blees et al. 2014).

15 The dominance of type I over type II methanotrophs has been frequently reported in various
16 stratified freshwater (Sundh et al. 1995, Blees et al. 2014) or marine environments (Schubert
17 et al. 2006, Schmale et al. 2012), but this recurrent observation is still difficult to explain. In a
18 recent review, Ho et al. (2013) attempted to classify several genera of methanotrophs
19 according to their life strategies, using the competitor/stress tolerator/ruderal functional
20 classification framework (Grime 1977). Since type I methanotrophs dominate the active
21 community in many environments and are known to respond rapidly to substrate availability,
22 they classified them as competitors, or competitors-ruderals. In contrast, they proposed that
23 type II members would be more tolerant to environmental stress, and thus classified them as
24 stress tolerator, or stress tolerator-ruderal. Relatively large availability of CH₄ and O₂ (O₂:CH₄
25 ratio close to 1, Figures 2 and 6) at the bottom of the oxycline of Lake Kivu is a favourable
26 environment for the competitor-ruderal bacterial communities that could explain the
27 dominance of type I methanotrophs over type II methanotrophs in this lake.

28 A significant MBP rate (1.3 μmol L⁻¹ d⁻¹) was measured in O₂-depleted waters (< 3 μmol L⁻¹)
29 at 60 m during the rainy season (February 2012). Moreover, the PLFA labelling pattern was
30 drastically different, with a more important specific ¹³C incorporation into 10Me16:0 and C17
31 MUFA instead of the C16 MUFA, relative to their concentrations. This different labelling
32 pattern suggests that a different population of methanotrophs was active in CH₄ oxidation

1 deeper in the water column. Archaea lack ester-linked fatty acids in their membrane and are
2 therefore undetectable in PLFA analysis. However 10Me16:0 and C17 MUFA are known to
3 be especially abundant in sulphate-reducing bacteria (Macalady et al. 2000, Boschker and
4 Middelburg 2002), one of the syntrophic partner of anaerobic CH₄ oxidizing archaea (Knittel
5 and Boetius 2009). Hence, the specific labelling of 10Me16:0 and C17 MUFA in O₂-depleted
6 waters could indicate that a fraction of the upward flux of CH₄ was oxidized syntrophically
7 by an archaea/bacteria consortium, and might support the hypothesis that the bacterial partner
8 grow on CH₄-derived carbon source supplied by anaerobic methane oxidizers within the
9 consortium, as already suggested by the results of an in vitro labelling (¹³CH₄) study
10 (Blumenberg et al. 2005). However, our data does not necessarily imply that anaerobic
11 methane oxidation would be coupled with SO₄²⁻ reduction, as some sulphate-reducing bacteria
12 have been also found to be able to reduce iron (Coleman et al. 1993). Furthermore, the
13 phylogenetic resolution of SIP-PLFA analyses is rather low (Uhlík et al. 2009), and recent
14 studies showed that anaerobic methane oxidation could be carried out syntrophically by
15 consortium between methanotrophic archaea and denitrifying bacteria (Raghoebarsing et al.
16 2006), or between methanotrophic archaea and manganese reducing bacteria (Beal et al.
17 2009). Further investigations would be needed to address more accurately which is the
18 electron acceptors coupled to anaerobic CH₄ oxidation.

19

20 **5. Conclusions**

21 We provide conclusive evidences on the occurrence of CH₄ oxidation in the oxycline of Lake
22 Kivu using stable isotopic characterisation of a suite of carbon pools (CH₄, POC, PLFA) as
23 well as rate measurements (MBP). Vertically integrated MBP ranged between 8 and 29 mmol
24 m⁻² d⁻¹, and was higher than previously reported in other lakes (Bastvinken et al. 2003,
25 Kankaala et al. 2013). MBP was equivalent to 16-60% of the average annual phytoplankton
26 primary production, a fraction distinctly higher than previously reported in other lakes,
27 usually < 10% (Bastvinken et al. 2003, Kankaala et al. 2006). Hence, methanotrophic bacteria
28 could potentially sustain a significant fraction of the pelagic food-web in this oligotrophic
29 CH₄-rich lake. Lake Kivu ranks globally among the lakes with the lowest CH₄ emissions to
30 the atmosphere (Borges et al. 2011), despite the huge amount of CH₄ dissolved in its deep
31 waters and a relatively high upward flux of CH₄ to the mixed layer (9.38 mmol m⁻² d⁻¹,
32 Pasche et al. 2009). This apparent paradox is linked to its strong meromictic nature that on

1 one hand promotes a long residence time of deep waters and the accumulation of CH₄, and on
2 the other hand leads to very slow upward diffusion of solutes, promoting the removal of CH₄
3 by microbial oxidation as it diffuses to the surface.

