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Methanogenesis by CO_2 reduction dominates lake sediments with different organic matter compositions

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Abstract

Microbial methane production is a respiration reaction involved in the terminal step of anaerobic degradation of organic matter. Due to the dependency of methanogenic substrate production on fermentation reactions that produce different end productions, different sources and compositions of organic carbon (OC) may impact the methanogenic potential in lake sediments. Here, we investigate the sources and compositions of OC in sediments of Lake Geneva and how both are potentially linked to methane production. Differences in dominant long-chain fatty acid abundances and carbon isotopic compositions suggest the predominance of diagenetically altered phytoplankton-derived OC at a profundal site and temporally highly variable sources of both aquatic and terrestrial OC in a deltaic location. Despite these differences, radiotracer-based methanogenesis rate measurements and stable isotopic signatures of methane indicate significant methane production that is dominated by CO_2 reduction (>95% of total methanogenesis) in both locations. Matching this interpretation, members of well-known CO_2-reducing *Methanoregula* sp. dominate both sites. No clear effect of OC source on methane production rates was evident. Our data demonstrate that OC of diverse sources and diagenetic states support microbial methane production, but do not indicate a clear impact of the OC source on the dominant methanogenic pathway or the community structure of methanogenic microorganisms in lacustrine sediments.

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1. Introduction

Lakes represent an important source of methane (CH_4) to the atmosphere (Bastviken et al., 2011), which is a potent greenhouse gas with a global warming potential more than 27 times that of carbon dioxide on a 100-year basis (GWP-100) (Masson-Delmotte et al., 2021). A large fraction of CH_4 produced in lakes is produced during the anaerobic decomposition of organic carbon (OC) in sediments, from where it escapes by ebullition or diffusion into the bottom waters. Methane formation is the final step in the degradation of organic matter, and is catalyzed by anaerobic methanogenic archaea, capable of using several substrates including H_2/CO_2, acetate, and methylated compounds (Lyu et al., 2018). It is still poorly understood how methane production in lake sediments is regulated, and to what extent the methanogenic potential is related to OC quality. In this regard, microbial organic matter degradation reactions (e.g., hydrolysis, fermentation, and anaerobic oxidation) play a critical role by sequentially breaking down organic macromolecules, such as proteins, carbohydrates, and lipids, to acetate and H_2/CO_2, which are then used as substrates by methanogenic archaea (Demirel and Scherer, 2008).

Freshwater lakes cover only a small portion of the Earth's surface (< 3%) (Downing et al., 2006), compared to oceans (71%), yet, the annually accumulated OC in lakes represents nearly half of what is stored in the oceans (Mendonça et al., 2017). In many lakes, most of organic matter in sediments is derived from autochthonous aquatic organisms like phytoplankton and aquatic macrophytes (Dean and Gorham, 1998). On the other hand, allochthonous organic carbon such as detritus of terrestrial vegetation can account for a significant fraction of the lacustrine sedimentary organic matter (Larsen et al., 2011), for example within river deltas (Randlett et al., 2015). In small or oligotrophic lakes and/or high-latitude/altitude lakes, OC sedimentation may in fact be dominated by land-derived organic matter, and it has previously been observed that high carbon burial efficiency in sediments is linked to a high proportion of allochthonous OC (Sobek et al., 2009). In general, OC from aquatic biomass such as phytoplankton mainly comprises relatively labile compounds (Parsons et al., 1961). In

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contrast, allochthonous OC (e.g., terrestrial plants) contains more complex structural and biochemically recalcitrant compounds, such as cellulose and lignin (Opsahl and Benner, 1995), which are more effectively preserved over time (Han et al., 2020, 2022). The different biochemical compositions and characteristics of OC in lake sediments, and the associated differential susceptibility to hydrolytic attack and microbial breakdown into smaller carbon compounds (Lehmann et al., 2002a, 2020), might substantially affect CH_4 production and emission, as has been suggested in previous studies (Grasset et al., 2018; West et al., 2012). However, laboratory experiments performed at short-time scales (i.e., within weeks or months), involving the spiking of sediments with fresh algal and/or plant organic materials, do not accurately reflect effects of enhanced OC contribution to natural sediments on CH_4 production because the compositions and lability of original organic materials may vary greatly after sedimentation due to oxidative destruction and microbial alteration during sinking in the water column (Kawamura et al., 1987). Moreover, allochthonous OC found in lake sediments is often the less-reactive (i.e. less bio-degradable) remnant of land-derived debris, where the more reactive fractions have already been removed on land or during fluvial transport (Raymond and Bauer, 2001). Lastly, while it is known that organic matter bioavailability decreases over time, as labile components are selectively remineralized (Grasset et al., 2018), the effects of organic matter quality and OC degradation state, and their interactions, on CH_4 production remain uncertain.

Lipid biomarkers and their stable carbon isotopic composition can be used to infer sources and diagenetic state of lacustrine organic matter (Dai et al., 2005; Meyers and Ishiwatari, 1993). Different chain lengths of fatty acids and n-alkanes can be used for the distinction between aquatic phytoplankton and terrestrial plants (Cranwell, 1976; Eglinton and Hamilton, 1967). In addition, primary producers growing in different habitats (e.g., terrestrial plants and freshwater phytoplankton) have distinct isotopic compositions, due to the differences in C sources and biochemical pathways, through which inorganic carbon is assimilated and incorporated into biomass (Cloern et al., 2002). For example, terrestrial plants incorporate carbon dioxide from the atmosphere using the C3 Calvin-Benson pathway and consequently have an average bulk isotopic value of ca -28‰, while isotopic values of various aquatic plants are often significantly less negative (e.g., an average of -20‰ for benthic diatoms) (Cloern et al., 2002; O'Leary, 1981). Moreover, stable carbon isotope measurements can be used to trace the carbon flow from organic matter degradation to CH_4 in lake sediments. The isotopic

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signatures of methane and methane precursors (e.g., dissolved inorganic carbon, bulk organic carbon) can be used to assess the relative contribution of the major pathways (i.e., hydrogenotrophic and acetoclastic methanogenesis) to total CH_4 production (Conrad, 2005; Whiticar, 1999). To date it remains unclear, which organic compounds represent the main precursors of molecules that are ultimately converted to methane in older sediments, in which more labile OC fractions, such as OC from microalgal cells, have largely been remineralized already. Previous research in Lake Geneva revealed higher benthic CH_4 fluxes, but lower total mineralization rates of organic matter in deltaic sediments, compared to profundal sites with reduced riverine impact (Randlett et al., 2015). The observed differences imply that despite less efficient total remineralization that leads to elevated OC burial rates in deltaic sediments, high CH_4 production is sustained due to the high input of allochthonous OC (Sollberger et al., 2014).

Here we investigated relationships between methanogenesis rates and pathways, and the sources and degradation state of sedimentary organic matter in profundal and deltaic sediments of Lake Geneva. We combined methanogenic rate measurements by radiotracer incubations with ^{14}C-labeled bicarbonate and acetate with compositional (lipids, pyrolysis-GC/MS) and stable carbon isotopic analyses of organic carbon and CH4, and sedimentation rate measurements (based on Pb-210, Cs-137). Additional quantitative analyses of a methanogenic marker gene (*mcr*A) and analyses of methanogenic community structure (16S rRNA gene sequences) provided insights into the abundances and identities of in situ methanogenic populations. Based on this multi-disciplinary data set, we identified potential relationships between sediment OC sources and degradation status, rates, pathways, and the organisms involved in the microbial production of methane.

2. Material and methods

2.1. Study sites

Lake Geneva is the largest western European lake. The Rhone River, the main tributary to the lake in the northeastern part, has a catchment area of 5220 km^2 and accounts for about 68% of the total water discharge and large amounts of suspended sediment and fine-particle loading to the lake (Burrus et al., 1989). The Rhone River inflow brings in large amounts of terrestrial OC, which is mostly deposited near the

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river mouth as an important contribution to deltaic sediments. On the other hand, sedimentation in the deeper part of the lake is usually dominated by phytoplankton-derived OC (Gallina et al., 2017).

