Organic Geochemistry 66 (2014) 174–181

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Tracing the methane cycle with lipid biomarkers in Lake Rotsee (Switzerland)

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ARTICLE INFO

Article history:
Received 26 May 2013
Received in revised form 17 October 2013
Accepted 1 November 2013
Available online 11 November 2013

ABSTRACT

We analysed the distributions of glycerol dialkyl glycerol tetraethers (GDGTs) and other lipid biomarkers [glycerol dialkyl diethers (DGDs), fatty acids (FAs), sterols, hopanoids and phytol] in the water column and sediments of Lake Rotsee (Switzerland) to understand the processes and organisms involved in CH_4 cycling. In the sediment we found substantial amounts of GDGT-0. This originates mainly from acetoclastic methanogens, as indicated by microbial data, high GDGT-0/crenarchaeol ratio and $\delta^{13}C$ signature of the isoprenoid alkyl chains (ca. −35‰ to −30‰). The more depleted $\delta^{13}C$ values of archaeol (as low as −62‰) can be attributed to hydrogenotrophic methanogens, with a potential contribution from methanotrophic archaea (anaerobic CH_4 oxidising archaea). An increase in GDGT-0 in sediment layers deposited in the early 1920s [driven by an increase in organic matter supply to the sediment] indicates a maximum in methanogenic biomass and thus a potential peak in CH_4 production, which fits with the eutrophication history of the lake. Excess methanogenesis most probably led to CH_4 liberation to the water column and subsequent aerobic CH_4 oxidation (MOx) as indicated by a higher concentration of diploptene and 17β,21β-homohopanoic acid, with $\delta^{13}C$ values as low as −60‰ and −64‰, respectively. Variation in these markers in the sediment indicated changes in the abundance of aerobic CH_4 oxidising bacteria (MOB), which thrive at the oxic/anoxic interface in the water column. In the water column, the presence of the $C_{16:1\omega8}$ FA indicated that the MOx community was dominated by Type I MOB. Incorporation of CH_4-derived carbon into microbial biomass was also indicated by ^{13}C-depleted diagnostic FAs with $\delta^{13}C$ values as low as −53‰ (10-methyl-$C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{18:1\omega7}$, $C_{18:1\omega5}$). In addition, CH_4-derived carbon could also be traced into the biomass of photosynthetic organisms. In the water column, the sterols and phytol, originating from photosynthetic organisms, were ^{13}C-depleted. This indicates significant CH_4 turnover in the water column, leading to a ^{12}C enrichment in the dissolved organic carbon (DIC)/CO_2 pool, which was subsequently fixed by primary producers.

1. Introduction

CH_4 is the second most important greenhouse gas after CO_2 and has a 25× higher global warming potential over a 100 yr period (IPCC, 2007). Lakes are typically a natural source of CH_4, emitting between 8 and 48 Tg/yr (total freshwater emissions at least about 103 Tg/yr), which represents up to 16% of all natural CH_4 emissions to the atmosphere (Bastviken et al., 2004, 2011). Consequently, lakes have a potentially significant impact for climate.

In order to reconstruct past changes in CH_4 cycling in aquatic systems, sedimentary indicators can be used: CH_4 oxidation can be traced via depleted isotopic signatures of bulk organic carbon (Rudd et al., 1976; Rudd and Taylor, 1980; Hollander and Smith, 2001), authigenic carbonate (Peckmann and Thiel, 2004) and lipid biomarkers (Hinrichs et al., 2000, 2003; Niemann and Elvert, 2008). As the activity of bacteria and archaea involved in CH_4 oxidation lead to a significant depletion in ^{13}C relative to the substrate, carbon isotopic excursions of related lipid biomarkers in the sediment are indicators of past CH_4 oxidation (Hinrichs et al., 2000, 2003).

Potential lipid indicators are, for instance, glycerol dialkyl glycerol tetraethers (GDGTs; structures of these and other lipids in Fig. A.1), membrane constituents of many archaea and some bacteria (De Rosa and Gambacorta, 1988; Sinninghe Damsté et al., 2011; Schouten et al., 2013). The archaeal isoprenoid GDGTs (isoGDGTs)

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http://dx.doi.org/10.1016/j.orggeochem.2013.11.002

are produced by Thaumarchaeota (formerly Crenarchaeota) and Euryarchaeota (De Rosa and Gambacorta, 1988; Schouten et al., 2002) and include planktonic (DeLong, 1992; Wuchter et al., 2005; Blaga et al., 2009), as well as methanogenic and methanotrophic species (Garcia, 1990; Hinrichs et al., 2000; Blumenberg et al., 2004; Sundh et al., 2005). The isoGDGTs produced by methanogenic and methanotrophic archaea can thus serve as indicators for CH_4 cycling in aquatic environments (Blumenberg et al., 2004; Schouten et al., 2013).

Other archaeal indicators of CH_4 cycling in aquatic systems are DGDs such as archaeol and hydroxyarchaeol. Archaeol is the most common and ubiquitous archaeal diether and has been found in halophiles, thermophiles, methanogens and methanotrophs (Koga et al., 1998; Hinrichs et al., 2000; Pancost et al., 2001; Niemann and Elvert, 2008). In contrast, hydroxyarchaeol has been observed in the halophile genus *Natronobacterium* (Upasani et al., 1994), some thermoacidophiles (Sprott et al., 1997) and methanogenic archaea (*Methanococcales*, *Methanosarcinales*, *Methanosphaera*, *Methanobrevibacter smithii*, as well as some *Methanobacteriaceae*; Koga et al., 1998; Sprott et al., 1999; Hinrichs et al., 2000; Pancost et al., 2001). Crocetane and pentamethylicosanes are usually depleted in ^{13}C and have been considered to be derived from anaerobic methanotrophs (e.g. reviewed by Peckmann and Thiel, 2004).