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20 **7. References**

21 Amaral, J. A., and Knowles, R. (1995). Growth of methanotrophs in methane and
22 oxygen counter gradients. *FEMS Microbiology Letters*, 126: 215-220.

23 Bastviken D, J Ejlertsson, I Sundh and L Tranvik (2003) Methane as a Source of
24 Carbon and Energy for Lake Pelagic Food Webs, *Ecology*, 84: 969-981

25 Bastviken, D, Tranvik, L.J, Downing J.A., Crill P.M., and Enrich-Prast, A. (2011).
26 Freshwater methane emissions offset the continental carbon sink. *Science* 331: 50-50

27 Beal, E. J., House, C. H., & Orphan, V. J. (2009). Manganese- and iron-dependent
28 marine methane oxidation. *Science*, 325:184-187

29 Blees, J., Niemann, H., Wenk, C. B., Zopfi, J., Schubert, C. J., Kirf, M. K., Veronesi,
30 M. L., Hitz, C., and Lehmann, M. F. (2014). Micro-aerobic bacterial methane oxidation in the

1 chemocline and anoxic water column of deep south-Alpine Lake Lugano (Switzerland).
2 *Limnology and Oceanography* 59: 311-324.

3 Blumenberg, M., Seifert, R., Nauhaus, K., Pape, T., and Michaelis, W. (2005). In vitro
4 study of lipid biosynthesis in an anaerobically methane-oxidizing microbial mat. *Applied and*
5 *Environmental Microbiology*, 71: 4345-4351.

6 Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A.,
7 Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O. (2000). A marine microbial
8 consortium apparently mediating anaerobic oxidation of methane. *Nature*, 407: 623-626.

9 Borges, A. V., Abril, G., Delille, B., Descy, J. P., and Darchambeau, F. (2011).
10 Diffusive methane emissions to the atmosphere from Lake Kivu (Eastern Africa). *Journal of*
11 *Geophysical Research*, 116. G03032, doi:10.1029/2011JG001673.

12 Boschker H. T. S., and Middelburg J. J. (2002). Stable isotopes and biomarkers in
13 microbial ecology. *FEMS Microbiology Ecology*, 40:85-95

14 Boschker, H. T. S. (2004). Linking microbial community structure and functioning:
15 stable isotope (^{13}C) labeling in combination with PLFA analysis. In: *Molecular Microbial*
16 *Ecology Manual II*. Kowalchuk, G. A., de Bruijn, F. J., Head, I. M., Akkermans, A. D., and
17 van Elsas, J. D. (eds.). Kluwer Academic Publishers, The Netherlands. 1673–1688, 2004.

18 Coleman, D. D., Risatti, J.B., and Schoell, M. (1981). Fractionation of carbon and
19 hydrogen by methane-oxidizing bacteria. *Geochimica Cosmochimica Acta* 45:1033–1037.

20 Coleman, M. L., Hedrick, D. B., Lovley, D. R., White, D. C., and Pye, K. (1993).
21 Reduction of Fe(III) in sediments by sulphate-reducing bacteria. *Nature*, 361:436-438

22 Damas, H. (1937) La stratification thermique et chimique des lacs Kivu, Edouard et
23 Ndalaga (Congo Belge) *Verhandlungen der Internationalen Vereinigung für Theoretische und*
24 *Angewandte Limnologie*, Schweizerbart science publishers, Stuttgart. 8, 51-68

25 Darchambeau, F., Sarmiento, H., and Descy, J. P. (2014). Primary production in a
26 tropical large lake: The role of phytoplankton composition. *Science of The Total*
27 *Environment*, 473: 178-188.

28 Darchambeau, F., Sarmiento, H., and Descy, J. P. (2014). Primary production in a
29 tropical large lake: The role of phytoplankton composition. *Science of the Total Environment*,
30 473: 178-188.

1 Degens, E. T., vos Herzes, R. P., Wosg, H-K., Deuser, W. G., and Jannasch, H. W.
2 (1973), Lake Kivu: Structure, chemistry and biology of an East African rift lake, *Geol.*
3 *Rundsch.*, 62, 245-277.