2.2. Sample collection and processing

Sediment cores were collected using a larger gravity corer (14 cm inner diameter) at a profundal site (46°25′54″N, 6°47′33″E, water depth: 240 m) and a smaller gravity corer (6.5 cm inner diameter) at a deltaic site (46°24′58″N, 6°51′34″E, water depth: 128 m) in August and December 2019, respectively. Profundal samples for different analyses were obtained from a single sediment core at 2-cm vertical resolution. Samples for the analysis of dissolved methane concentrations were collected on site with cut-off syringes through pre-drilled holes in the core liner covered with adhesive tape. Sediment samples of 2 cm^3 were fixed with 5 mL 10% NaOH in 120 mL serum bottles, which were capped immediately with thick butyl rubber stoppers and an aluminum crimp cap. Additionally, two replicate samples were taken from the same depth for methanogenesis rate measurements (described below). Sediment porewater was extracted with Rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands) connected to 20-mL syringes through predrilled small holes, with 2 cm distance between them. Porewater samples for DIC concentration measurements and stable carbon isotope analyses were stored in 4-mL glass vials without headspace at 4 °C. Separate porewater sample aliquots for the quantification of acetate and other volatile fatty acids were stored in combusted (450 °C for 5 h) glass vials at -20 °C until further analysis. The sediment core was then extruded in the lab and sectioned into 2 cm segments. Samples were taken from each segment and stored frozen (-20 °C) until further analysis for bulk parameters and lipid biomarkers. Using the gravity corer equipped with the smaller core liner, four sediment cores were obtained from the deltaic site. One core was used for methane concentration measurements, a second one for rate measurements (both at 2-cm resolution, sample collection as described above). The third core was used for porewater extraction in the lab using Rhizon samplers, and the last one was split open for the determination of porosity, analysis of bulk parameters and lipid biomarkers. An additional small-diameter sediment core was taken at the profundal site during the second sampling campaign for porosity analysis.

2.3. Rate measurements of methanogenesis using radiolabeled substrates

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Methanogenesis rates (MGR) were determined using a radioisotope-based approach. We determined the modes of methane production and activity rates in incubation experiments with radio-labeled acetate and bicarbonate. At each depth, samples (2.5 cm^3) were collected through pre-drilled holes using 3 mL cut-off plastic syringes, which were closed with rubber stoppers and stored at 4 °C. Upon arrival of the samples in the laboratory, ten microliters of anoxic ^{14}C-labeled bicarbonate (~16 kBq, Perkin-Elmer) or 2-^{14}C-labeled (i.e., 14C label in the carboxyl group) acetate solution (~18 kBq, Perkin-Elmer) were injected for the MGR measurements to determine hydrogenotrophic (MGR_{DIC}) and acetoclastic (MGR_{Ac}) methanogenesis rates, respectively. Immediately after the tracer injection through the stoppers, all samples including killed controls (i.e., samples that were transferred to 10 mL 5% NaOH solution immediately after tracer injection) were incubated under an N_2 atmosphere at in situ temperature (4 °C) in the dark for 48 h.

To stop microbial activity in the incubations with ^{14}C-labeled substrates, samples were transferred into 120-mL serum bottles containing 10 mL aqueous NaOH (5% wt:wt), immediately crimp-sealed with butyl rubber stoppers and vigorously shaken. The ^{14}C activity in the different carbon pools was determined as previously described (Su et al., 2019). In brief, the headspace of a fixed sample is purged with air (30 mL min^{-1} for 30 min) through a heated (850 °C) quartz tube filled with copper oxide, where the $^{14}CH_4$ (product of methanogenesis during incubation) is combusted to $^{14}CO_2$. The $^{14}CO_2$ is then captured in two sequential traps of scintillation vials (20 mL) containing 8 mL of 1:7 phenylethylamine and methoxyethanol. The cumulative radioactivity of both traps is then determined by liquid scintillation counting (2200CA Tri-Carb Liquid Scintillation Analyzer) after adding 8 mL of a scintillation cocktail (Ultima Gold, PerkinElmer) to each vial, and thorough mixing using a vortex-mixer. For incubations with bicarbonate, the residual ^{14}C-bicarbonate was measured as $^{14}CO_2$, released from the alkaline liquid phase after adding 2.5 mL of 32% HCl. The remaining radioactivity (possibly explained by inorganic carbon assimilation into biomass) was determined in a 1-mL aliquot of the acidified mixture (amended with 4 mL Ultima Gold) by liquid scintillation counting. Incubation bottles with ^{14}C-acetate were also acidified with 2.5 mL of 32% HCl after extraction of $^{14}CH_4$, and $^{14}CO_2$ from microbial acetate oxidation was subsequently purged and trapped as described above. To determine the residual ^{14}C-acetate in the incubation vial, 1 mL of the acidified mixture was mixed with 4 mL Ultima Gold for scintillation counting (Beulig et al., 2018). The control samples were

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processed in the same way as the incubated samples after the termination of incubation. The methanogenesis rates with DIC (nmol cm^{-3} d^{-1}) and acetate (nmol cm^{-3} d^{-1}) as substrates were calculated using Eq. 1 and Eq. 2, respectively, modified from a previous study (Beulig et al., 2018).

$$MGR_{DIC} = 1.08 \times \varphi \times [DIC] \times \frac{A_{CH_4}}{A_{CH_4} + A_{DIC} + A_R} \times t^{-1} \quad (1)$$

$$MGR_{Ac} = 1.08 \times \varphi \times [Ac] \times \frac{A_{CH_4}}{A_{CH_4} + A_{DIC} + A_{Ac}} \times t^{-1} \quad (2)$$

$[DIC]$ and $[Ac]$ are concentrations of DIC and acetate in the sediment porewater, respectively. A_{CH_4} and A_{DIC} represent the activities of produced CH_4 and DIC (i.e., residual DIC in bicarbonate-amended incubations, product DIC in acetate-amended incubations) at the end of the incubation (in CPM). A_R and A_{Ac} represent the remaining radioactivity of DIC-incubated samples (i.e., biomass and metabolic intermediates) and the residual ^{14}C-acetate radioactivity (possibly also including a small fraction of ^{14}C in metabolic intermediates and incorporated into biomass), respectively. φ is the porosity of the sediment samples. The factor 1.08 accounts for the isotopic fractionation of ^{14}C (Hansen et al., 2001). t represents the incubation time in days. Measured $^{14}CH_4$ activities in all incubation samples were blank-corrected by subtracting the $^{14}CH_4$ activity measured in the killed control (typically close to background radioactivity) incubated with the same amounts of ^{14}C-labeled substrates. The activities in the $^{14}CH_4$ pool were considered zero if the blank-corrected value was negative.

2.4. Pore water methane and nutrient analyses

Methane concentrations were measured in the headspace of NaOH-preserved samples using a gas chromatograph (GC, Agilent 6890N) with a flame ionization detector, and helium as a carrier gas. CH_4 concentrations in the wet sediments were then calculated based on the headspace-to-sample volume ratio. The C isotopic composition ($^{13}C/^{12}C$) of methane was determined in the same samples using a pre-concentration unit (TraceGas, Micromass, UK) connected to an isotope ratio mass spectrometer (IRMS; GV Instruments, Isoprime). Stable C-isotope values are reported in the conventional δ notation (in ‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB), with a reproducibility of 0.5‰ based on replicate measurements of methane standards. A carbon analyzer (TOC-L, Shimadzu, Kyoto, Japan) was used to quantify dissolved inorganic carbon (DIC) concentrations in sediment porewaters. Samples were

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manually injected into the DIC reaction vessel and measured with a non-dispersive infrared detector (NDIR) after acidification and volatilization to CO_2. To determine the carbon isotopic composition of DIC, a porewater aliquot of 1-2 mL was introduced into a He-purged exetainer (Labco Ltd) and acidified with ~200 µL 85% H_3PO_4. After 2 h equilibration at 37 °C, the CO_2 released from the aqueous phase was subsequently analyzed using a preparation system (MultiFlow, Isoprime) coupled to an IRMS (Micromass, Isoprime). The standard deviation for replicate measurements of samples and standards was < 0.2‰. Porewater samples for acetate and other volatile fatty acids were analyzed with a two-dimensional ion chromatography (2D IC), as described previously (Glombitza et al., 2014), with modifications reported in (Schaedler et al., 2018). Samples were filtered through pre-washed (10 mL Ultrapure Type I water) disposable syringe filters (Acrodisc® IC grade, 0.2 µm pore size, 13 mm diameter). The first 0.5 mL sample after filtration was discarded before collecting the samples for 2D IC analysis. We used a dual Dionex ICS6000 instrument (Thermo Scientific) equipped with a Dionex AS24 column (2 mm diameter) for the first dimension, and a Dionex AS11HC column (2 mm diameter) for the second dimension. Quantification was done from the conductivity detector signal with a series of 5 external standards between 0.5 and 100 µM.