Bacterial lipids can also be indicative of CH_4 cycling in lakes: For instance, the $C_{16:1\omega8}$ fatty acid (FA) and $C_{18:1\omega8}$ FA are specific markers for Type I and Type II CH_4 oxidising bacteria (MOB), respectively (Bowman et al., 1991; Hanson and Hanson, 1996; Elvert and Niemann, 2008). The carbon isotopic depletion of other FAs and hopanoids is also indicative of MOB (e.g. reviewed by Peckmann and Thiel, 2004).

One example of a eutrophic, CH_4-rich lake is the Swiss Lake Rotsee, in which a CH_4 concentration of up to 1 mM CH_4 has been detected in the hypolimnion, and up to 7 mg CH_4 sq./m/day are emitted from the lake (Schubert et al., 2010). In this study, we have traced the CH_4 cycle following a biomarker approach, and have reconstructed past changes in methanogenesis and CH_4 oxidation in the lake. To this aim, we analysed GDGTs and other lipid biomarkers (DGDs, FAs, sterols, hopanoids and phytol) in particulate organic matter (POM) in the water column and in a sediment core covering the period between ca. 1860 and the present. Since the mid-19th century, the lake has experienced eutrophication due to excessive sewage and related nutrient supply (Stadelmann, 1980). The eutrophication history has been determined by Naeher et al. (2012), and can be used to assess its influence on the CH_4 cycle. Our results indicate that GDGT concentration and carbon isotopic signatures, as well as $\delta^{13}C$ values of FAs, sterols and phytol, can be used as indicators of past and present CH_4 cycling in lakes.

2. Material and methods

2.1. Study site and sample collection

Lake Rotsee is a small (0.46 km^2), prealpine, monomictic and eutrophic lake in Switzerland (Fig. 1; hydrographical and limnological parameters summarised in Naeher et al., 2012). It has a stable stratified water column with a strong chemocline between ca. 6 and 10 m and an anoxic hypolimnion for most of the year (Schubert et al., 2010). The sedimentation rate is ca. 0.38 cm/yr (Naeher et al., 2012).

At the maximum depth of 16 m, a 56 cm core was recovered with a gravity corer in October 2009 (N47°4.251 E8°18.955, WGS84; Fig. 1). Shortly after recovery, the core was sliced in continuous 1 cm intervals and frozen at −20 °C until analysis.

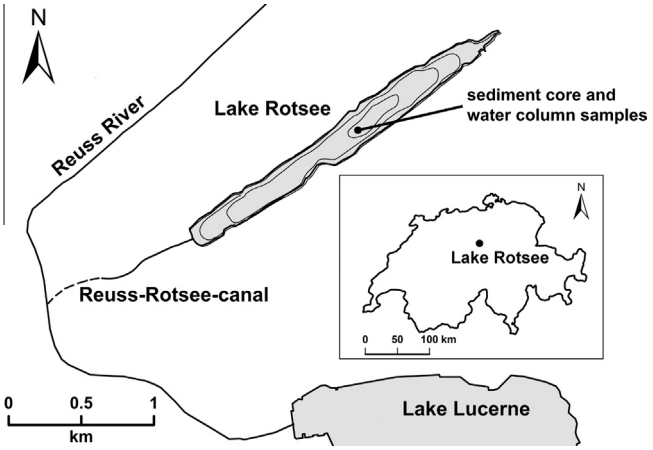

Fig. 1. Map with Lake Rotsee, the Reuss River and its connection with the lake by the Reuss-Rotsee-canal (partly below ground level, dashed line) and the northwest corner of Lake Lucerne. The sampling station of core and water column particulate organic matter (POM) is at the maximum lake depth (16 m).

We sampled water column POM at the core location (Fig. 1) via in situ filtration (2–13 l; McLane WTS-142 filtration system; particle retention down to 0.7 μm with GFF filters) at 9, 10, 11 and 13 m in October 2004 and at 8, 9, 10, 11, 12 and 14 m in November 2007. A sample from 4 m depth was obtained in October 2012. During these sampling campaigns, the chemocline was at 8–10 m water depth. The filters were freeze dried prior to analysis. The samples from 2004, which had been used for microbial investigations (Schubert et al., 2010), were also used for GDGT analysis. Concentrations and $\delta^{13}C$ values of FAs and sterols were determined for the samples from 2007. CTD (conductivity–temperature–depth) profiles showed that the chemocline was at ca. 8–10 m during both sampling campaigns.