4 Denman, K.L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P.M., Dickinson, R.E.,
5 Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., da
6 Silva Dias, P. L., Wofsy, S. C., and Zhang, X. (2007). Couplings between changes in the
7 climate system and biogeochemistry. p. 499-587, in: *Climate Change 2007: The Physical*
8 *Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the*
9 *Intergovernmental Panel on Climate Change.* Solomon, S., D. Qin, M. Manning, Z. Chen, M.
10 Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.). Cambridge University Press,
11 Cambridge, United Kingdom and New York, NY, USA.

12 Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W.,
13 Haywood, J., Lean, J., Lowe, D. C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M.,
14 and Van Dorland, R. (2007). Changes in atmospheric constituents and in radiative forcing. p.
15 499-587, 129-234, in: *Climate Change 2007: The Physical Science Basis. Contribution of*
16 *Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate*
17 *Change.* Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and
18 H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York,
19 NY, USA.

20 Grime, J.P. (1977). Evidence for the existence of three primary strategies in plants and
21 its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.

22 Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Otsuki, A., and
23 Ichimura, S. (1983). Measurement of photosynthetic production of a marine phytoplankton
24 population using a stable ¹³C isotope. *Marine Biology*, 73: 31-36.

25 Hanson, R. S., and Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological*
26 *reviews*, 60: 439-471.

27 Ho, A., Kerckhof, F. M., Luke, C., Reim, A., Krause, S., Boon, N., and Le Bodelier,
28 P. L. (2013). Conceptualizing functional traits and ecological characteristics of
29 methane-oxidizing bacteria as life strategies. *Environmental microbiology reports*, 5: 335-
30 345.

1 Isumbisho, M., Sarmiento, H., Kaningini, B., Micha, J. C., and Descy, J-P. (2006).
2 Zooplankton of Lake Kivu, East Africa, half a century after the Tanganyika sardine
3 introduction. *Journal of Plankton Research*, 28: 971-989.

4 Jannasch, H. W. (1975). Methane oxidation in Lake Kivu (central Africa), *Limnology
5 and Oceanography*, 20: 860–864

6 Jones, R. I., and Grey, J. (2011). Biogenic methane in freshwater food webs.
7 *Freshwater Biology*, 56: 213–229.

8 Kalyuzhnaya, M. G., Yang, S., Rozova, O. N., Smalley, N. E., Clubb, J., Lamb, A.,
9 Nagana, G. A., Gowda, D., Raftery, D., Fu, Y., Bringel, F., Vuilleumier, S., Beck, D. A. C.,
10 Trosenko, Y. A., Khmelenina, V. N., and Lidstrom, M. E. (2013). Highly efficient methane
11 biocatalysis revealed in a methanotrophic bacterium. *Nature communications*, 4, 2785. doi:
12 10.1038/ncomms3785

13 Kankaala, P., Bellido, J. L., Ojala, A., Tulonen, T., and Jones, R. I. (2013). Variable
14 production by different pelagic energy mobilizers in boreal lakes. *Ecosystems*, 16: 1152-1164.

15 King, G. M. (1992). Ecological aspects of methane oxidation, a key determinant of
16 global methane dynamics. Pages 431- 468 in K. C. Marshall, editor. *Advances in microbial
17 ecology*. Plenum Press, New York, New York, USA.

18 Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J.,
19 Bergamaschi, P., Bergmann, D., Blake, D. R., Bruhwiler, L., Cameron-Smith, P., Castaldi, S.,
20 Chevallier, F. Feng, L., Fraser, A., Heimann, M., Hodson, E. L., Houweling, S., Josse, B.,
21 Fraser, P. J., Krummel, P. B., Lamarque, J-F., Langenfelds, R. L., Le Quéré, C., Naik, V.,
22 O'Doherty, S., Palmer, P. I., Pison, I., Plummer, D., Poulter, B., Prinn, R. G., Rigby, M.,
23 Ringeval, B., Santini, M., Schmidt, M., Shindell, D. T., Simpson, I. J., Spahni, R., Steele, L.
24 P., Strode, S. A., Sudo, K., Szopa, S., van der Werf, G., Voulgarakis, A., van Weele, M.,
25 Weiss, R. F., Williams J. E., and Zeng G. (2013) Three decades of global methane sources
26 and sinks, *Nature Geoscience*, 6:813-823. doi: 10.1038/NGEO1955

27 Knittel, K., and Boetius, A. (2009). Anaerobic oxidation of methane : progress with an
28 unknown process. *Annual Review of Microbiology*, 63: 311-334

29 Le Bodelier, P. L., Gillisen, M. J. B., Hordijk, K., Damsté, J. S. S., Rijpstra, W. I. C.,
30 Genevasen, J. A., and Dunfield, P. F. (2009). A reanalysis of phospholipid fatty acids as
31 ecological biomarkers for methanotrophic bacteria. *The ISME journal*, 3: 606-617.

