J Paleolimnol (2017) 58:391–402
DOI 10.1007/s10933-017-9985-8

ORIGINAL PAPER

# Sources of organic matter for bacteria in sediments of Lake Rotsee, Switzerland

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Received: 9 July 2016 / Accepted: 20 June 2017 / Published online: 26 June 2017


**Abstract** Determination of carbon sources and microbial activity in lake sediment is important for understanding organic carbon preservation and methane production. This study aimed to determine the organic carbon sources and microbial activity over the last 140 years in sediments of methanotrophic Lake Rotsee (Switzerland). We investigated phospholipid-derived fatty acid biomarkers and their stable carbon isotope signatures in the sediments of this eutrophic lake. Strong bacterial activity in the sediment deposited during the 1920s–1960s could account for the relatively low ratio of long-chain to short-chain fatty acid ((C24 + C26 + C28)/(C14 + C16 + C18), $TAR_{FA}$) values, which is consistent with low TOC/TN ratios in the sediment deposited during that interval. The carbon stable isotope records, both bulk and compound-specific, showed greater values at such times, although the offset between the bulk and fatty acids decreased. This implies that the microbial community residing at sediment depths deposited in the 1960s preferentially utilised the compounds derived from the enhanced surface-water productivity at that time. This observation contrasts with data from the depth intervals before and after, when a major portion of the labile organic matter was derived from methane-sourced production. In sediments deposited before ca. 1964, the overall very low fatty acid $\delta^{13}C$ values suggest that labile carbon was primarily derived from methanotrophs.

**Keywords** Biomarker · Phospholipid fatty acids · Stable isotopic analysis · Methanotrophs

**Electronic supplementary material** The online version of this article (doi:10.1007/s10933-017-9985-8) contains supplementary material, which is available to authorized users.

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## Introduction

Carbon storage in lake sediments plays a major role in the global carbon cycle because lake sediments probably sequester 30–60% of the total stored carbon on only 2% of the Earth's surface (Cole et al. 2007). This implies that carbon production and/or preservation rates (per $m^2$) in lakes are much higher than those in the marine environment. Even so, organic matter (OM) degradation in lake sediment accounts for an appreciable fraction of global methane production

(Bastviken 2009; Sanseverino et al. 2012). Both the preservation and degradation of OM in lake sediment depends on the microbial activity in the water column and the underlying sediment. The composition of OM can be altered by microbial activity because of the variable decomposition rates amongst different types of OM (Meyers 1994). Heterotrophs in the freshwater column depend on the supply of carbon from different sources: (1) local primary production (autochthonous sources), (2) terrestrial input from plants and soils (allochthonous sources) and (3) methanotrophic biomass. As a consequence, unravelling carbon sources and microbial activity in lake sediment is important for understanding carbon preservation and methane production.

During the mid-twentieth century, many European lakes became eutrophic and thus potentially altered the corresponding microbial communities and substrates. Subsequent restoration again modified the trophic states, but not necessarily to the original state. In many lakes, the food webs and carbon cycling primarily depend on autochthonous sources as a supplier of labile OM (Richardson et al. 2010). Although allochthonous carbon is generally considered to be largely refractory, this carbon can still be subjected to microbiological co-metabolism during the processing of the autochthonously produced OM (Dalton and Stirling 1982). Recently, the input of allochthonous sources has been reported to be important in subsidising secondary production in lakes (Cole et al. 2006; Battin et al. 2008). The relative rates of primary and secondary production and respiration determine the flux of OM to the sediment (Kritzberg et al. 2004). Organic carbon derived from different sources ends up in the sediment and, in turn, provides the substrate for the sediment microbial community. Degradation of OM in lake sediments often results in methane production under anoxic conditions. Most of the methane produced in lake sediments is, however, efficiently oxidized by methane-oxidising bacteria in the subsurface and never reaches the overlying water column (Bastviken 2009; Sanseverino et al. 2012). In some lakes with anoxic hypolimnia, however, methane escapes to the water column and aerobic methanotrophs thrive at the chemocline (Schubert et al. 2010). These methanotrophs often play a critical role in determining carbon production in eutrophic lakes (Deines et al. 2007). For oceanic ecosystems, methanotrophs may contribute appreciably to the carbon turnover in anoxic sediments (Hinrichs and Boetius 2002). For lakes, the relative contribution of methanotrophs to secondary productivity is also important, as methanotrophy is a form of chemosynthetic production (Trotsenko and Murrell 2008; He et al. 2012; Grey 2016).

We applied molecular and stable carbon isotopic analyses to explore past and present-day carbon cycling in Lake Rotsee, investigating sediments deposited before, during and after a pronounced episode of lake eutrophication. Bulk carbon isotopes were used to reconstruct overall lake productivity, whereas compound-specific carbon isotopes were used to infer dietary sources for bacteria. Fatty acids (FAs) or phospholipid-derived fatty acids (PLFAs) allowed the identification of microbial communities, including methanotrophs (Hinrichs et al. 1999; Boschker and Middelburg 2002). The specific monounsaturated 16:1ω8, 16:1ω6 and 18:1ω8 FAs or PLFAs were interpreted as being derived from methanotrophs (Bowman et al. 1991; Boschker and Middelburg 2002).

### Environmental setting

Lake Rotsee (47°04′11″N, 8°18′51″E), located close to Lake Lucerne in Switzerland (Fig. 1), is 0.48 $km^2$ in area and has an average depth of 9 m, with a maximum depth of 16 m. The lake is currently eutrophic and has high rates of methane production (Kohler et al. 1984; Schubert et al. 2010). Recently, Naeher et al. (2012) reported the effects of eutrophication on microbial community changes using high-resolution lipid biomarker and trace metal data from Rotsee sediments. The authors showed that higher sewage and nutrient input repeatedly stimulated the growth of diatoms, other primary producers and methanogens.