2.2. Lipid biomarkers

Concentrations and carbon isotopic signatures of lipid biomarkers were determined according to the procedures and instrumentation reported by Naeher et al. (2012). In short, sediment samples were extracted successively by ultrasonication with mixtures of MeOH and dichloromethane (DCM). Half of each filter (POM samples) was Soxhlet extracted with DCM/MeOH (7:3, v/v) for 24 h. Internal standards (5α-cholestane, *n*-C_{19} FA, *n*-C_{19} alcohol) of known concentration were added to the total lipid extract (TLE) for quantification and to monitor instrumental precision. After saponification of the TLE, and separation into a neutral and a FA fraction, the neutral fraction was separated into an apolar and a polar fraction over a NH_2 column (Hinrichs et al., 2003). The polar fraction was derivatised with BSTFA [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide] for 1 h at 80 °C and the FA fraction with 14% BF_3/MeOH. FA double bond positions were determined from the dimethyl disulfide (DMDS) adducts according to Nichols et al. (1986).

GDGTs in POM and sediments were determined as described by Naeher et al. (this issue). In short, each sediment TLE in hexane/isopropanol (99:1, v/v) was filtered through 0.45 μm PTFE filter prior to analysis with high performance liquid chromatography (HPLC) as reported by Bechtel et al. (2010). The TLEs from POM were first separated over a 5% water deactivated silica column using hexane/DCM (9:1, v/v) and DCM/MeOH (1:1, v/v). GDGTs in the latter, polar fraction were analysed. An Agilent 1260 Infinity series HPLC–atmospheric chemical ionisation mass spectrometry (HPLC–APCI–MS) instrument equipped with a Grace Prevail Cyano column (150 mm × 2.1 mm; 3 μm) was used for GDGT analysis. The GDGTs were eluted isocratically with 90% A and 10% B for

5 min and then with a linear gradient to 18% B for 34 min at 0.2 ml min^{-1}, where A = hexane and B = hexane/isopropanol (9:1, v/v). We used single ion monitoring of $[M+H]^+$ to detect GDGTs.

Compound specific $\delta^{13}C$ values of core GDGTs were determined via ether cleavage with HI followed by a reduction step with $LiAlH_4$ to afford GC-amenable biphytanes (Kohnen et al., 1992; Blumenberg et al., 2004; Niemann et al., 2005). The resulting compounds were analysed as reported by Naeher et al. (2012).

The $\delta^{13}C$ values of derivatised lipids (DGDs, FAs, 17β,21β-homohopanoic acid, sterols and phytol) were corrected for the introduction of external carbon. The values were not corrected for the Suess effect as it is considered to be of minor importance in this lake, as discussed by Naeher et al. (2012). The analytical reproducibility was within 15% for lipid concentration based on duplicate measurements. The analytical precision was 1–2‰ for compound specific carbon isotope measurements.

3. Results and discussion

3.1. Sedimentary indicators of present and past CH_4 cycling

3.1.1. Present archaeal CH_4 production

A study of the sedimentary microbial community structure indicated that 98% of the archaeal community in Lake Rotsee consists of methanogenic archaea (91% acetoclastic methanogens related to *Methanosaeta* spp., and 7% hydrogenotrophic methanogens related to a methanogenic endosymbiont of *Plagiopyla nasuta*; Zepp Falz et al., 1999).

Dominant modes of methanogenesis can be inferred from the carbon isotopic signature of CH_4 (Whiticar, 1999). Acetoclastic methanogenesis is characterised by ε values between −35‰ and −9‰ (Valentine et al., 2004; Penning et al., 2006; Londry et al., 2008; Goevert and Conrad, 2009). $\delta^{13}C_{CH4}$ values of ca. −70‰ in the water column and sediment (Fig. 2; Schubert et al., 2010) of the lake, together with the microbiological data, indicate an isotopic overprint of acetoclasticity by hydrogenotrophic methanogenesis, which is characterised by a comparably strong fractionation

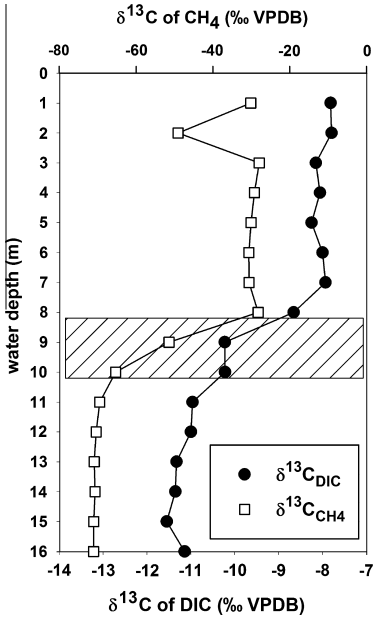

Fig. 2. $\delta^{13}C$ values (‰ VPDB) of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and CH_4 ($\delta^{13}C_{CH4}$; data from Schubert et al., 2010) in the water column from October 2004. Shaded area shows location of the chemocline (8–10 m).

of −79‰ to −28‰ (Valentine et al., 2004; Londry et al., 2008; Penger et al., 2012).

Although we have no porewater $\delta^{13}C_{DIC}$ values, the ^{13}C-depleted hypolimnetic $\delta^{13}C_{DIC}$ of ca. −12‰ (Table 2, Fig. 2) indicated that the C substrate for hydrogenotrophic methanogenesis (pore water DIC) could already be ^{13}C-depleted, which would consequently, lead to very low $\delta^{13}C$ values of hydrogenotrophic CH_4. Admixture of small amounts of hydrogenotrophic to acetoclastic CH_4 in the lake would thus result in a comparably strong overprint of the acetoclastic $\delta^{13}C_{CH4}$ values. Alternatively, a (partially) closed cycle involving the turnover of OM from methanogens and the release of ^{13}C-depleted acetate, which in return may be reused by acetoclastic methanogens, may also account for the very low $\delta^{13}C_{CH4}$ values.