1 Lehmann, M. F., Bernasconi, S. M., McKenzie, J. A., Barbieri, A., Simona, M., and
2 Veronesi, M. (2004). Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved
3 carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic
4 lake. *Limnology and Oceanography* 49: 415-429.

5 Macalady, J. L., Mack, E. E., Nelson, D. C., and Scow, K. M. (2000). Sediment
6 microbial community structure and mercury methylation in mercury-polluted Clear Lake,
7 California. *Applied and Environmental Microbiology*, 66:1479-1488.

8 Pasche, N., Dinkel, C., Müller, B., Schmid, M., Wüest, A., and Wehrli, B. (2009).
9 Physical and bio-geochemical limits to internal nutrient loading of meromictic Lake Kivu.
10 *Limnology and Oceanography*, 54:1863-1873.

11 Pasche, N., Schmid, M., Vazquez, F., Schubert, C. J., Wüest, A., Kessler, J. D., Pack,
12 M. A., Reeburgh, W. S., and Bürgmann, H. (2011). Methane sources and sinks in Lake Kivu.
13 *Journal of Geophysical Research*, 116. G03006. doi:10.1029/2011JG001690

14 Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig,
15 K. F., Rijpstra, W. I. C., Schouten, S., Sinninghe Damsté, J. S., Op den Camp, H. J. M., Jetten
16 M. S. M., & Strous, M. (2006). A microbial consortium couples anaerobic methane oxidation
17 to denitrification. *Nature*, 440: 918-921

18 Roslev, P., and King, G. M. (1995). Aerobic and anaerobic starvation metabolism in
19 methanotrophic bacteria. *Applied and Environmental Microbiology*, 61:1563-1570

20 Rudd, J. W., Hamilton, R. D., and Campbell, N. E. R. (1974). Measurement of
21 microbial oxidation of methane in lake water. *Limnology and Oceanography*, 19: 519-524.

22 Sanseverino AM, Bastviken D, Sundh I, Pickova J, and Enrich-Prast A (2012).
23 Methane Carbon Supports Aquatic Food Webs to the Fish Level. *PLoS ONE* 7(8): e42723.
24 doi:10.1371/journal.pone.0042723

25 Sarmiento, H., Isumbisho, M., and Descy, J-P. (2006). Phytoplankton ecology of Lake
26 Kivu (Eastern Africa), *Journal of Plankton Research*, 28: 815-829.

27 Schmale, O., Blumenberg, M., Kießlich, K., Jakobs, G., Berndmeyer, C., Labrenz, M.,
28 and Rehder, G. (2012). Aerobic methanotrophy within the pelagic redox-zone of the Gotland
29 Deep (central Baltic Sea). *Biogeosciences*, 9: 4969-4977

1 Schmid, M., Halbwachs, M., Wehrli, B., and Wüest, A. (2005). Weak mixing in Lake
2 Kivu: new insights indicate increasing risk of uncontrolled gas eruption. *Geochemistry,*
3 *Geophysics, Geosystems*, 6. Q07009. doi:10.1029/2004GC000892

4 Schoell, M., Tietze, K., and Schoberth, S. M. (1988). Origin of methane in Lake Kivu
5 (east-central Africa). *Chemical geology*, 71: 257-265.

6 Schubert, C. J., Coolen, M. J., Neretin, L. N., Schippers, A., Abbas, B.,
7 Durisch-Kaiser, and Kuypers, M. M. (2006). Aerobic and anaerobic methanotrophs in the
8 Black Sea water column. *Environmental Microbiology*, 8: 1844-1856.

9 Sundh, I., Bastviken, D., and Tranvik, L. J. (2005). Abundance, activity, and
10 community structure of pelagic methane-oxidizing bacteria in temperate lakes. *Applied and*
11 *environmental microbiology*, 71: 6746-6752.

12 Thiery, W., Martynov, A., Darchambeau, F., Descy, J., Plisnier, P., Sushama, L., and
13 Van Lipzig, N. (2014). Understanding the performance of the FLake model over two African
14 Great Lakes. *Geoscientific Model Development*, 7: 317-337.

15 Uhlík, O., Jecná, K., Leigh, M. B., Macková, M., & Macek, T. (2009). DNA-based
16 stable isotope probing: a link between community structure and function. *Science of the Total*
17 *Environment*, 407: 3611-3619.