2.5. Bulk sediment analyses

The total organic carbon (TOC) contents of sediment samples were determined by the difference between total carbon and total inorganic carbon. Samples for total carbon were measured with an Elementar Vario Pyro Cube CN elemental analyser (Elementar, Germany), and samples for total inorganic carbon were analysed by coulometry using a UIC CM5015 coulometer (Joliet, IL, USA). Sediment cores were dated by gamma spectrometry using ^{137}Cs on freeze-dried and ground sediment for the determination of sedimentation rates, as described previously (Randlett et al., 2015). The $\delta^{13}C$-TOC was determined on decalcified samples (Schubert and Nielsen, 2000). Briefly, freeze-dried and homogenized samples were treated with 5 mL 10% HCl in 15 mL Falcon tubes overnight. After centrifugation, the supernatant was discarded, and the solid phase was washed/centrifuged three times with 5 mL MilliQ water. Samples were dried at 50 °C for 48 h prior to analysis. The $\delta^{13}C$-TOC was then assessed by elemental analysis-isotope ratio mass spectrometry (EA, Pyro Cube, Elementar and IRMS, Isoprime, UK).

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The reproducibility based on replicate measurements of standards and samples was better than 0.2‰.

2.6. Pyrolysis gas chromatography-mass spectrometry

The sedimentary organic matter composition was characterized at the molecular level using a pyrolyzer equipped with an autosampler (EGA/PY-3030D and AS-1020E, FrontierLabs, Japan) connected to a gas chromatograph (Trace 1310, Thermo Scientific) and a mass spectrometer (ISQ 7000, Thermo Scientific), following the optimized method (Tolu et al., 2015). Depending on the sample, an aliquot of 2-3 mg of dry sediment was pyrolyzed at 450°C. A data-processing pipeline, including chromatogram smoothing, alignment background correction and multivariate curve resolution by alternate regression was used to automatically detect and integrate the peaks and extract their mass spectra under "R" computational environment (R Core Team 2014). To optimize the number of detected peaks, data processing was performed independently for the sediments from the deltaic site (DS) and the profundal site (PS). Individual peaks were identified using "NIST MS Search 2" software which includes the library "NIST/EPA/NIH 2011", complemented by spectra from published studies. The relative abundances of these identified pyrolytic organic compounds were calculated for each sample by normalization to the sum of their peak areas set at 100%.

2.7. Lipid extraction, separation, and quantification

Based on methanogenesis rate and geochemical profiles, sediment samples from five selected depths of both locations were lyophilized and homogenized for subsequent lipid extraction. Prior to extraction, an internal standard mix (5α-androstane, 3-eicosanone, n-C19:0 fatty acid and n-C19 alkanol) was added to each sample for the quantification of single biomarkers. Lipids were extracted in 20 mL of 7:3 Dichloromethane/Methanol (DCM/MeOH) in a Microwave Reaction System (SolvPro, Anton Paar, Graz, Austria), as described previously (Ladd et al., 2018). Total lipid extracts (TLEs) were obtained by successively rinsing the samples with DCM after centrifugation, and then concentrated using a Multivapor P-6 (Büchi Labortechnik AG, Switzerland). TLEs were further evaporated to dryness, and saponified in 3 mL methanolic KOH-solution (~1 N) at 80 °C for 3 h. Neutral compounds were extracted by liquid-liquid extraction using hexane, fatty acids (FAs) were then extracted from the remaining aqueous phase with hexane after acidification (pH < 2). The fatty acid

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fraction was treated using 1 mL of BF_3 in methanol (14% v/v, Sigma Aldrich) at 80 °C for 2 h, and converted to fatty acid methyl esters (FAMEs). Neutral compounds were further separated into four different fractions using 500 mg/6mL pre-packed Si gel columns (filling quantity/volume, Biotage, Uppsala, Sweden). Briefly, the neutral fraction was dissolved in 4 mL hexane and transferred onto the column, followed by 4 ml hexane/DCM (2:1 v/v), then 4 mL DCM/MeOH (19:1 v/v) and finally 4 mL MeOH, with the elution of hydrocarbon, ketone, alcohol and remaining polar compounds, respectively. The alcohol fraction was acetylated in 25 µL acetic anhydride and 200 µL pyridine at 70 °C for 30 min.

All fractions were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID) (GC-2010 Plus, Shimadzu, Japan). Samples were injected onto an InertCap 5MS/NP column (0.25 mm × 30 m × 0.25 µm, GL Sciences, Japan) using an AOC-20i autosampler (Shimadzu) through a split/splitless injector operated in splitless mode at 280 °C. The column was heated from 70 °C to 130 °C at 20 °C min^{-1}, then to 320 °C at 4 °C min^{-1}, and held at 320 °C for 20 min. FAMEs were identified by comparing their retention times to those of laboratory standards (i.e., a fatty acid methyl ester mix and bacterial acid methyl ester from Supelco, reference no. 47885-U and 47080-U, respectively), and were quantified by normalization to the internal n-C19:0 fatty acid standard. Identification of hydrocarbons was performed by comparing their retention times to those of an external standard containing C14 to C40 n-alkanes (Sigma-Aldrich). The alcohols were characterized using a gas chromatography-mass spectrometer (GC-MS, QP2020, Shimadzu, Japan) under identical chromatographic conditions. Acquired mass spectra were identified through comparison with published data.

2.8. Compound-specific stable carbon isotope analysis

The stable carbon isotope composition of FAMEs, n-alkanes and alcohols was determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS), using a Delta V Advantage IRMS (Thermo Scientific) with a ConFlow IV (Thermo Scientific). Samples were injected with a TriPlus RSH autosampler to a PTV inlet operated in splitless mode at 280 °C on a GC-1310 gas chromatograph (Thermo Scientific, Bremen, Germany). The GC was equipped with a 30 m DB-5MS fused silica capillary column (0.25 mm i.d., 0.25 µm film thickness). The GC oven was heated from 80 °C to 215 °C at 15 °C min^{-1}, then to 320 °C at 5 °C min^{-1}, and held at 320 °C for 10

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min. Column effluent was combusted at 1020 °C. Compound-specific $\delta^{13}C$ values were reported relative to the V-PDB scale, and calibrated externally using known $\delta^{13}C$ values of an alkane mixture (n-$C_{17,\ 19,\ 21,\ 23,\ 25,\ 28\ and\ 34}$, Arndt Schimmelmann, Indiana University, USA), which were run at the beginning and the end of each sequence, as well as after every 6th sample injection. The standard deviation for replicate measurements for these standards averaged 0.4‰, with the average offset from their known values of less than 0.5‰. The isotopic values of FAMEs and acetylated alcohols were additionally corrected for the introduction of carbon atoms during the derivatization step.