GDGT-0 (Fig. 3c) is the predominant isoGDGT (or commonly the only isoGDGT) produced by methanogens, based on culture studies (e.g. reviewed by Schouten et al., 2013). Thus, methanogens are likely the predominant source of GDGT-0 in the lake (see also Naeher et al., this issue). This is supported by GDGT-0/crenarchaeol ratio values > 2 in the water column and sediment (Naeher et al., this issue; Fig. 3d), which, according to Blaga et al. (2009), is diagnostic for methanogens. In contrast to GDGT-0, substantial amounts of crenarchaeol were present in the catchment soils (Naeher et al., this issue).

The high $\delta^{13}C$ values of acyclic (−35‰ to −32‰) biphytanes derived from GDGT-0 and GDGT-1, and monocyclic biphytanes (−31‰ to −30‰) derived from GDGTs-1 to -3, in the sediment (Table 1) indicate an acetoclastic–methanogenic origin of the compounds, rather than a hydrogenotrophic–methanogenic or methanotrophic origin (Summons et al., 1994; Hinrichs et al., 2000; Blumenberg et al., 2004; Niemann et al., 2005), in good agreement with microbiological data (Zepp Falz et al., 1999). However, additional sources of GDGTs 1–3 from Thaumarchaeota in the catchment soils cannot be excluded (Naeher et al., this issue). Naeher et al. (this issue) showed that the branched GDGTs and crenarchaeol in the sediments derive partly from the surrounding soils, but in situ production of isoGDGTs also takes place. They demonstrated that the eutrophic conditions in the lake promote methanogenic activity and cause a subsequent contribution of isoGDGTs of methanogenic origin to the sediments.

DGDs such as archaeol can also derive from methanogenic archaea (Koga et al., 1993, 1998), but have frequently been observed in anaerobic methanotrophic archaea (Niemann and Elvert, 2008). However, the presence of a dominant methanotrophic archaeal community in the sediments of Lake Rotsee seems unlikely because the SO_4^{2-} concentration is very low and therefore not sufficient in this water body (Schubert et al., 2010). Schubert et al. (2010) observed that SO_4^{2-} might play a role in CH_4 oxidation only later in the year (up to 170 μM in the epilimnion), but more research would be required to answer which electron acceptor is used to oxidise CH_4 anaerobically in the lake.

In comparison with GDGT-derived biphytanes, $\delta^{13}C$ values of archaeol in the lake were much lower and ranged between −62‰ and −55‰ (Table 1, Fig. 3g), indicating a different source organism. With respect to the generally higher isotope fractionation associated to hydrogenotrophic methanogenesis, we suggest that the origin of the ^{13}C-depleted archaeol lies in hydrogenotrophic methanogens. However, admixture of archaeol from acetoclastic methanogens (which may also produce archaeol; e.g. Koga et al., 1998) and a minor contribution from methanotrophic archaea cannot be excluded (Section 3.1.4).

3.1.2. Present bacterial CH_4 oxidation

Sedimentary proxies for CH_4 oxidation by MOB in the water column are diploptene and 17β,21β-homohopanoic acid, based on their strongly negative $\delta^{13}C$ signature (Fig. 3e and f), in agreement

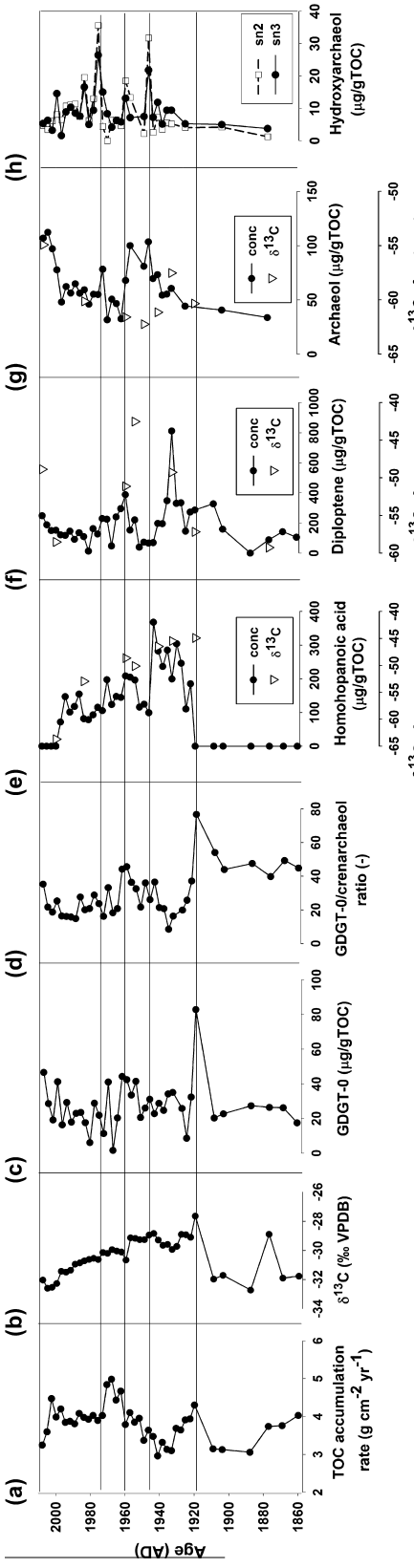

Fig. 3. (a) TOC accumulation rate (g sq. m/day) and (b) $\delta^{13}C$ values (‰ VPDB) of TOC (data from Naeher et al., 2012). Concentration (µg/g TOC) of lipid biomarkers and the GDGT-0/crenarchaeol ratio in the sediment vs. age (AD): (c) GDGT-0, (d) GDGT-0/crenarchaeol ratio, (e) 17β,21β-homohopanoic acid, (f) diploptene, (g) archaeol, (h) *sn*2- and *sn*3-hydroxyarchaeol. The $\delta^{13}C$ values (‰ VPDB) of 17β,21β-homohopanoic acid (e), diploptene (f) and archaeol (g) are also shown. These data were obtained from samples of the sediment core recovered in October 2009.