18 Weiss, R. F. (1981). Determinations of carbon dioxide and methane by dual catalyst
19 flame ionization chromatography and nitrous oxide by electron capture chromatography.
20 *Journal of Chromatographic Sciences*, 19: 611-616.

21 Whiticar, M. J., Faber, E., and Schoell, M. (1986) Biogenic Methane formation in
22 marine and freshwater environments: CO₂ Reduction vs acetate fermentation—isotope
23 evidence. *Geochimica Cosmochimica Acta*, 50: 693-709.

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1 8. Figure captions

2 Figure 1. Map of Lake Kivu.

3 Figure 2. Vertical profiles of dissolved O₂ concentration ($\mu\text{mol L}^{-1}$), CH₄ concentration (mmol
4 L⁻¹), $\delta^{13}\text{C-CH}_4$ (‰) and $\delta^{13}\text{C-POC}$ (‰) in Lake Kivu, in September 2012 (dry season) in the
5 Southern Basin (a) and Northern Basin (b), and in February 2012 (rainy season) in the
6 Northern Basin (c). Information about the precision of measurement can be found in the
7 material and methods section. Vertical profiles of methanotrophic bacterial production rates
8 (MBP, $\mu\text{mol L}^{-1} \text{d}^{-1}$) in September 2012 in the Southern Basin (d) and Northern Basin (e) and
9 in February 2012 in the Northern Basin (f). Symbols in (d), (e), and (f) represent mean values,
10 vertical bars represent standard deviation of replicates. The grey zone corresponds to waters
11 with dissolved O₂ concentration $< 3 \mu\text{mol L}^{-1}$.

12 Figure 3. Vertical profiles of the relative abundance of phospholipid fatty acids (PLFA, %) and their respective carbon isotopic signature ($\delta^{13}\text{C-PLFA}$, ‰) in (a, b) the Southern Basin in
13 September 2012 (dry season) and (c, d) in the Northern Basin in February 2012. The grey
14 zone corresponds to waters with dissolved O₂ concentration $< 3 \mu\text{mol L}^{-1}$.
15

16 Figure 4. Example (62.5 m) of relationship between the $\delta^{13}\text{C-CH}_4$ and the fraction of CH₄
17 remaining in the bottles during the incubation (%) to determine the isotope fractionation
18 factor carried out in September 2012 in the Southern Basin. Data points were gathered at a 24
19 h interval.

20 Figure 5. Specific CH₄-derived C incorporation pattern into phospholipid fatty acids (PLFA)
21 (incorporation rates of C into PLFA normalized on PLFA concentration, d⁻¹) in (a) September
22 2012 (dry season) in the Southern Basin and (b) in February 2012 (rainy season) in the
23 Northern Basin. Dissolved O₂ concentration was lower than $3 \mu\text{mol L}^{-1}$ at 67.5 m and 70 m
24 (a), and 50 m and 60 m (b).

25 Figure 6. In Lake Kivu, relationship between the methanotrophic bacterial production rates
26 (MBP, $\mu\text{mol C L}^{-1} \text{d}^{-1}$) and the *in situ* CH₄: O₂ molar ratio. The ratio was calculated with an
27 O₂ concentration value of $3 \mu\text{mol L}^{-1}$ when observed *in situ* values were below the detection
28 limit of the sensor ($3 \mu\text{mol L}^{-1}$).

29 Figure 7. In Lake Kivu, relationship between the methanotrophic bacterial growth efficiency
30 and the the *in situ* CH₄: O₂ molar ratio. The ratio was calculated with an O₂ concentration

1 value of $3 \mu\text{mol L}^{-1}$ when observed in situ values were below the detection limit of the sensor
2 ($3 \mu\text{mol L}^{-1}$).