2.9. DNA extraction, PCR amplification, Illumina sequencing and data analysis

DNA was extracted from selected sediment samples of Lake Geneva, where high methanogenic rates were detected (see below), using FastDNA SPIN Kit for Soils (MP Biomedicals) following the manufacturer's instructions. A two-step PCR approach was applied to prepare the library for Illumina sequencing at the Genomics Facility Basel, as described in detail previously (Su et al., 2020, 2023). PCR was performed using universal primers 515F-Y and 926R targeting the V4 and V5 regions of the 16S rRNA gene. These primers cover the majority (~84.7%) of the 16S rRNA gene sequences of methanogenic archaea and are well-suited for assessing community structures of methanogens in environmental samples (Table S4). Data were then analyzed with Phyloseq (McMurdie and Holmes, 2013) in the R environment (R Core Team 2014). Raw sequence data were deposited at NCBI Short Read Archive under the Bioproject ID PRJNA736863 with accession numbers from SAMN19667760 to SAMN19667767.

2.10. Quantitative PCR (qPCR)

The abundance of methanogens in Geneva sediments was determined by qPCR with the primer set mcrIRD and 2 µL DNA as template (Lever and Teske, 2015). qPCR reactions of all DNA samples were performed using the SensiFAST SYBR No-ROX Kit (Bioline) on a Mic (Magnetic Induction Cycler) real time PCR machine (Bio Molecular Systems, Inc). An initial denaturing step of 95 °C for 3 min was followed by 40 cycles of 5 s at 95 °C, 10 s at 56 °C, and 22 s at 72 °C. The specificity of the amplification was assessed by examining the melting curves from 72 °C to 95 °C. The calibration curve was generated using a serial of 10-fold dilutions of pGEM-T Easy plasmid DNA (Promega, USA) carrying a single copy of the target gene (mcrIRD-

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F/mcrIRD-R). The number of gene copies in plasmid DNA was calculated using the equation reported previously (Ritalahti et al., 2006).

3. Results

3.1. Sediment geochemistry at the two study sites

The two sites displayed different hydro- and geochemical characteristics (Fig. 1 and Table S1 and Fig. S1). Dating of the sediments revealed that the deltaic site had a slightly higher sedimentation rate (0.58 cm yr^{-1}) than the profundal site (0.43 cm yr^{-1}). At the deltaic site (DS), two distinct peaks in methane concentration (~7 mM) were observed at 9 and 23 cm, respectively (Fig. 1A). At the profundal site (PS), concentrations of CH_4 increased with depth and remained relatively constant at ~4 mM below 15 cm (Fig. 1E). At both sites, the pore water concentrations of dissolved inorganic carbon (DIC) increased with depth, and considerable amounts accumulated in the sediment pore water. The depth-integrated DIC concentrations in the top 30 cm were 1964 mmol m^{-2} at PS and 3490 mmol m^{-2} at DS, however, based on the sedimentation over the past 50 years, the depth-integrated DIC concentrations were 1302 mmol m^{-2} at PS (21 cm) and 3490 mmol m^{-2} at DS (29 cm). Acetate, as a potential methanogenic substrate, showed concentration maxima of 25.9 µM (DS) and 3.8 µM (PS) close to the sediment surface. The methane $\delta^{13}C$ values decreased with depth in both deltaic sediments (from -64.9‰ to -72.2‰, Fig. 1B) and profundal sediments (from -72.7‰ to -74.6‰, Fig. 1F). Overall, the observed methane carbon isotope values at both sites fall within a range that is typical for biogenic production (Whiticar, 1999). Vertical profiles of $\delta^{13}C$-DIC show similar pattern at the two sites, with the lowest values of -5.5‰ at 5 cm at DS (Fig. 1B) and -5.2‰ at 3 cm at PS (Fig. 1F). At PS, TOC concentrations increase slightly with depth, with the mean content almost doubled compared to DS (Fig. 1C and G, Table S1). With respect to its stable carbon isotope composition, $\delta^{13}C$-TOC increased slightly with depth at PS, from -28‰ in surficial sediments to -26‰ below 7 cm whereas $\delta^{13}C$-TOC values remained relatively constant at DS throughout the sampled sediment column (~ -26‰). The elemental C/N ratios in the sediments of DS show a very high variability with depth, and range from 6.7 to 16.8, with a mean value of 10.7 (Fig. 1D and Table S1), while the values at PS are lower and less variant along the sediment core (7.9 ± 0.3, Fig. 1 H).

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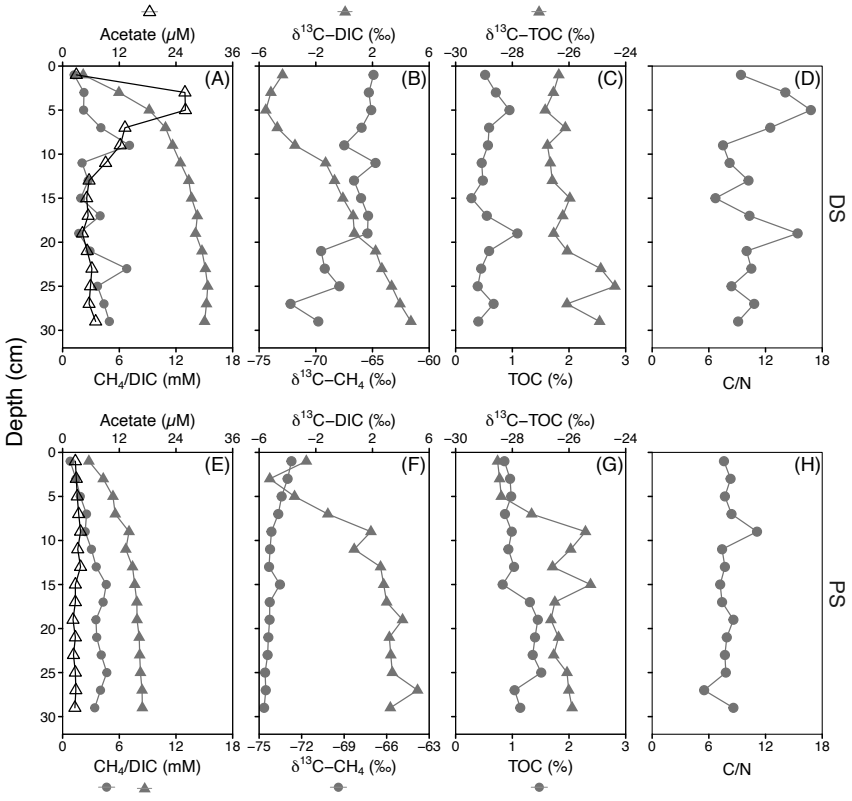

Figure 1. Sediment geochemistry as function of depth at a deltaic site (DS, A-D) and a profundal site (PS, E-H) in Lake Geneva. (A, E) Profiles of dissolved methane, dissolved inorganic carbon (DIC) and acetate concentrations. (B, F) Stable carbon isotopic signatures ($\delta^{13}C$) of CH_4 and DIC (in ‰ vs. V-PDB). (C, G) Concentrations of total organic carbon (TOC, % of dry weight) and its carbon isotopic composition (in ‰ vs. V-PDB). (D, H) Molar ratios of total organic carbon to total nitrogen (C/N).

3.2. Composition of sedimentary organic matter

To investigate the molecular composition of sedimentary organic matter at the two different sites, sediment samples were further analyzed using Py-GC/MS. We identified a total of 65 individual organic compounds (Table S2), which can be classified into the following compound groups: carbohydrates, N-compounds, *n*-alkenes, *n*-alkanes, phenols, lignin oligomers, and (poly)aromatics. Both carbohydrates and N-compounds were the most abundant organic compound groups among the pyrolysis products, with no statistical difference between DS and PS (Fig. 2 A and B). At both sites, the

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carbohydrates consisted mainly of compounds such as butenal, methylfuraldehyde and furanone, and N-compounds were dominated by pyrrole, pyridine, methyl-pyrrole and methyl-pyridine (Table S2), which are indicative of degraded products of carbohydrates and proteins (Schellekens et al., 2009; Tolu et al., 2017). Most strikingly, sediments at DS displayed significantly higher relative abundances of lignin ($p < 0.05$) and phenols ($p < 0.01$) compared to profundal sediments (Fig. 3D and E). In addition, we observed significantly higher relative abundances of *n*-alkenes at PS (Fig. 3C). This group contains monounsaturated short-chain *n*-C_{15} to *n*-C_{18} (Table S2), which were the most abundant organic compounds among the *n*-alkenes.