Table 1
$\delta^{13}C$ values of isoprenoid alkyl chains from ether cleavage and of archaeol in sediments (n.a., not analysed; n.d., not detected).

Depth (cm)	Age (AD)	$\delta^{13}C$ acyclic biphytane (‰)	$\delta^{13}C$ monocyclic biphytane (‰)	$\delta^{13}C$ archaeol (‰)
0–1	2006–2009	n.a.	n.a.	−54.9
9–10	1982–1985	n.a.	n.a.	−60.1
17–18	1961–1964	−34.7	n.d.	n.a.
18–19	1958–1961	−33.6	n.d.	−61.6
20–21	1953–1956	−32.9	−29.6	n.a.
22–23	1948–1950	n.a.	n.a.	−62.2
25–26	1940–1942	−31.7	−31.0	−61.1
28–29	1932–1934	−32.4	n.d.	−57.5
33–34	1918–1921	n.a.	n.a.	−60.3
Max.		−31.7	−29.6	−54.9
Min.		−34.7	−31.0	−62.2

with other studies (Huang et al., 1996; Pancost et al., 2000; Thiel et al., 2003; Birgel and Peckmann, 2008; Elvert and Niemann, 2008). The $\delta^{13}C$ values of diploptene ranged between −60‰ and −43‰ throughout the core (Fig. 3f), whereas values for 17β,21β-homohopanoic acid were slightly more depleted (−64‰ to −45‰; Fig. 3e). Schubert et al. (2010) observed that Type I MOB (closely related to *Methylomonas*) are abundant in the chemocline, while Type II MOB could not be detected, so we can presume that diploptene and 17β,21β-homohopanoic acid in the sediment are derived from Type I MOB.

In addition to a MOB source, diploptene may also originate from aerobic bacteria such as cyanobacteria, purple non-sulfur bacteria, NH_4^+ oxidisers and ferns (Bottari et al., 1972; Ageta and Arai, 1983; Rohmer et al., 1984; Ourisson et al., 1987; Prahl et al., 1992; Elvert et al., 2001; Werne et al., 2002). In contrast to diploptene, 17β,21β-homohopanoic acid was not detected in the water column, the surface sediment (0–4 cm) and lowermost sediment (below 33–34 cm) (Fig. 3e). The limitation of 17β,21β-homohopanoic acid to the sediment suggests that it is only produced within the sediment via degradation of intact hopanoids (bacteriohopanepolyols), in agreement with previous observations (Jaffe et al., 1988; Sinninghe Damsté et al., 1995; Nascimento et al., 1999; Saito and Suzuki, 2007).

3.1.3. Past changes in CH_4 cycling

Past changes in CH_4 production are indicated by the sedimentary profiles of GDGT-0 concentration and the GDGT-0/crenarchaeol ratio (Fig. 3c and d). Distinct maxima in both profiles at around 1920 and during the 1960s (Fig. 3c and d) suggest that the CH_4 production rate was high, coinciding with times of higher productivity, as indicated by a higher TOC accumulation rate (Fig. 3a; Naeher et al., 2012). The higher productivity was a result of eutrophication, which also led to enhanced stratification and photic zone euxinia (Bloesch, 1974; Lotter, 1989; Naeher et al., 2012; Naeher et al., 2013). The presumed increase in OM supply to the sediment at these times led to a greater methanogen biomass and enhanced methanogenesis in the sediment (Naeher et al., 2012).

A clear onset of increased CH_4 production can be observed in the 1920s, with the highest concentration of 17β,21β-homohopanoic acid in the 1930s. Although the decrease after 1940 could be real, it may also have been caused by in situ production of 17β,21β-homohopanoic acid in the sediment, as described above (Section 3.1.2). In contrast, the concentration of diploptene peaked at ca. 1933 (Fig. 3f). An elevated availability of CH_4 since the 1920s probably promoted aerobic methanotrophy in the oxic water column, with a peak in the early 1930s leading to an elevated abundance of MOB lipids in the sediment.

The disagreement between the concentration profiles of diploptene and 17β,21β-homohopanoic acid (Fig. 3e and f) may be related

to different communities of MOB. Alternatively, both lipids could originate from sources other than MOB. Remarkable is the gradual decrease in $\delta^{13}C$ value of 17β,21β-homohopanoic acid from ca. −45 ‰ in the 1920s and 1930s (Fig. 3e) to −65‰ around 2000. In contrast, $\delta^{13}C$ values of diploptene are much more variable, although least depleted values are also found around and after the maximum in the 1930s. Differences in $\delta^{13}C$ values can be explained in different ways. First, the balance between acetoclastic and hydrogenotrophic CH_4 may have changed through time, with the latter pathway becoming more important. Indeed, the concentration of archaeol, which appears to be primarily related to hydrogenotrophic methanogenesis (see Section 3.1.2), shows a general increase through time, although the maximum in the 1940s does not match well with the relatively high $\delta^{13}C$ values of the hopanoids at that time. Alternatively, MOB-derived diploptene and precursor hopanoids of 17β,21β-homohopanoic acid could be mixed with non-MOB counterparts. In this regard, the peak in diploptene at ca. 1933 might be partly due to a higher non-methanotrophic bacterial biomass, as indicated by a higher concentration of hopanoids at that time (Naeher et al., 2012).