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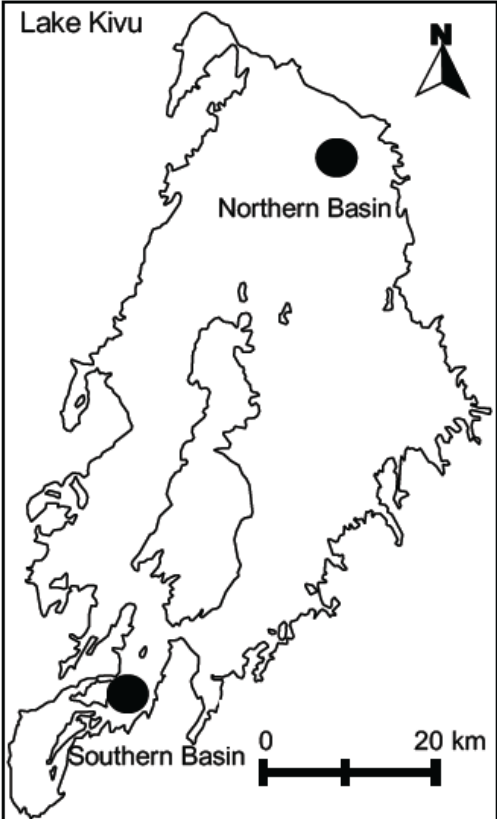
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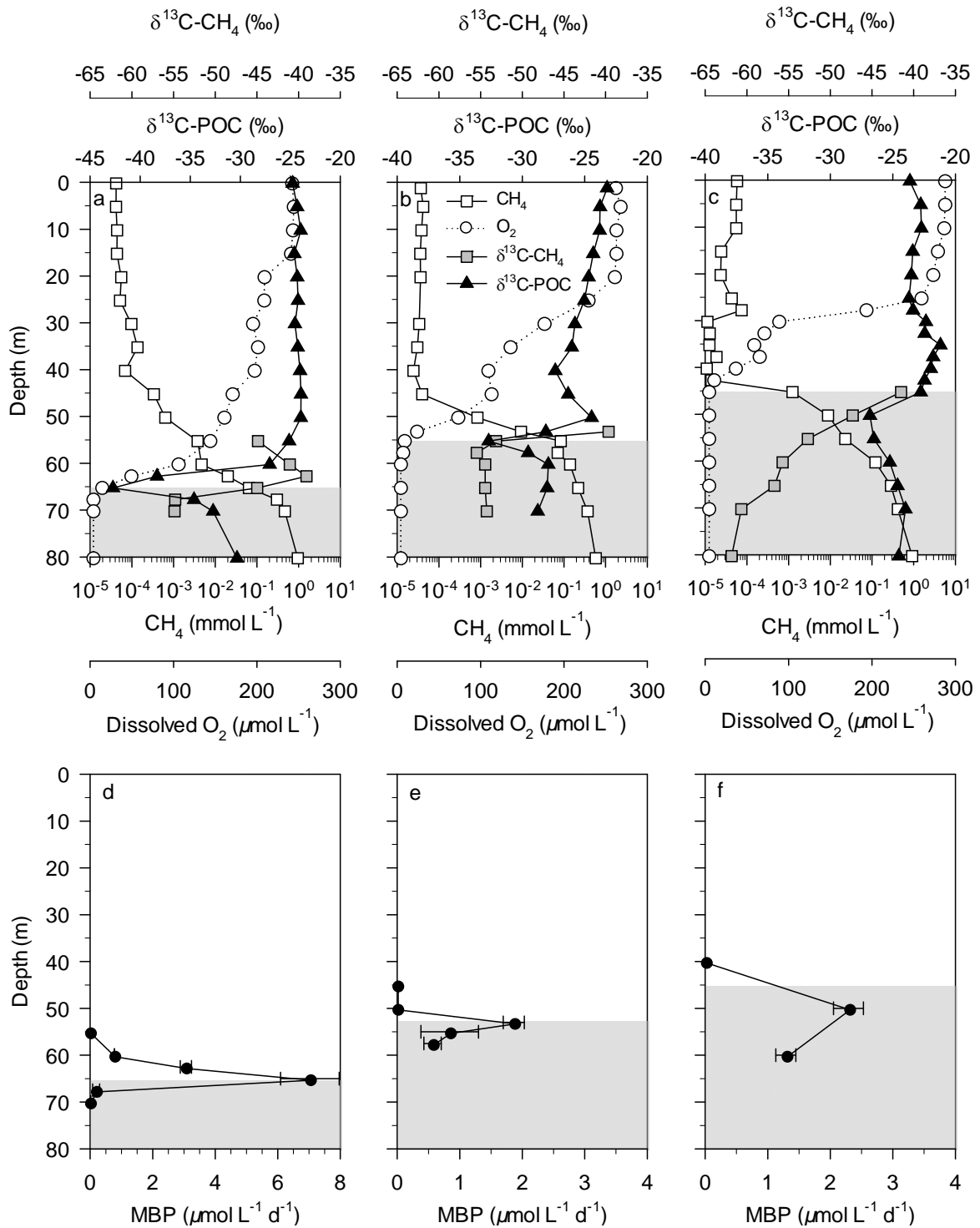
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1 Figure 1.