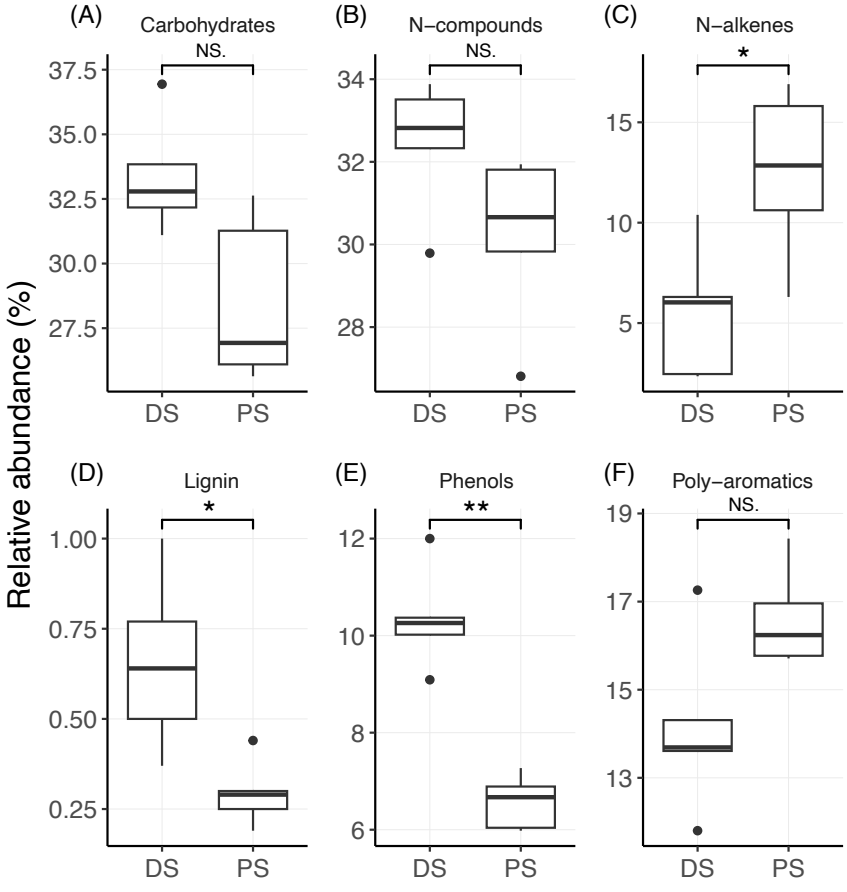

Figure 2. Relative abundances (in %) of different biochemical classes of organic compounds in sediments at the deltaic (DS) and profundal site (PS) of Lake Geneva. (A) Carbohydrates, (B) N-compounds, (C) N-alkenes, (D) Lignin, (E) Phenols and (F)

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Poly-aromatics. Statistical differences of compound abundances between the two sites were determined with the Wilcoxon signed-rank test. Significance levels are: ** $p<0.01$; * $p<0.05$; NS. no significant differences between the two sites ($p>0.05$).

3.3. Lipid concentration, distribution, and stable carbon isotopic signature

Typical lipid biomarkers derived from aquatic phytoplankton (e.g., short-chain fatty acids) and terrestrial plants (e.g., long-chain n-alkanes) were present in both profundal and deltaic sediments, yet concentrations of some of these biomarkers were strikingly different between the two sites, as well as between different depths within the same site (Fig. 3 and Table S3). Among the fatty acids, *n*-$C_{16:0}$ was by far the most abundant in all measured samples, with a slight increase in $\delta^{13}C$ values with depth at both sites. At DS, the unsaturated fatty acids *n*-$C_{16:1\omega7}$ was the most abundant monounsaturated fatty acids with the highest concentration observed at 11cm. At PS, *n*-$C_{16:1}$ ($\omega7$ and $\omega5$) and *n*-$C_{18:1}$ ($\omega9$ and $\omega7$) decreased in concentration with depth and were two to four times more abundant in the upper sediment layers (0-2 cm, 4-6 and 10-12 cm) than in the lower parts (18-20 and 28-30 cm). The short-chain fatty acids were generally depleted in ^{13}C, with stronger ^{13}C-depletions observed in surface sediments, particularly for the unsaturated fatty acids *n*-$C_{16:1\omega5}$ at PS.

In contrast to short-chain fatty acids, $C_{24:0}$ was most abundant among the long-chain fatty acids (i.e., $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$), with relatively higher concentrations found in profundal sediments. Most strikingly, an apparent increase in the concentrations of these long-chain fatty acids was observed in the sediments of PS, with consistently high concentrations in the lower parts (10-30 cm). Meanwhile, the $\delta^{13}C$ values of these compounds decreased dramatically with depth. By comparison, concentrations of long-chain fatty acids at DS were variable at different sediment depths, but their $\delta^{13}C$ values remained relatively constant (e.g., $C_{28:0}$, Fig. 3B) and showed less variability with depth compared with PS (e.g., $C_{24:0}$ and $C_{26:0}$). Although the concentrations of long-chain n-alkanes at DS were twice as high as those at PS, their $\delta^{13}C$ values (particularly for C_{29}, C_{31} and C_{33}) were very similar at the two sites (Fig. 3B).

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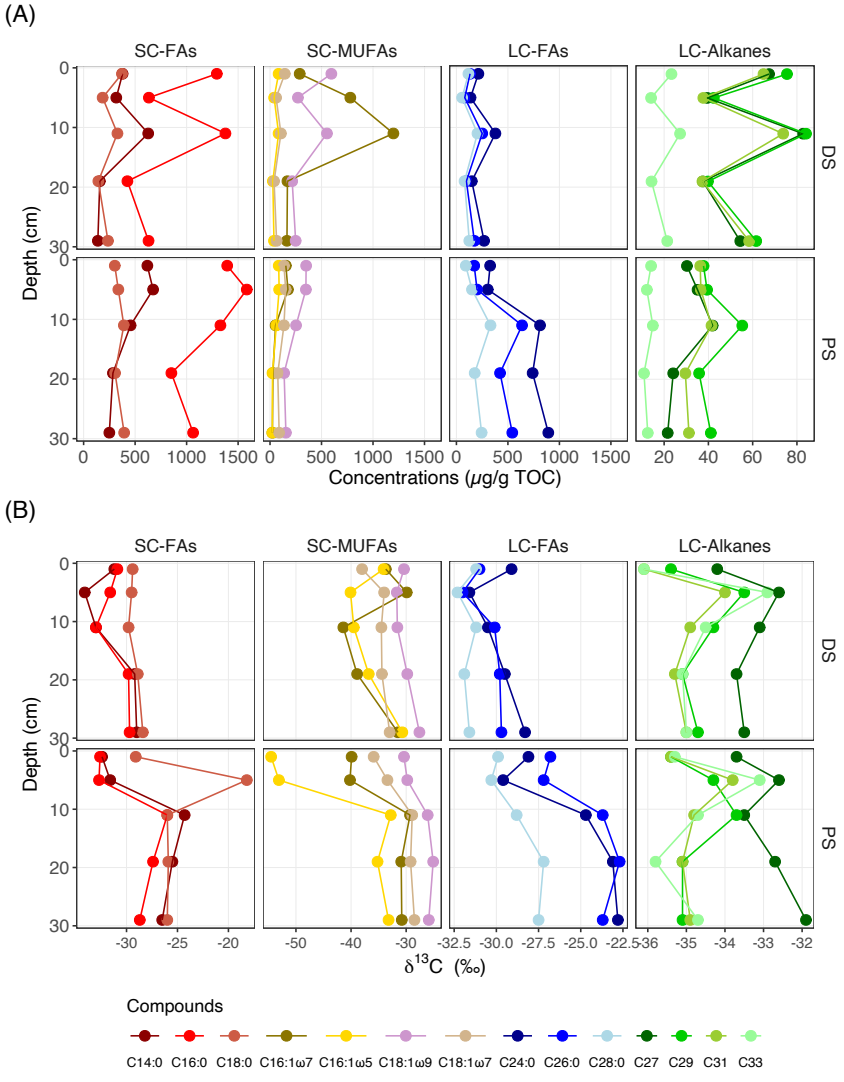