3.1.4. CH_4 oxidation by anaerobic methanotrophic archaea?

Schubert et al. (2010) showed that aerobic CH_4 oxidation rate was highest in the chemocline, coinciding with a peak in the abundance of MOB in this part of the water column. Therefore, MOB are likely the predominant CH_4 oxidisers in Lake Rotsee. However, the presence of methanotrophic archaea in the water column and/or sediment, i.e. organisms oxidising CH_4 anaerobically, cannot be excluded. Since methanotrophic archaea might also be a source of archaeol, the increasing archaeol concentration in the 1930s, in combination with decreasing $\delta^{13}C$ values (Fig. 3g), suggests a potential contribution from archaeal methanotrophs. An explanation for the ca. 10 yr delay after the onset of the higher CH_4 production rate may be the low growth rate of methanotrophs with a doubling time of several months (Nauhaus et al., 2007). Naeher et al. (2012) also observed a second peak in CH_4 productivity during the 1960s. Again, with a delay of ca. 10 yr after this productivity maximum, the archaeol concentration peaked at ca. 1975 (Fig. 3g). These observations suggest that archaeol might also originate partly from methanotrophs and that archaeal CH_4 oxidation is also coupled to changes in productivity.

Based on these data, like MOB, the methanotrophic archaea were likely present in the water column, so the observations for the sediments may represent a fossil water column signal and not a signal formed in the sediment. However, it cannot be excluded that the anaerobic oxidation of CH_4 by methanotrophs also occurs in the sediment. Porewater profiles for the sediments may answer this question, but the data were not available.

The similarity between the profiles of archaeol and *sn2*- and *sn3*-hydroxyarchaeol, with peaks in the 1940s and 1970s (Fig. 3g and h), suggests a common origin from methanogens and/or methanotrophs. However, the concentration was too low to reliably measure $\delta^{13}C$ values, so we cannot attribute them to a specific source organism.

3.2. Lipids indicating current CH_4 cycling in the water column

At the times of sampling, the chemocline was at 8–10 m depth (Figs. 2 and 4). Diploptene concentration peaked at 9 m (Fig. 4a), supporting the interpretation for diploptene in the sediment (Section 3.1.2), that it is produced mainly by MOB living in the chemocline. In contrast, archaeol increased with water depth (Fig. 4a), indicating a predominant methanogenic origin in the bottom water of the lake. Diploptene and archaeol concentrations in the water column were too low to allow determination of reliable $\delta^{13}C$ values, so our interpretations of their respective sources remain tentative. However, assuming the high abundance of both compounds in the sediment as an indicator of water column processes, such interpretations about their sources seem valid.

Owing to the predominant, photoautotrophic origin of OM in the water column, organic compounds are typically characterised by $\delta^{13}C$ values $\geqslant -30‰$ (e.g. Schidlowski, 1988; Lamb et al., 2006). Notably, the values for some FAs (10-methyl-$C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{18:1\omega7}$, $C_{18:1\omega5}$) were significantly lower than −30‰, and ranged between −53‰ and −46‰ (Fig. 4c). These relatively ^{13}C depleted values indicate that these FAs originate partially from MOB thriving in or below the chemocline. This is supported by the presence of $C_{16:1\omega8}$ FA, a marker for Type I MOB (Bowman et al., 1991; Hanson and Hanson, 1996; Elvert and Niemann, 2008). The Type II MOB-specific $C_{18:1\omega8}$ FA was not detected, corroborating the findings of Schubert et al. (2010), who also found that the MOx community in the lake is dominated by Type I MOB.