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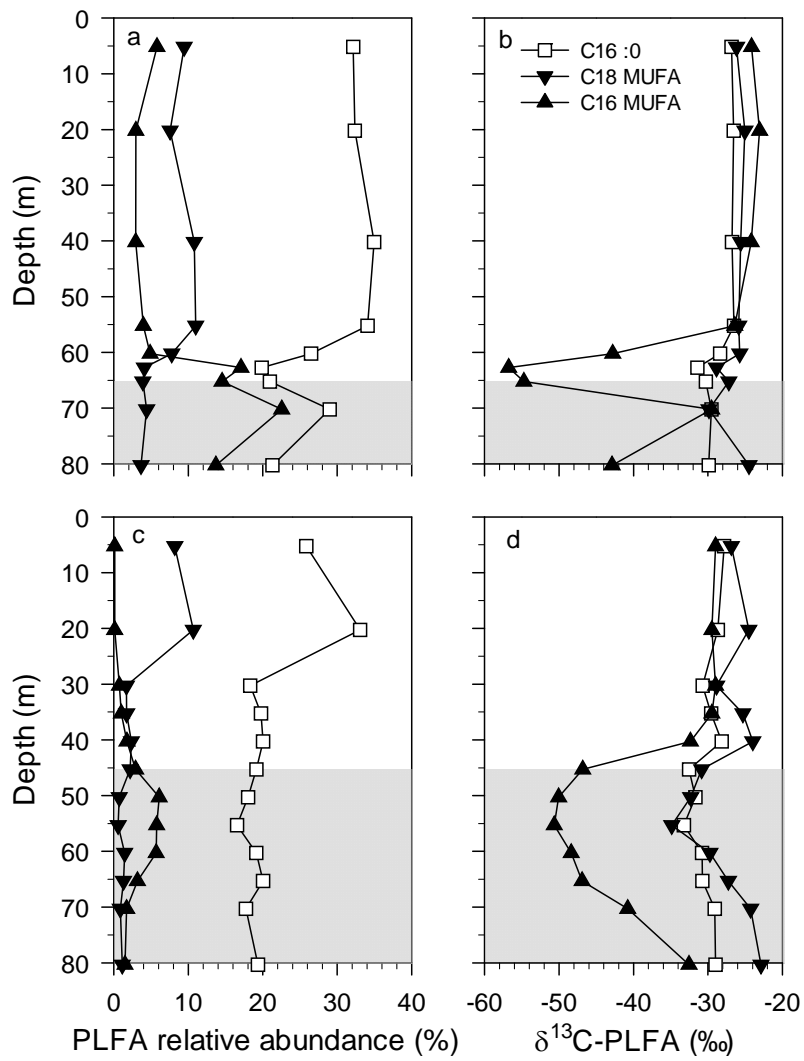
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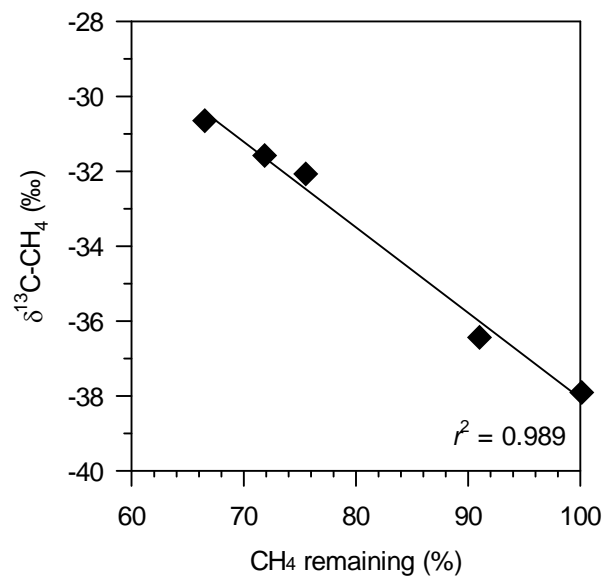
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1 Figure 4.