## Materials and methods

Using a gravity corer, we collected a 55-cm-long sediment core at a 16-m-deep site at the centre of Lake Rotsee in November 2010 (Fig. 1). This particular coring location was chosen because of previous sampling work in Rotsee in October 2009. Recognisable changes in lithology and colour are described as follows: dark mud with lighter (grey-brown) stains at 54–41 cm; greyish mud, olive green with light brown stains at 40–21 cm; black mud, leaves and fragments

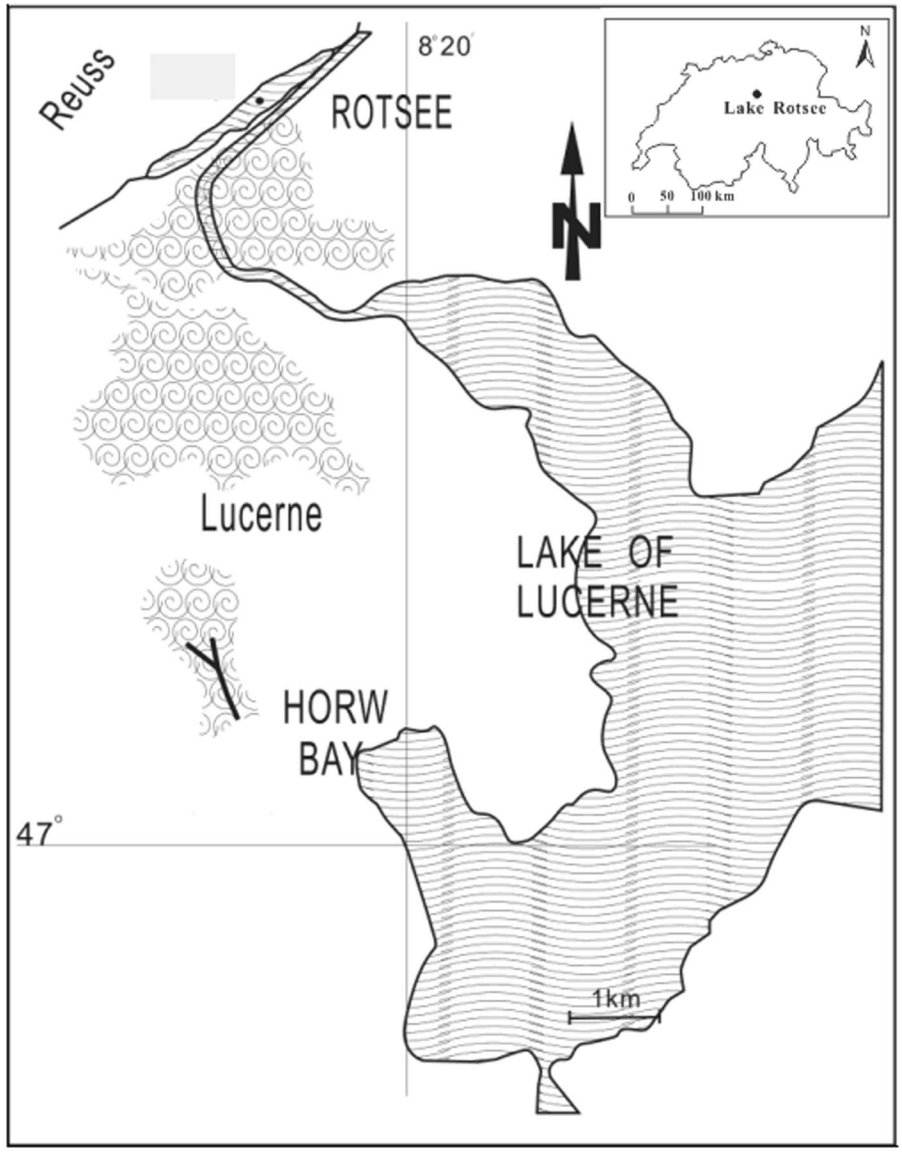



**Fig. 1** Location map of Lake Rotsee and adjacent Lake Lucerne (Switzerland). *Inset* map shows location of Rotsee in Switzerland

from 20 cm depth to the surface. There were abundant gas bubbles throughout the whole core. The core was sub-sampled into 1 cm slices. The collected samples were then stored at −20 °C and freeze-dried in the laboratory.

## Gamma-ray spectroscopy

Activity of the naturally occurring radionuclide $^{210}$Pb was determined on sediment slices using standard gamma-ray spectroscopy (Canberra BeGe, broad energy Germanium detector). Before measurement, sliced samples were freeze-dried and homogenised. Pre-weighed samples were transferred to and evenly distributed and compacted in Petri dishes. Immediately after weighing, the dishes were sealed into 50-µm-thick polyethylene bags and stored for at least 2 weeks. Sediment ages and accumulation rates were calculated using a model that assumes constant initial $^{210}$Pb concentration (Robbins and Edgington 1975).

## Lipid extraction and separation

Lipids were extracted by a modified Bligh and Dyer procedure (Dickson et al. 2009) in a dichloromethane (DCM)–methanol (MeOH)–phosphate (5:10:4 v/v/v)

solution and separated by silica gel column chromatography (Dickson et al. 2009) into three fractions as follows: neutral lipids, glycolipids and phospholipids. In organisms, FAs are bound to a polar headgroup, e.g. a phospho-group function such as membrane lipids. These polar headgroups, however, are quickly lost during degradation, leaving free FAs (FFAs) behind. FFAs can be used to infer past changes in productivity (Naeher et al. 2012), whereas intact FAs are used as an indicator for fresh material.