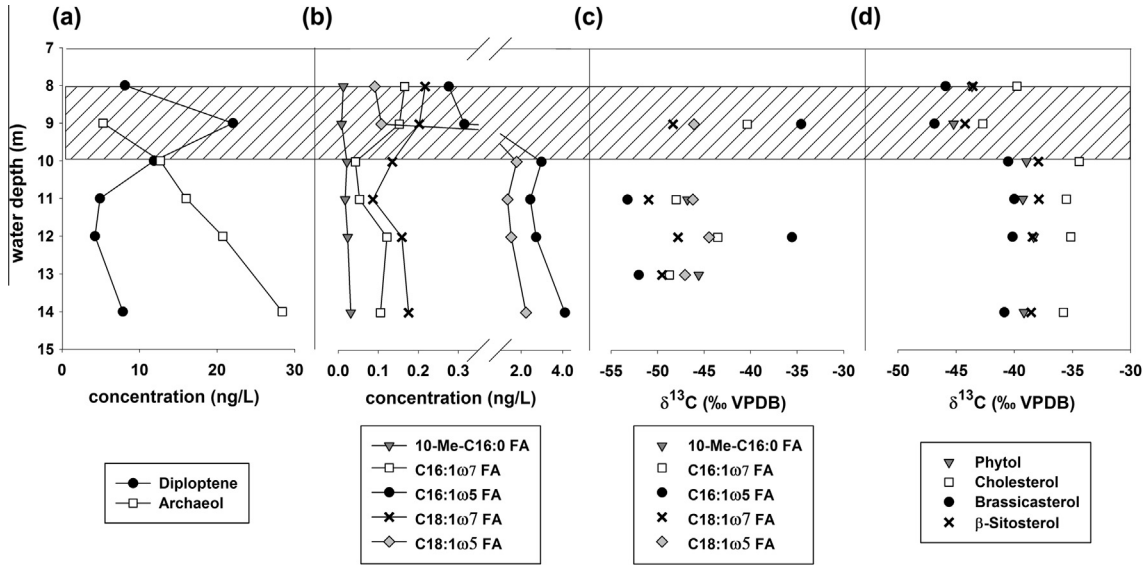

Fig. 4. Concentration (ng/l) of (a) diploptene and archaeol in and below the chemocline from October 2004. From November 2007, water column concentration and $\delta^{13}C$ values (‰ VPDB) of selected FAs (b and c), sterols and phytol (d) are shown.

Table 2
$\Delta\delta^{13}C$ values ($\Delta\delta^{13}C_{DIC-lipid}$) for water column of Lake Rotsee.

Depth (m)	$\delta^{13}C_{DIC}$ (‰ VPDB)	10Me-$C_{16:0}$	$C_{16:1\omega7}$ FA	$C_{16:1\omega5}$ FA	$C_{18:1\omega7}$ FA	$C_{18:1\omega5}$ FA	Phytol	Cholesterol	Brassicasterol	Sitosterol
8	−8.7						35	31	37	35
9	−10.2		30	24	38	36	35	32	37	34
10	−10.2						29	24	30	28
11	−11.0	36	37	42	40	36	28	25	29	27
12	−11.0		33	25	37	33	27	24	29	27
13	−11.3	34	37	41	38	36				
14	−11.4						28	24	29	27

Of these FAs, only $C_{16:1\omega7}$ was found in the sediment. In general, the $\delta^{13}C$ values of FAs in the sediment were more enriched than in the water column (all higher than −37‰), indicating that an initial MOB signal seems to be overprinted by OC from sources other than MOx. This is probably related to bacterial in situ production and an allochthonous source.

To some degree, the ^{13}C depleted FAs in the water column (Fig. 4c) may also originate from primary producers that utilise ^{13}C-CO_2, comprising a mixture from various sources, including heterotrophic respiration and MOx. This also explains the negative isotope excursion of ca. −5‰ in phytol, cholesterol, brassicasterol and β-sitosterol in the chemocline vs. the hypolimnion (Fig. 4d). These compounds are typically derived from primary producers and higher plants (Volkman, 1986, 2003; Rontani and Volkman, 2003). Naeher et al. (2012) showed that the OM in the sediments of Lake Rotsee is mainly autochthonous, so these compounds likely originate to a large degree from primary producers. However, their results also indicated that β-sitosterol is produced predominantly by emergent macrophytes, but the similar stable carbon isotopic composition to phytol, cholesterol and brassicasterol (Fig. 4d) suggests a common predominant phytoplanktonic source.

The calculated $\Delta\delta^{13}C$ values between DIC and the lipids ($\Delta\delta^{13}C_{DIC-FA}$, $\Delta\delta^{13}C_{DIC-sterol}$) ranged between 24‰ and 42‰ (Table 2), providing evidence for a contribution of CH_4-derived carbon (CH_4-C) to photosynthetic biomass. While $\Delta\delta^{13}C_{DIC-FA}$ values were generally higher below (11–13 m) than within (8–9 m) the chemocline, the opposite was observed for $\Delta\delta^{13}C_{DIC-sterol}$ (Table 2). This indicates that the ^{13}C-depleted sterols originate from phototrophs that fix ^{13}C depleted CO_2 in the chemocline. In contrast, the FAs originate from microbes that accumulate CH_4 and other depleted carbon sources at and below the chemocline, resulting in higher $\Delta\delta^{13}C_{DIC-FA}$ values for 10-methyl-$C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$ and $C_{18:1\omega5}$ FAs in the hypolimnion.

3.3. Estimating CH_4 carbon contribution to lipids

To roughly estimate the contribution of CH_4-C to the lipid biomass, we used an end member approach (e.g. Bianchi and Canuel, 2011):

$$X_{non\text{-}CH4\text{-}C} = \frac{\delta^{13}C_{lipid} - \delta^{13}C_{CH4\text{-}C}}{\delta^{13}C_{non\text{-}CH4\text{-}C} - \delta^{13}C_{CH4\text{-}C}} \times 100 \quad (1)$$

where

$X_{non\text{-}CH4\text{-}C}$ is the fraction of non-CH4 carbon sources in the lipid (%).
$\delta^{13}C_{lipid}$ the measured carbon isotopic signature of the lipid.
$\delta^{13}C_{CH4\text{-}C}$ the carbon isotopic signature of CH_4 carbon end member (i.e. OC of lipid is 100% CH4 derived).
$\delta^{13}C_{non\text{-}CH4\text{-}C}$ the carbon isotopic signature of non-CH_4 carbon end member (i.e. CH_4 contribution to lipid biomass is 0%).