Figure 3. Vertical distribution of specific fatty acids and n-alkanes in deltaic (DS) and profundal (PS) sediments of Lake Geneva. (A) Concentrations (expressed as µg lipid g^{-1} TOC), and (B) compound-specific $\delta^{13}C$ values (in ‰ vs. V-PDB). SC-FAs, short-chain fatty acids ($C_{14:0}$+$C_{16:0}$+$C_{18:0}$); SC-MUFAs, short-chain monounsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$); LC-FAs, long-chain fatty acids ($C_{24:0}$+$C_{26:0}$+$C_{28:0}$); LC-Alkanes, long-chain n-alkanes (C_{27}+C_{29}+C_{31}+C_{33}).

3.4. Relative importance of methanogenic pathways

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Methane production through CO_2 reduction (i.e., hydrogenotrophic methanogenesis) was observed at both sites and at all depths. At the deltaic site, highest activity of hydrogenotrophic methanogenesis was found at the lower part of the sediment core (19-29 cm), while in the profundal sediment, maximum rates of hydrogenotrophic methanogenesis were detected within the upper sediments at depths between 7 and 17 cm (Fig. 4B and E, Table S1). In contrast, the rates of methane production via acetate fermentation (i.e., acetoclastic methanogenesis) were very low, with maxima of 2.8 and 1.0 nmol cm^{-3} d^{-1} observed at 3 cm (PS) and 7 cm (DS), respectively. The areal methanogenesis rates by CO_2 reduction within the top 30 cm, at both sites, were two orders of magnitude higher than acetoclastic methanogenesis rates (Table S1). Acetate oxidation activity was very low at both PS and DS, with the depth-integrated (0-30 cm) rates of 0.1 and 0.2 mmol m^{-2} d^{-1}, respectively (Table S1). At both sites, the fractionation factor α_c, which is approximated based on the difference between the measured δ^{13}C-DIC and δ^{13}C-CH_4 values, increased with depth (Fig. 4F), ranging from 1.064-1.071 (DS) and 1.073-1.084 (PS).

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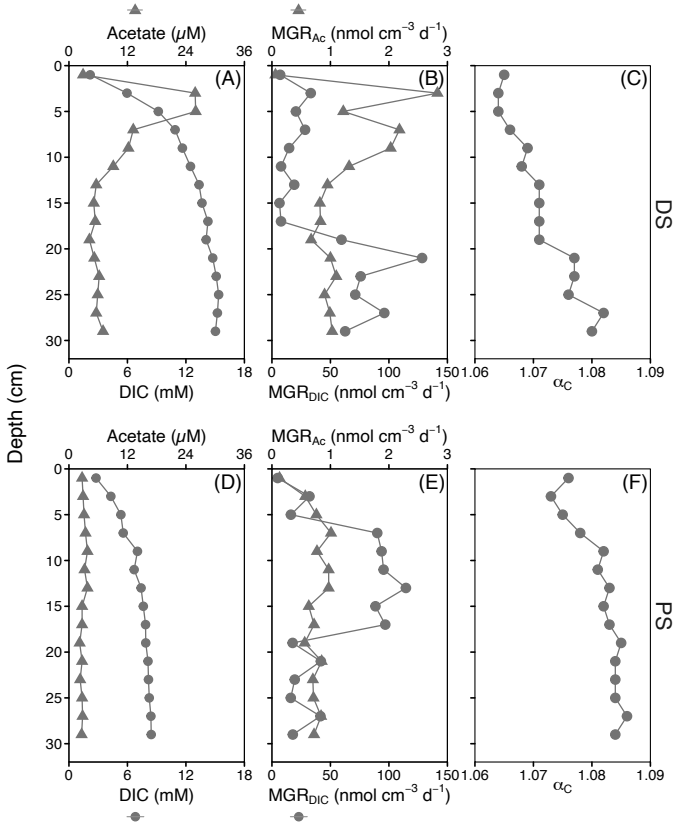

Figure 4. Depth profiles of (A, D) porewater dissolved inorganic carbon (DIC) and acetate, (B, E) CO_2 reduction (MGR_{DIC}) and acetoclastic (MGR_{Ac}) methanogenesis rates, (C, F) apparent fractionation factor α_C in sediments of deltaic (DS) and profundal site (PS) in Lake Geneva. α_c is an approximation of the apparent C-isotope fractionation during methanogenesis, calculated from δ^{13}C-CH_4 and δ^{13}C-DIC values, where α_c = (1000 + δ^{13}C-DIC)/(1000 + δ^{13}C-CH_4). Values of α_c > 1.065 indicate predominance of CO_2 reduction over aceticlastic methanogenesis (Conrad, 2005b).

3.5. Abundance and diversity of methanogenic archaea

On average, gene copy numbers of the methyl coenzyme M reductase gene (*mcrA*) in the deltaic sediments of Lake Geneva were significantly higher than those in the profundal sediments (p < 0.05; Fig. 5A). At both sites, *Methanoregula* and *Methanothrix* dominated the methanogenic guild, followed by *Methanosarcina* and *Methanobacterium* (Fig. 5B). Within the methanogenic community, the mean relative

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abundances of *Methanothrix* and *Methanosarcina* were both higher at DS than those at PS. By comparison, *Methanoregula* was the most abundant genus in the methanogenic community at PS. This matches recent studies on other lakes in Switzerland, in which Methanoregula was found to dominate methanogenic communities in the sediments (Bartosiewicz et al., 2024; Meier et al., 2024). Both *Methanoregula*, *Methanobacterium* and *Methanosphaerula* use H_2/CO_2 as the main substrates for methanogenesis (Oren, 2014a). While *Methanothrix* have long been considered to use exclusively acetate as substrate for methanogenesis (Jetten et al., 1992; Smith and Ingram-Smith, 2007), there is now evidence that some species can also perform CO_2 reduction via direct interspecies electron transfer (DIET) (Rotaru et al., 2014; Zhou et al., 2023). Also, *Methanosarcina* can utilize a broader range of substrates, including CO_2 reduction with H_2 or via DIET, acetate, as well as some methylated compounds (Oren, 2014b).

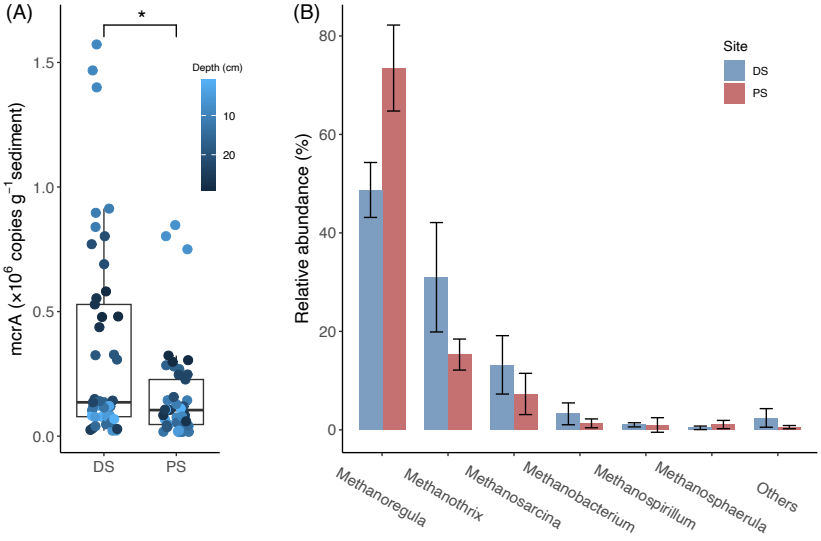

Figure 5. Abundance and diversity of methanogens at the deltaic (DS) and the profundal site (PS) in Lake Geneva. (A) Absolute abundances of the *mcrA* gene encoding the α-subunit of the methyl-coenzyme M reductase. Statistical difference between the two sites was determined with the Wilcoxon signed-rank test and asterisk denotes significance level (*: $p<0.05$). (B) Mean relative abundances of different methanogenic groups (% of the total methanogens). Data are based on read abundances of 16S rRNA gene sequences.