Neutral lipids were separated into two fractions using short columns with activated $Al_2O_3$ and subsequent solvent mixtures of hexane:DCM (9:1, v/v) and DCM:MeOH (1:1, v/v) corresponding to apolar and polar fractions, respectively. The polar fraction, containing FFAs was derivatised with $BF_3$–MeOH to transform FAs into methyl esters and with acetic anhydride to convert alcohols into esters. Short-chain FFAs (SCFAs) ($C_{14}$–$C_{20}$) are the dominant lipids in bacteria and algae; meanwhile, the FFAs with longer chain lengths (LCFAs, $C_{20}$–$C_{30}$) are derived mainly from terrestrial higher plants (Volkman et al. 1998; Millar et al. 1999). Both short-chain and long-chain FFAs are produced by the other group as well, albeit in much smaller amounts (Volkman et al. 1980; Cranwell et al. 1987).

The phospholipid fraction, containing PLFAs, was derivatised by mild alkaline transmethylation to form FA methyl esters (FAME) (Boschker et al. 2005). Concurrently, an internal FAME (19:0) standard was added to the extracts.

### Gas chromatography (GC)/GC mass spectrometry (MS) analyses

Lipid concentrations were measured with an HP GC–flame ionisation detection (FID) apparatus. The free FAs were separated over a CP-Sil 5CB fused silica capillary column (30 m, 0.32 mm internal diameter [i.d.]). The oven was programmed starting at 70 °C, heated up to 130 °C at 20 °C/min and to 320 °C at 4 °C/min, a temperature that was maintained for 20 min. The PLFAs were separated using a DB-5 MS column. The oven was programmed starting at 80 °C (2 min), then increased to 290 °C at 4 °C/min, with the final temperature maintained for 15 min. Helium was used as the carrier gas, at a flow rate of 1.0 ml/min. Compounds were quantified by integrating peak areas of the FID traces in the chromatograms relative to a co-injected standard (squalane for FFA fractions and C19:0 for PLFA fractions).

GC–MS (Thermo Finnigan Trace) was used to identify compounds using the same temperature program and capillary column, but with constant pressure instead of constant flow. Identification was based on comparing retention times to those of a known mixture and mass spectra.

### $\delta^{13}C$ measurements

Carbon isotope ratios of individual FAMEs of total FFAs and PLFAs were analysed by GC combustion isotope ratio mass spectrometry (GC-c-IRMS, using a Trace GC Ultra GC apparatus, Thermo Finnigan) coupled to a GC PAL auto-sampler. The Trace GC Ultra was fitted with an Agilent HP-5 column (50 m, 0.2 mm i.d.). Identification of peaks was based on retention times. On the basis of FAME carbon number and known isotopic composition of the derivatising agent, we corrected the carbon isotope ratios of the individual FAs for the one introduced carbon atom in the methyl group during derivatisation, with the derivation agent being analysed off line. Stable carbon isotope data are expressed as $\delta^{13}C$ in ‰ relative to Vienna Peedee Belemnite (VPDB).

### Total organic carbon (TOC) and total nitrogen (TN) analyses

Aliquots of sediment (1.5 g) were treated with HCl to remove inorganic carbon. Concentrations of TOC and TN were subsequently measured using a N, C and S analyser (Fison NA 1500). Carbon isotope ratios ($\delta^{13}C$) of TOC were determined using a Deltaplus Advantage isotope ratio mass spectrometer (Finnigan MAT). Carbon isotope values were expressed in ‰ relative to VPDB. Analytical precision was ±0.1‰.

## Results

### Age model and average sedimentation rates

$^{210}Pb$ activity values were used to construct an age model (Fig. 2a). On the basis of the $^{210}Pb$ record, the average linear sedimentation was 0.49 cm $year^{-1}$ (Fig. S1). This value is somewhat higher than the 0.38 cm $year^{-1}$ reported by Naeher et al. (2012). This

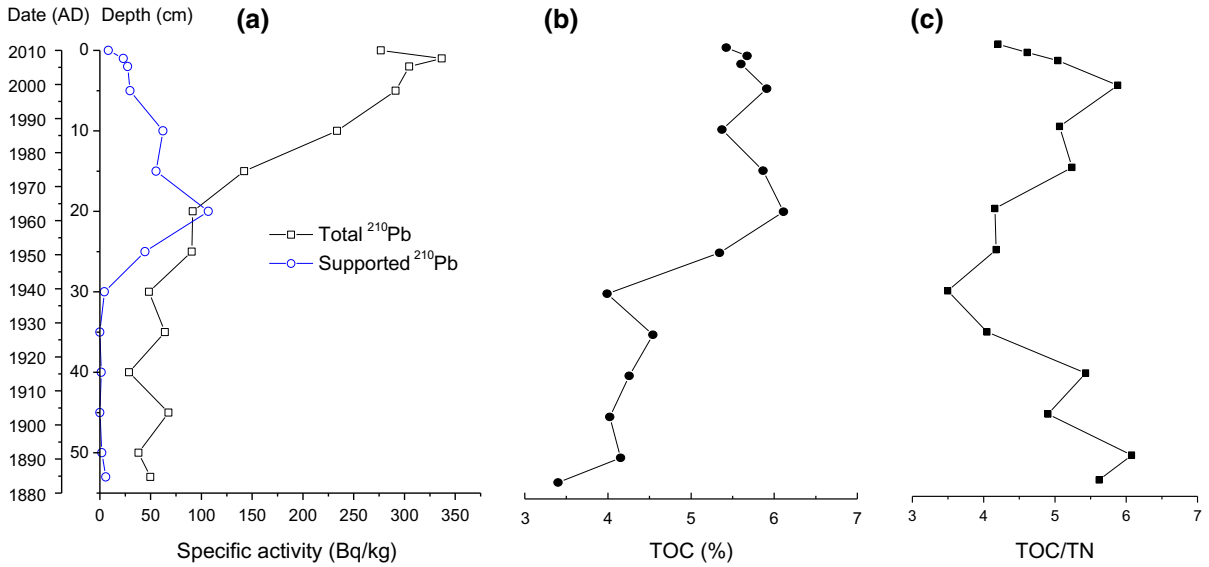



**Fig. 2** Profiles of **a** $^{210}$Pb activities; **b** the content of TOC; and **c** the ratio of TOC/TN versus depth in the Lake Rotsee sediment core

discrepancy possibly resulted from the lower sample resolution (5 cm) in our work. Nevertheless, our age model overall matches well with the age model constructed by Naeher et al. (2012). They calibrated their age model with TOC concentrations and derived a 4-year time resolution for a sampling resolution of 1 cm (Naeher et al. 2012).