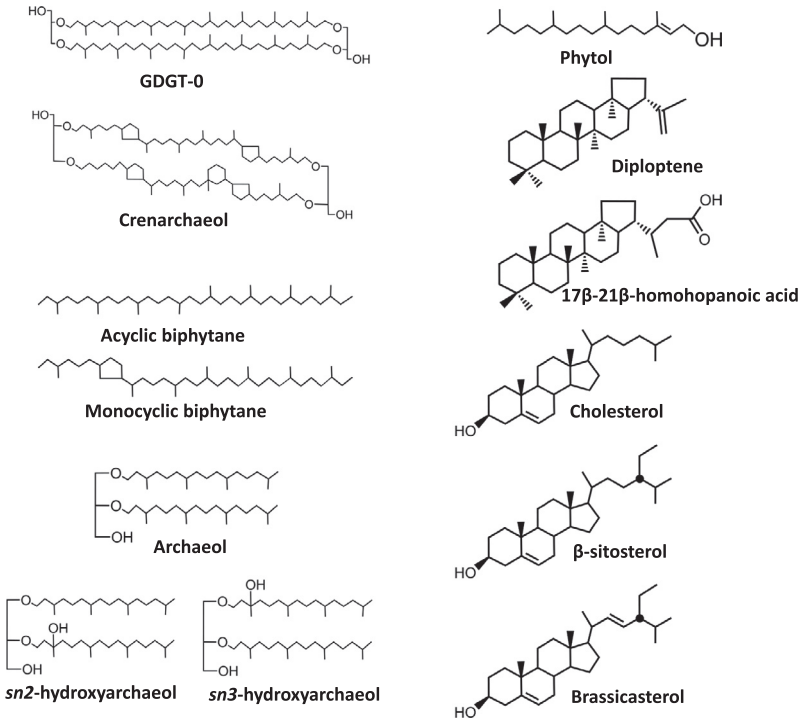

Fig. A.1. Nomenclature and structures of lipids discussed in this study.

For end member values, we assume a Calvin–Benson–Bassham cycle (CBB) derived OC $\delta^{13}C$ value of −30‰ (e.g. Schidlowski, 1988; Lamb et al., 2006), a CH_4 source in the sediment of, at present, −70‰ (Schubert et al., 2010), and a large range of ε-values for MOB between 3‰ and 39‰ (Jahnke et al., 1999; Templeton et al., 2006). According to Eq. (1), the lipid $\delta^{13}C$ values of 17β,21β-homohopanoic acid (−64‰ to −45‰) and diploptene (−60‰ to −43‰) in the sediment indicate that ca. 20–80% and 10–70%, respectively of these compounds originate from MOB. This wide range of values is a result of the relatively high uncertainty regarding end member definition and fractionation factors, while a different relative contribution of acetoclastic and hydrogenotrophic methanogenesis may also cause different values for CH_4. Nevertheless, the values indicate that CH_4-C contribute to a large extent to the lipid biomass.

In the water column, similar calculations indicate that the contribution of CH_4 derived carbon in phytol, cholesterol, brassicasterol and sitosterol was up to 60% at 9 m water depth, whereas it was < 20% in the hypolimnion. Similarly, the fraction of FAs derived from MOB or from ^{13}C-depleted carbon sources was up to 50% in 10-methyl-$C_{16:0}$, $C_{16:1\omega7}$ and $C_{16:1\omega5}$ FAs and up to 70% in the case of $C_{18:1\omega7}$ and $C_{18:1\omega5}$ FAs.

4. Summary and conclusions

GDGT-0 is the predominant isoGDGT in Lake Rotsee and originates mainly from (acetoclastic) methanogens in the sediment. Archaeol and probably hydroxyarchaeol are also of a methanogenic origin (mainly hydrogenotrophic), but their sedimentary profiles suggest a potential contribution from methanotrophs. An increase in GDGT-0 in sediment layers deposited in the early 1920s indicates a productivity-driven methanogens maximum and thus a peak in CH_4 production. In response to an increase in CH_4 availability, MOB biomass and thus MOx activity increased, as suggested by a higher concentration of ^{13}C-depleted diploptene and 17β,21β-homohopanoic acid.

The $\delta^{13}C$ values of several FAs (10-methyl-$C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{18:1\omega7}$, $C_{18:1\omega5}$) attest to a mixed origin, dominated by MOB, with contributions from non-methanotrophic sources. The ^{13}C-depleted values for phytol, cholesterol, brassicasterol and β-sitosterol indicate that the source organisms used ^{13}C-depleted carbon sources.

Determination of the GDGT concentration, together with $\delta^{13}C$ values of the GDGTs obtained from ether cleavage, adds valuable insights into the distributions and role of archaea involved in CH_4 cycling in lakes. Although our results from an end member approach have to be regarded with caution and are only tentative, they show that CH_4 may provide > 50% of the carbon utilised by the lake ecosystem.

Acknowledgements

The study was funded by the European Union project "Hypox – In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas and land-locked water bodies" (EC Grant 226213) and internal Eawag funds. Additional funding came from ETH Fellowship FEL-36 11-1 (F. Peterse). We thank the late G. Nobbe and A. Zwyssig (both Eawag) for support during sampling and laboratory work. We thank two anonymous reviewers for comments and critical reviewing of the manuscript.

Appendix A

See Fig. A.1.

Associate Editor—**Philip A. Meyers**

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