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3.6. Correlation analysis between geochemical parameters, methane production rates, and microbial communities

The alpha diversity measures indicate that the community structure of microorganisms in the sediments at DS were more diverse than at PS (Fig. S2). Principal coordinate analysis (PCoA) revealed significantly different microbial community compositions at the two investigated sites, as indicated by the clear separation of the data by the first principal coordinates, explaining 50.8% and 44.5% of the observed variance for archaea and bacteria, respectively (Fig. S3). For both archaeal and bacterial communities, PCoA plots show a very close aggregation of the deep sediment samples at PS (17, 23 and 29 cm), indicating that their community structures are highly similar. Conversely, there is quite some variance among the samples at the more dynamic deltaic site DS, with its variable deposition history. The measured rates of both CO_2 reduction and acetoclastic methanogenesis tend to be higher in sediment samples harboring a more diverse microbial community (Fig. S4), but the relation is weak and particularly for acetoclastic methanogenesis not significant. Pearson correlation analysis between methane production rates and environmental parameters show that methane production rates from both MGR_{DIC} and MGR_{Ac} was positively but not significantly correlated with the concentration of short-chain n-alkanes (correlation coefficients of 0.46 and 0.51 for MGR_{DIC} and MGR_{Ac}, respectively, Fig. S5). $\delta^{13}C\text{-}CH_4$ values showed a positive correlation with the abundance of carbohydrates, lignin, and long-chain n-alkanes, and significant negative correlation with the abundance of long-chain fatty acids and total organic carbon. C/N ratios were negatively correlated with the concentrations of both short-chain and long-chain fatty acids (Fig. S5).

4. Discussion

Our results indicate a dominance of methane production by CO_2 reduction in both profundal and deltaic sediments of Lake Geneva. This inference is supported by radiotracer measurements, the observed apparent fractionation factors, and methanogenic community analyses, which revealed members of CO_2-reducing *Methanoregula* as the dominant group of methanogens. Thus, CO_2 reduction was observed as the primary MGR process both in sediments at PS, which predominantly contained diagenetically altered phytoplankton-derived OC, and sediments at DS

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characterized by variable sources of aquatic and terrestrial OC. We conclude, therefore, that the dominant pathway of methanogenesis does not primarily depend on the chemical composition of sedimentary organic matter. Other factors that affect production rates of different electron donors (e.g., H_2, acetate) could play a more important role, and will be discussed below.

4.1. Difference in organic carbon sources and diagenetic alteration

The deltaic sediments in this study displayed a strikingly large range of C/N ratios (6.7-16.8), whereas C/N ratios of profundal samples remained relatively constant throughout the sediment core (7.9 ± 0.3). Fresh organic matter from lacustrine phytoplankton, which is protein- rich (Parsons et al., 1961), typically has low C/N ratios of 6-9 (Meyers and Ishiwatari, 1993). In contrast, bulk sediment containing large portions of terrestrial vascular plants often displays much higher C/N ratios, sometimes at least three times greater, due to its high content of high-carbon structural components and refractory organics such as lignin (Hedges et al., 1986). A comparison of carbon isotope and bulk C/N values to data from previous studies (Lamb et al., 2006) confirmed the presence of both aquatic and terrestrial sources in the deltaic sediments, and the dominance of organic matter of autochthonous origin (e.g., algal biomass) at PS (Fig. 6). At DS, variable microbial community structures and significant changes in C/N ratios with depth reflect a highly variable depositional history, indicating differing origins of sedimentary organic matter. Specifically, peak C/N ratios of 16.8 at 5 cm and 15.4 at 19 cm were likely derived from terrestrial organic matter in these intercalated layers, while relatively lower ratios (~10) at other depths suggest a mixture of aquatic and terrestrial sources. Even lower values (~7) in some deltaic sediment layers may indicate predominantly autochthonous deposition of phytoplankton particles.

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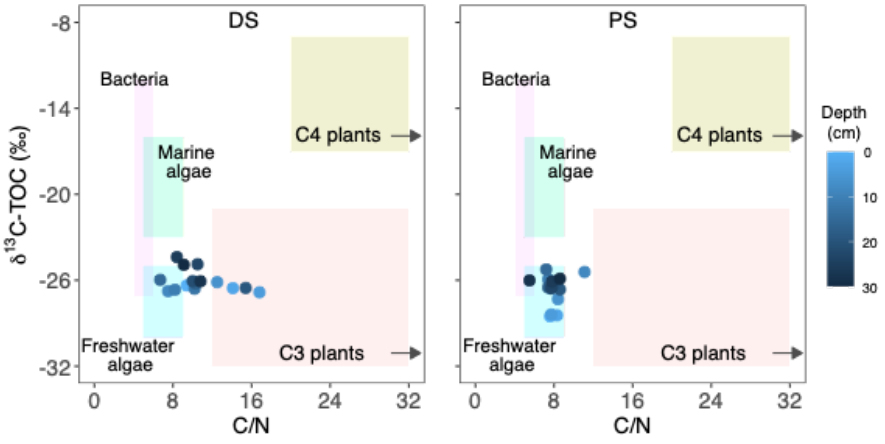

Figure 6. Comparison of $\delta^{13}C$ and bulk C/N values of Lake Geneva sediments from the deltaic (DS, left) and profundal site (PS, right) to elemental and isotopic indicators of bulk organic matter produced by bacteria, marine and freshwater algae, as well as terrestrial C3 and C4 plants (Lamb et al., 2006).

Indeed, at both sites, sediments contained OC from aquatic biomass, as verified by the short-chain fatty acids, indicative of freshwater algae (Cranwell, 1976), and/or in situ production by bacteria (Volkman et al., 1998). However, contrary to the findings from the combined carbon isotope and bulk C/N ratios, both lignin/phenols (Fig. 2) and long-chain n-alkanes (Fig. 3A) derived from terrestrial plants were detected in profundal sediments, although the concentrations of these compounds were lower at PS than at DS. Long-chain fatty acids (i.e., $C_{24:0}$ and $C_{26:0}$), which are typically assumed to originate from terrestrial plant waxes (Cranwell, 1974), make up a proportionally more important fraction of lipids in deeper sediment layers, particularly below 5 cm depth. However, the distinct carbon isotope compositions of these long-chain fatty acids between the two sites (Fig. 3B and Table S3, $\delta^{13}C$ on average ~7‰ lower at DS than at PS) suggest different OC sources.

Indeed, Chikaraishi et al. (2004) reported that long-chain fatty acids (i.e., $C_{24:0}$ and $C_{26:0}$) have $\delta^{13}C$ values of -36.3±2.6‰ for a variety of terrestrial vascular plants, whereas the $\delta^{13}C$ for aquatic plants is significantly less negative (-25.5±0.9‰). At PS, $\delta^{13}C$ values for $C_{24:0}$ and $C_{26:0}$ fatty acids (below 5 cm depth) were close to those indicative of freshwater aquatic plants (Chikaraishi et al., 2004). Previous studies have shown that both aquatic macrophytes and microalgae may represent potential sources

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of long-chain fatty acids (Ficken et al., 2000; Volkman et al., 1998). However, it seems somewhat contradictory to conclude that long-chain fatty acids were primarily derived from aquatic vascular plants, simply because the observed C/N ratios at PS were too low. Indeed, microalgae such as diatoms were estimated to contribute from 30-80% of the $C_{24:0}$ to $C_{28:0}$ fatty acid pool in intertidal sandy sediment (Volkman et al., 1980). Thus, the long-chain fatty acids observed in the profundal sediments may also have been derived from microalgae (Volkman et al., 1998). This, in turn, could explain why TOC was more depleted in 13C in the surface sediments at PS, which does not necessarily indicate the origins of terrestrial plants (Cloern et al., 2002).