## TOC content and TOC/TN

TOC concentrations (Fig. 2b) range between 3.3 and 7.3 wt%, which is close to values (4.3–6.8 wt%) previously reported by Naeher et al. (2012). TOC concentrations are low and relatively stable until ca. 1933, after which they increase to a maximum of 6.1% in the mid-1960s. Afterwards, concentrations fluctuated further, but did not decrease to previous levels. This result is in line with previous reports on the eutrophication history of Rotsee (Naeher et al. 2012).

TOC/TN ratios (Fig. 2c) varied between 3.5 and 6.1. Similar values were found for another eutrophic Swiss lake, Lake Lugano. The eutrophication in Lake Lugano began in the second part of the last century, which led to significantly greater primary productivity (Bechtel and Schubert 2009). The TOC/TN ratios in Lake Rotsee show a decreasing trend until ca. 1940, after which the ratios slowly increased until ca. 1998, when values subsequently decreased again.

## FFAs and terrigenous/aquatic FA ratios ($TAR_{FA}$)

FFAs are a generally highly abundant type of lipid found in sediment. In this work, FFAs with chain lengths of 14–30 carbon atoms were found, and concentrations of SCFAs and LCFAs in this work are shown in Fig. 3. The amount of SCFAs shows a pronounced maximum at the top and a period of higher concentrations between the 1920s and 1960s. The LCFAs show maximum concentrations between the 1920s and 1960s, but are missing the peak values observed near the sediment surface for the SCFAs. SCFAs and LCFAs are largely composed of even-carbon FAs, i.e. they display remarkable even carbon-number predominance. The $C_{14}$, $C_{16}$ and $C_{18}$ chain lengths of SCFAs are highly predominant (Fig. S2).

To further investigate the source of organic carbon in Rotsee sediments, the $TAR_{FA}$ (C24 + C26 + C28)/(C14 + C16 + C18) was calculated to determine the relative contribution of terrigenous versus aquatic FAs (Bourbonnier and Meyers 1996). The $TAR_{FA}$ values ranged from 0.27 to 19.56 (Fig. 3c). In the 20th century (1920s–1990s), the $TAR_{FA}$ values were fairly constant, with a minimum of 0.57 during the 1960s, which is still larger than the $TAR_{FA}$ values in oligotrophic Lake Lugu, China (0.01–0.48) (Zhang et al. 2016). The strong peak ca. 1880 is likely a mathematical artefact, a consequence of division of two very low numbers

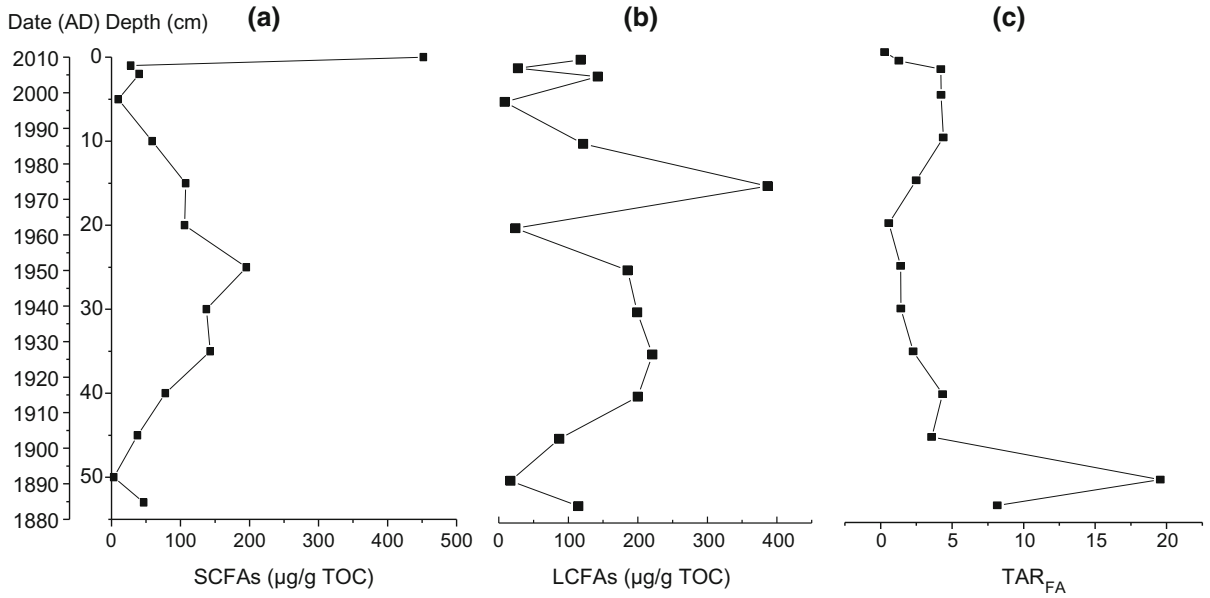



**Fig. 3** TOC-normalized concentrations of **a** SCFAs (C14–C23); **b** LCFAs (C24–C30); and **c** $TAR_{FA}$ index calculated as (C24 + C26 + C28)/(C14 + C16 + C18) versus depth in the Lake Rotsee sediment core

(concentrations) leading to a large number, but one with large uncertainty.