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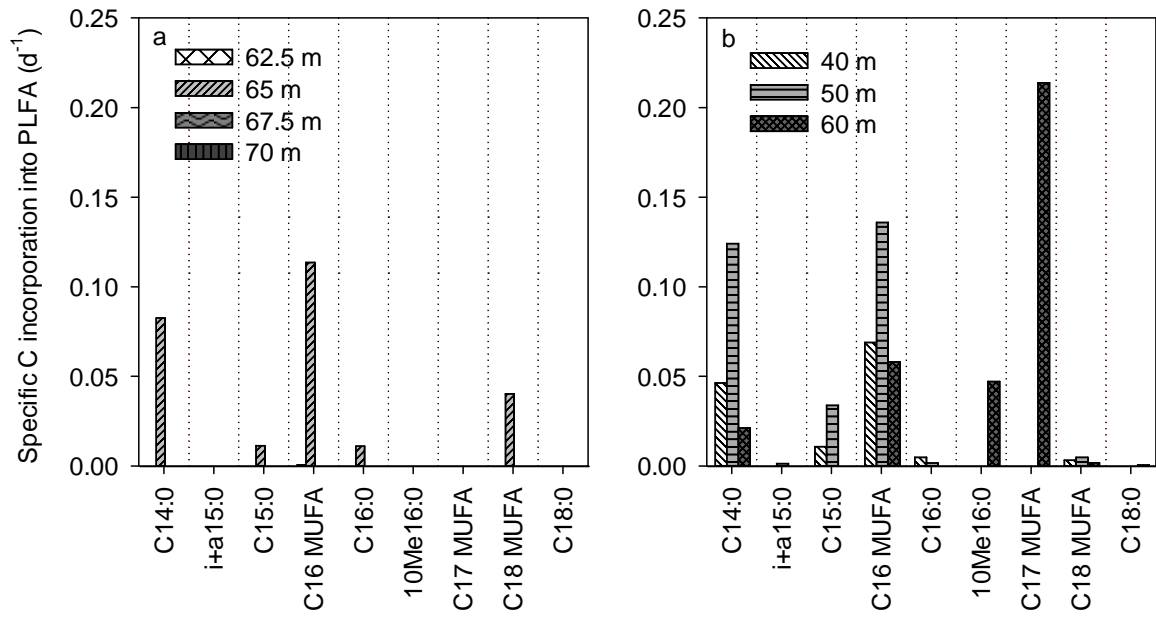
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1 Figure 5.



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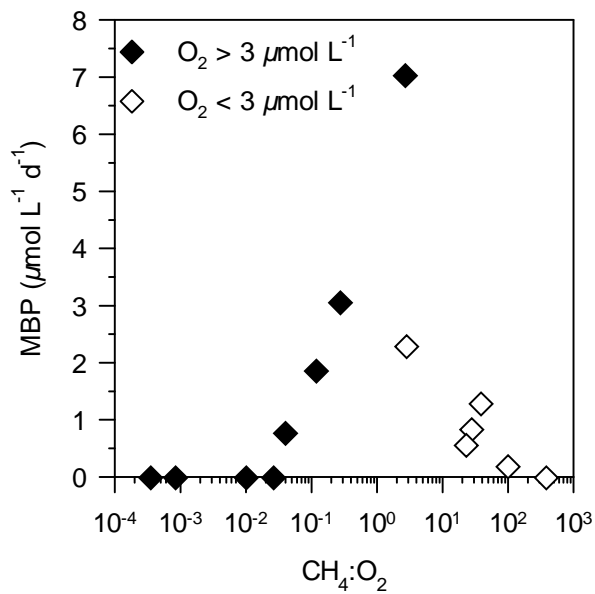
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1 Figure 6.



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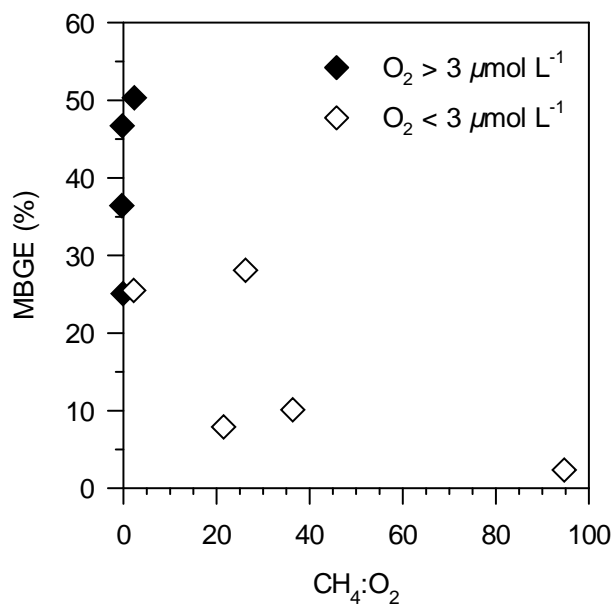
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1 Figure 7.



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