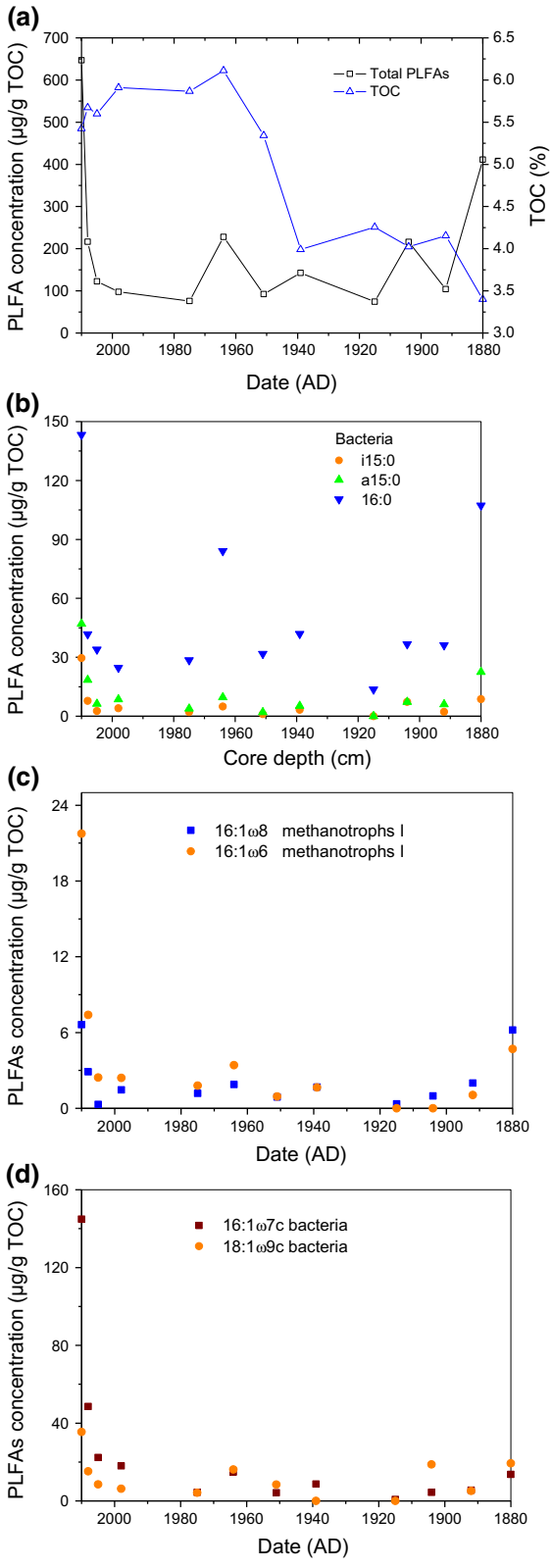
## PLFAs

PLFAs are good variables for detecting rapid changes in microbial communities. Because of their rapid decomposition after cell death, they are mainly associated with living biomass (Boschker et al. 2005). In this study, PLFAs are dominated by 16:0 (16:1) and 18:0 (18:1), which are mainly derived from algal and bacterial biomass and the total concentration of PLFAs displays a fluctuating distribution (Fig. 4a). The high concentration at the sediment surface (ca. 2010) declined rapidly down-core to low values at a depth corresponding to ca. 1975, underlain by two minor peaks at depths corresponding to ages of ca. 1964 and ca. 1904. The concentration of PLFAs after ca. 1975 shows a trend different from that of TOC.

Several compounds of PLFAs can be considered specific biomarkers. The methyl-branched PLFAs (i14:0, i15:0, a15:0 and i16:0) found in this work (Fig. 4b) pertain mainly to bacteria (Dijkman and Kromkamp 2006). The specific monounsaturated PLFAs (16:1ω8 and 16:1ω6) detected in this work (Fig. 4c) are interpreted as methanotrophs I (Bowman et al. 1991; Boschker and Middelburg 2002). We found that the methanotrophs were dominated by the type I group (c-Proteobacteria), consistent with the finding of Schubert et al. (2010). Biomarkers 16:1ω7c and 18:1ω9c (Fig. 4d) also indicate the presence of bacterial PLFA (Boschker and Middelburg 2002).

## $\delta^{13}C$ of PLFAs and FFAs

Total FFAs of 16:0, 16:1, 18:0, 18:1 and 18:2 and PLFA of 16:0 were sufficiently abundant for isotope analyses. Most $\delta^{13}C$ values of PLFAs and FFAs increased before decreasing with age (Fig. 5). Generally, i + a15:0 PLFAs are representative bacterial biomarkers (Boschker et al. 2005). In this work, 16:0 PLFA instead of i + a15:0 was employed to characterise bacterial PLFAs because the former is present at higher concentrations and could hence be analysed with better precision for its $\delta^{13}C$ values, and eukaryote biomass is likely small in these sediments. The values of $\delta^{13}C$ for 16:0 PLFA remained high during ca. 1904–1975 (−29.1 to −34.4‰), but low $\delta^{13}C$ values appeared from ca. 1998–2010 (−41.1 to −45.8‰) (Fig. 5a). These $\delta^{13}C$ values are within or close to the typical range of $C_3$ plant lipids (−34 ∼ −23‰) (Cifuentes and Salata 2001). The top three and the bottom values of the record are less than −40‰ and




◀**Fig. 4** Concentrations in the sediment core from Lake Rotsee of: **a** total PLFA and TOC; **b** bacterial PLFA; **c** 16:1ω6 and 16:1ω8 PLFA derived from methanotrophs; and **d** 16:1ω7 and 18:1ω9 PLFA derived from algae and bacteria, respectively. All profiles are plotted versus date

seem to indicate bacteria that utilized a carbon source strongly depleted in $^{13}C$.

The $\delta^{13}C$ values of 16:0 PLFA increased from ca. 1880–1939 and decreased from ca. 1939–recent, suggesting that the stable isotope composition of the carbon sources for heterotrophs changed. To highlight the impact of such a change, we plotted the correlations between $\delta^{13}C$ and TOC for the periods 1939–2010 and 1880–1939. A positive correlation was also observed between the $\delta^{13}C$ values for 16:0 PLFA and TOC (Fig. 5b). The relatively high correlation ($r = 0.84$) after ca. 1939 suggests that the isotopic ratio of bacterial biomass varied with that of the carbon source. The $\delta^{13}C$ values of 16:0 PLFA were depleted compared to that of the bulk TOC isotope values after ca. 1939. Afterwards, the $\delta^{13}C$ values of TOC decreased again in the 1960s/1970s. This result was likely caused by an effective recovery from eutrophication leading to a decrease in primary production and consequently a proportional decrease of atmospheric carbon input typically enriched in $^{13}C$. Otherwise, the relatively weak correlation ($r = 0.67$) before ca. 1939 suggests that the carbon source and metabolic pathway of bacteria differed from that during the later years.

The concentrations and $\delta^{13}C$ values of FAs of methanotrophs are presented in Fig. 5c to investigate the potential role of methane as a carbon source for the bacterial community in Lake Rotsee. Besides the 16:1ω6 FA of methanotroph I, the 18:1ω8 FA of methanotroph II was detected, albeit unexpectedly. This type II methanothroph was not detected before in the water column of Lake Rotsee (Schubert et al. 2010). The highest concentration of 16:1ω6 and 18:1ω8 FAs were found in the top of the sediment column, and were 523.0 and 992.6 µg/g TOC, respectively. Concentrations rapidly declined with depth in the sediment. The decreasing pattern observed for the 16:1ω6 and 18:1ω8 FAs deeper in the sediment is in line with the notion that methanotrophs grow mainly near the top of the sediment, with activity decreasing deeper in the sediment, as suggested by the pattern of 16:1ω6 and 16:1ω8 PLFA (Fig. 4c).

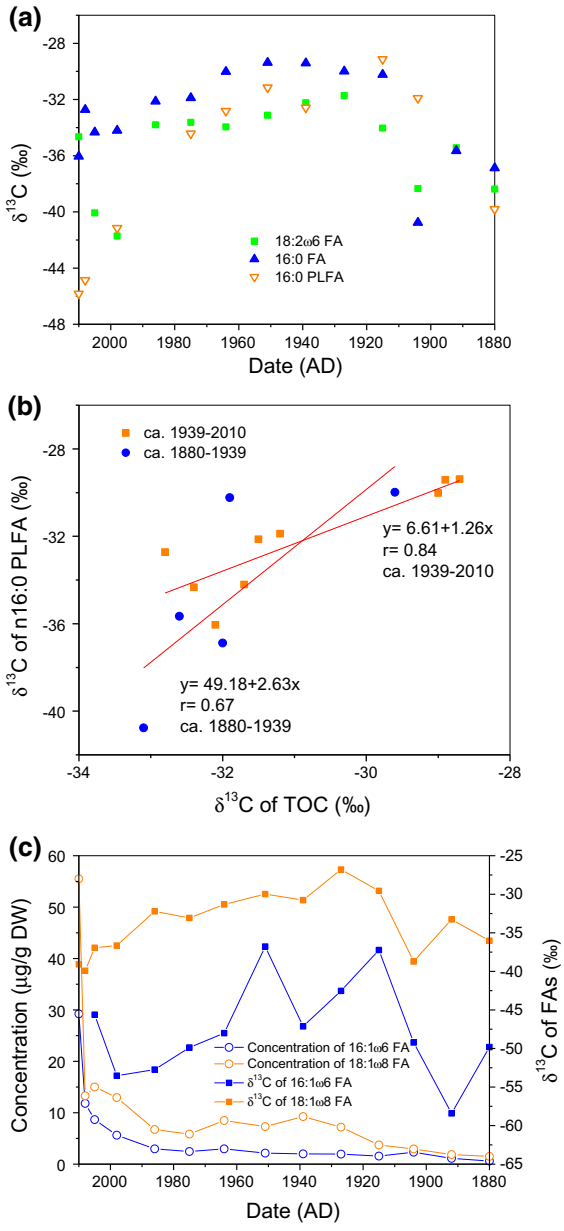



**Fig. 5** Carbon stable isotope ratios for FAs extracted from the Lake Rotsee sediment core: **a** $\delta^{13}C$ values of algal 18:2ω6 FFA, bacterial 16:0 FFA, and 16:0 PLFA; **b** the correlation between $\delta^{13}C$ values for 16:0 PLFA and TOC; and **c** $\delta^{13}C$ values and concentrations of 16:1ω6 and 18:1ω8 FFAs derived from methanotrophs. Profiles of **a**, **c** are plotted versus date

The $\delta^{13}C$ for 16:1ω6 FA (Fig. 5c) shows a bimodal distribution, with a minimum value at ca. 1892 (−58.4‰), but much higher values at ca. 1915 (−37.2‰) and ca. 1951 (−36.8‰). Meanwhile, the $\delta^{13}C$ for 18:1ω8 FA shows relatively enriched values compared to 16:1ω6 FA, with a peak value of −26.8‰ at ca. 1927 and a minimum of −39.9‰ at ca. 2008.

## Discussion

### Change of abundance and OM sources over time

Eutrophication is a paramount, widespread environmental problem for lakes in recent decades (Carpenter et al. 1999). Eutrophication has a major effect on lake ecosystems by fueling primary productivity and altering amounts and composition of OM (Naeher et al. 2012). Lake Rotsee is one of the best-characterized eutrophic lakes and can be considered a model system for eutrophic pre-alpine lakes. At the beginning of the 1960s, peak eutrophication occurred in Lake Rotsee, and a decrease in productivity ensued after the construction of a sewage treatment plant in 1974 (Naeher et al. 2012). The lack of a dramatic decrease in TOC accumulation after that period was attributed to continued, non-point-source input of OM from agriculture or internal loading of nutrients from the lake sediment (Matzinger et al. 2010; Naeher et al. 2012). Such a non-linear response of lakes to environmental management efforts is a commonly observed phenomenon and important to understand for planning future restoration projects.

The ratio of TOC/TN in lake sediments is commonly used to infer the biological source of the carbon (Meyers 1994). Generally, TOC/TN ratios of higher terrestrial plants vary in the range of 14–23, whereas the ratio is about 6–7 for plankton (Müller and Mathesius 1999) and 4–6 for bacteria (Goñi and Hedges 1995). In addition, soil OM can have a wide range of TOC/TN ratios (5–10.7), which generally tend to be lower than those of the overlying vegetation (Goñi et al. 1998; Holtvoeth et al. 2016). TOC/TN ratios in the core (Fig. 2c) fall into the range of bacteria, plankton and soil, implying a mixed source for the organic carbon in Rotsee sediments, with a relatively large contribution from bacterial biomass with low TOC/TN ratios in the sediment deposited between the 1920s and 1960s. Naeher et al. (2012) also indicated that higher bacterial activity during Lake Rotsee eutrophication, especially from the 1920s to 1960s, could be inferred from epicholestanol, which is an indicator of bacterial alteration. This might be a consequence of superior preservation of N-containing

OM and/or increased amounts of inorganic N in the sediment deposited in the 1920s–1960s. It was observed that there was a small peak of methanotrophic-related FFA or PLFA compounds during the 1920s–1960s, indicating relatively high methanotroph biomass. Methanotrophs can oxidize ammonia in addition to methane (O'Neill and Wilkinson 1977; Megraw and Knowles 1989; Bodelier and Frenzel 1999). Therefore, it is possible that there was a high concentration of inorganic nitrogen (ammonium) at the time, which results in a low TOC/TN ratio.

Numerous studies proved that TOC/TN ratios in lake sediment are associated with human activities (Routh et al. 2004; Bragée et al. 2013). A canal construction from the Reuss River to Lake Rotsee started in 1922 (Kohler et al. 1984). Afterwards, construction of an interceptor sewer in 1969 and a sewage treatment plant in 1974 were completed (Stadelmann 1980). Increasing sewage supply and soil erosion along the canal may have delivered a large amount of nutrients into this lake during the 1920s–1960s. In addition, animal waste could have been transferred by runoff from surrounding livestock farms into the lake and become another source of nutrients before construction of the sewage treatment plant (Naeher et al. 2012). Hence, low TOC/TN ratios in the 1920s–1960s may result from both changes in the microbial community structure and human activities in the catchment. Increasing values for $TAR_{FA}$ indicate increased terrigenous input relative to aquatic production. Conversely, this parameter can indicate the degradation of aquatic FAs relative to land-derived components (Bourbonnier and Meyers 1996). In Lake Rotsee, lower values for $TAR_{FA}$ were found during the 1920s–1960s, which is in agreement with the low TOC/TN ratios observed for this period. The fact that these variables changed at the same time is in line with the eutrophication and resulting high productivity that occurred during this period. Nevertheless, even in the 1920s–1960s, $TAR_{FA}$ values are not 0, i.e. some terrestrial OM with higher TOC/TN ratios must have been supplied.

Autochthonous and allochthonous subsidies could contribute to sustenance of bacterial carbon biomass, although selective degradation and/or diagenesis could overprint primary FA source signatures. Generally, SCFAs tend to be preferentially degraded by microbes during early diagenesis (Ho and Meyers 1994), and this phenomenon may lead to higher $TAR_{FA}$ values. Low $TAR_{FA}$ values during the 1920s–1960s, however, may have two explanations: (1) both aquatic and terrestrial inputs were similarly important, or (2) microbial recycling of sedimentary OM overprinted the FA source signatures. We observed that the abundance of SCFAs was relatively high during this period, which caused the low $TAR_{FA}$ values during the 1920s–1960s. This is possibly related to higher bacterial biomass, as supported by low TOC/TN ratios of sediment from that period. To evaluate the second possibility, we used PLFAs to explore in situ production and the consumption of available food sources.

## In situ production and the consumption of available OM sources

Microbial biomass reconstruction can be ascertained using PLFA biomarkers combined with other sediment variables (Naeher et al. 2012). The PLFAs in the top few cm of sediment from Lake Rotsee may be related to export from the water column and growth of heterotrophs on detritus. At these sediment depths, algal and bacterial PLFAs can be derived from the water column (sinking and re-working of the sediment) and in situ production, whereas bacterial PLFAs present at greater depths in the sediment are likely derived from in situ production because of rapid degradation of recently settled biomass. Within the sediment, the anoxic and dark conditions prevent in situ production of algal PLFAs. As shown by the total PLFA concentration (Fig. 4a), high bacterial biomass at 20–40 cm in the sediment deposited during the 1920s–1960s is consistent with the high SCFA concentrations during this period.

Inferred bacterial biomass in the sediment is, surprisingly, relatively low at 5–15 cm (after the 1970s), with the exception of the very top, where there is export from the water column. Besides, high TOC contents and low PLFA concentrations during ca. 1975–1998 (Fig. 4a) may indicate that the carbon accumulation resulted in weak microbial activity. The microbial growth does not appear to follow regular patterns. Bacterial growth is usually controlled by multiple environmental factors, such as pH, temperature and oxygen. Under stable conditions, growth rate depends primarily on the amount of substrate available. The amount of carbon available to microbes can, however, change abruptly under unstable conditions. For instance, when the micro-ecological environment is unsuitable or less suited for growth of the bacterial

population, microbial activity will be inhibited and accumulation of refractory substances will ensue (Wardle 1992).

### Relation between methanotrophs and OM in sediment

The contribution of methanotrophic PLFAs to total PLFAs ranged from 0.47 to 4.88%. Methanotrophic PLFAs constituted a greater contribution at 20 cm (ca. 1964) and at the core bottom than at other levels, with a similar trend being observed for the PLFA 16:0, likely derived primarily from bacteria. This suggests fairly high methane concentrations, resulting in high a contribution to PLFAs at these two depths, related to a high methanotrophic biomass and probably high methanogenesis (Amaral and Knowles 1995).

Methane-oxidising bacteria are currently classified into the Phyla Proteobacteria (methanotroph I) and Verrucomicrobia (methanotroph II) (Bowman 2006; Bodelier et al. 2009; Op den Camp et al. 2009). The predominance of methanotrophs of the type I group (c-Proteobacteria) observed in this work is reflected in the more depleted $\delta^{13}C$ values. The Lake Rotsee sediment has been shown to experience anoxic conditions year-round, with high production of methane throughout the entire year as well (Schubert et al. 2010). Methane concentrations on the sediment surface were found to reach 0.7–4.1 mmol/L, with a maximum value of 5.9 mmol/L observed ca. 1975 (Schubert et al. 2010). High production of phytoplankton in monomictic and eutrophic Lake Rotsee during the spring and summer results in anoxic conditions in the bottom waters and sediment. Accordingly, OM degradation relies on anaerobic processes, which lead to methane formation by methanogenesis (Conrad 1989). The anaerobic oxidation of methane is an essential, but poorly understood process in methane-rich sediments. Previous research showed that methanogenic archaea contributed 98% to the archaeal community in Lake Rotsee (Zepp Falz et al. 1999). Such high methane production supplies carbon with low $\delta^{13}C$ values for methanotrophs and stimulates their growth.

Notably, the maximum difference between the $\delta^{13}C$ of 16:0 PLFA and TOC reached around 7.6‰ (ca. 1904) (Fig. 5b). This can be explained by: (1) anoxic conditions, (2) relatively negative $\delta^{13}C$ values of more easily degradable substrates (such as methanol and acetic acid), and (3) existence of methanotrophs in the anaerobic environment (Cifuentes and Salata 2001). In addition, the $\delta^{13}C$ values of the 16:0 PLFA were particularly low compared with those of the 16:0 FA after ca. 1975 (Fig. 5a). The low $\delta^{13}C$ values of the 16:0 PLFA suggests utilisation of an OM source with $^{13}C$-depleted values in the upper layer, such as is typical of $^{13}C$-depleted methane values. In contrast, the $\delta^{13}C$ value of the 16:0 FA denotes a source from ordinary heterotrophic bacteria and algae. Meanwhile, coincident low $\delta^{13}C$ values for the 16:0 PLFA and 16:0 FA in deeper sediments indicates a major contribution of methanotrophs/methanogens to 16:0 FA and 16:0 PLFA. During ca. 1927–1975, the $\delta^{13}C$ values of PLFA 16:0 (−34.4 to −31.1‰) and FA 16:0 (−31.9 to −29.4‰) were similar. This implies a greater contribution of ordinary heterotrophic bacteria to 16:0 PLFA because methanotrophs/methanogens usually show more negative $\delta^{13}C$ signatures (−60 to −35‰) (Nusslein et al. 2003), although they probably make a contribution to PLFA 16:0 as well.

In Lake Rotsee, diatom blooms support bacterial biomass accumulation in the sediment (Naeher et al. 2012). The trend in $\delta^{13}C$ for the algal 18:2ω6 is very similar to that of the 16:0 PLFA, but shows more negative values than the latter. Furthermore, the isotopic fractionation between $\delta^{13}C$ values of 18:2ω6 and 16:0 PLFA was mostly >1‰, with the maximum offset being 11.2‰. This result suggests that the algae may partly utilise $^{13}C$-depleted $CO_2$ and/or dissolved inorganic carbon produced by methane oxidation (van Winden et al. 2010). Other heterotrophic processes that employ different metabolic pathways could result in similar isotopic patterns, but this is less likely in a lake that is known to be strongly affected by methane production and oxidation. Methanotrophs play a major role in the methane cycle and provide $C_1$ intermediates and various metabolites to other members of microbial communities in ecosystems (Trotsenko and Khmelenina 2002).

## Conclusions

We demonstrated the application of lipid biomarkers in investigating organic carbon sources and microbial processing in core sediment from Lake Rotsee (Switzerland). As shown by the total PLFA concentration, strong bacterial activity in the sediment

deposited during the interval from the 1920s to 1960s may explain the relatively low $TAR_{FA}$ values, which is consistent with the observed low TOC/TN ratios in the sediment deposited during that period. High methane production in Lake Rotsee supplied carbon for methanotrophs, stimulating their growth. The low $\delta^{13}C$ of PLFA 16:0 suggests the strong bacterial utilisation of OMs with $^{13}C$-depleted values in the surface layer of sediments (depth $< 15$ cm), such as methane, which is highly $^{13}C$-depleted. The simultaneous decline in $\delta^{13}C$ values for the 16:0 PLFA and 16:0 FA in deeper sediments indicates a major contribution of methanotrophs/methanogens to 16:0 FA and 16:0 PLFA. During the period ca. 1927–1975, the $\delta^{13}C$ values of 16:0 PLFA and 16:0 FA were similar, but the values of the former were slightly more negative. This suggests a greater contribution of ordinary heterotrophic bacteria to 16:0 PLFA because methanotrophs/methanogens usually show more negative $\delta^{13}C$ signatures ($-60$ to $-35$‰).

**Acknowledgements** We acknowledge Dr. Carsten J. Schubert for making field work possible, Dr. Johan W.H. Weijers for help with PLFA extraction and analysis, and Dr. Klaas G.J. Nierop (Utrecht University) for helpful discussions on fatty acids. Arnold van Dijk, Dominika Kasjaniuk, Michiel Kienhuis and Linda van Roij (Utrecht University) all provided technical and analytical assistance. The National Science Foundation of China (Nos. 31370478 and 31670461) and the Netherlands Organization for Scientific Research (NWO) Earth and Life Sciences (No. 820.02.017) supported this research.

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