The carbon isotopic composition of long-chain n-alkanes (i.e., $C_{27–31}$), characteristic for land-derived organic matter (Chikaraishi et al., 2004), was almost identical between PS and DS (Fig. 3B, ranging from -35‰ to -33‰), suggesting a similar terrestrial source. At DS, if long-chain n-alkanes and long-chain fatty acids were derived from the same terrestrial source, they should have similar C-isotopic values. However, the slightly lower $\delta^{13}C$ values of long-chain fatty acids at DS (on average, -30‰) imply a mixture of both aquatic and terrestrial sources, which is consistent with the C/N ratios observed at this site.

Turning to other OM biomarkers, phytol concentrations were considerably higher at PS (with no clear depth trend) compared to DS, whereas no clear difference in cholesterol concentrations was observed between the sites (with depth-dependent concentration decrease at both sites). For brassicasterol, there seems to be a clear depth-related decrease at both sites, with slightly higher concentrations in surface sediments (0-2 and 4-6 cm) at PS compared to DS. Phytol, a side chain of chlorophyll, can originate from both phytoplankton and terrestrial plants (Shi et al., 2001). Due to its rapid degradation under intense-light and oxic conditions in terrestrial environments, terrestrially derived phytol (or chlorophyll) is generally of minor importance in aquatic sediments (Meyers and Takeuchi, 1981), so it can be assumed that most of the sedimentary phytol is derived from aquatic sources (Ladd et al., 2018). As for cholestanol and brassicasterol (Table S3), the former can originate from both zooplankton and phytoplankton (Bechtel and Schubert, 2009), while the latter is a lipid biomarker mainly derived from phytoplankton in aquatic sediments (Volkman, 1986). Hence, based on at least two of the biomarkers presented here, we have putative evidence for a shift towards more autochthonous OM at the profundal site, as could be expected.

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4.2. Methanogenesis is mainly driven by CO_2 reduction

Our rate measurements using trace ^{14}C-labelled substrates clearly showed that CO_2 reduction played a dominant role in methane formation in both the PS and DS sediments, compared to acetoclastic methanogenesis. As we did not measure rates of methylotrophic methanogenesis, we are unable to determine the relative importance of this pathway. However, since we neither detected common methylated compounds such as methanol in the sediment porewater, nor methanogens that exclusively perform methylotrophic methanogenesis at any of the studied sites, we argue that this pathway is likely of a minor importance with regards to its the contribution to the overall methane production at these sites. Indeed, H_2/CO_2 and acetate are usually the main substrates in freshwater environments (Lyu et al., 2018), and to date, no direct evidence for the occurrence of methylotrophic methanogenesis has been documented for lake sediments. Methylotrophic methanogenesis has been shown to be an important pathway mainly in specific marine systems, where methanogens can utilize methylated compounds (e.g., methanol and trimethylamine) as non-competitive substrates for methane production (Xiao et al., 2018; Xu et al., 2021; Zhuang et al., 2018).

The finding that CO_2 reduction pathway dominated methane production throughout the sediment cores at both sites was further supported by the observed apparent fractionation factor α_c (DS: 1.071 ± 0.001 and PS: 1.081 ± 0.001; Fig. 4C, F and Table S1) (Conrad, 2005). Rates for CO_2 reduction in Lake Geneva were similar at the two studied sites, but were much higher than those previously reported in other lakes at similar depths (Kuivila et al., 1989). The observed low rates for acetoclastic methanogenesis on the other hand were comparable to those in other lake sediments (Kuivila et al., 1989; Schulz and Conrad, 1996). Methane production via acetate fermentation was traditionally believed to play a more important role than via CO_2 reduction in lake sediments (Whiticar, 1999; Whiticar et al., 1986). However, more recent evidence seems to contradict this paradigm, demonstrating that hydrogenotrophic methanogenesis can indeed be a much more significant methane-producing biogeochemical pathway than acetoclastic methanogenesis (Blair et al., 2018; Conrad et al., 2011; Meier et al., 2024). The predominance of methane formation via CO_2 reduction over acetoclastic methanogenesis is an important finding, which, in Lake Geneva, appears to apply to sediments across different sedimentary depositional regimes.

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At both sites, *Methanoregula* was the most abundant methanogenic genus, particularly in deep sediments where the highest CO_2 reduction rates were observed. This genus, commonly found in freshwater lakes (Bartosiewicz et al., 2024; Berberich et al., 2020; Meier et al., 2024), is known to perform hydrogenotrophic methanogenesis. *Methanothrix*, the second most abundant methanogen, primairly performs acetoclastic methanogenosis, although some species within this cluster can also produce methane via CO_2 reduction (Rotaru et al., 2014). At PS, *Methanoregula* dominated at all investigated depths, in accordance with high rates of autotrophic methanogenesis. While acetoclastic methanogenesis played only a minor role in total methane production, higher rates were observed for deltaic sediments, with lower apparent fractionation factor. This observation is consistent with higher concentrations of acetate and a higher relative abundance of acetate-comsuming *Methanothrix*, suggesting that the pathway of acetoclastic methanogenesis was influenced by both the availability of acetate and the functional methanogenic population.

Although overall methane production rates were similar at both sites, the qPCR data in hand indicated that bulk methane production was not primarily/directly controlled by (i.e., proportional to) the cell abundance of methanogens. Instead, it was likely regulated by other environmental factors, such as limited substrate availability/supply (i.e., H_2 and acetate), stemming from rates of hydrolysis (Kristensen et al., 1995) and/or fermentation rates (Valentine et al., 1994) within the sediments. Methanogens depend on syntrophic and other heterotrophic bacteria, which may significantly influence methane production rates (Beulig et al., 2018; Liu and Whitman, 2008).

In Lake Geneva sediments, rates of both CO_2 reduction and acetoclastic methanogenesis increased with microbial Shannon diversity. More diverse microbial communities tend to possess more diverse organic matter degradation capacities, resulting in higher production of acetate and H_2/CO_2, which in turn promotes overall methane production (Conrad, 2020). However, our microbial abundance and diversity data were insufficient to fully characterize the carbon metabolism of microorganisms involved in H_2 and acetate production, which could directly impact CO_2 reduction and acetoclastic methanogenesis and potentially explain the observed methane production differences. The very low acetate oxidation rates at both sites suggest that differences in methane generation via MGR_{DIC} versus MGR_{Ac} may be attributed to the relative availability of methane precursors available (i.e., H_2/CO_2 versus acetate) (Capone and

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Kiene, 1988). The acetate concentrations were low (below 26 µM) at both sites, but well within the range of what has been measured in profundal sediment of Lake Constance, where acetoclastic methanogenesis was dominant (Schulz and Conrad, 1996). Hence, the consistently low rates of acetoclastic methanogenesis throughout the sediment cores at both sites likely resulted from low turnover rates of acetate during organic matter degradation.

4.3. Effect of organic carbon characteristics on methanogenesis

Despite the differences in OC sources and quality between the two sites, the overall methane production rates were comparable (Fig. 4B, E and Table S1). However, the sedimentary zones of maximum methane production and the depth-dependent rates of both metabolic modes of methanogenesis varied. This likely reflects differences in OC quality and degradation pathways, which influence the balance between remineralization to H_2/CO_2 versus acetate (Conrad, 2020), resulting in spatial variability in sedimentary methane production.

Potential methane production rates have been shown to correlate positively with the quantity of sediment organic matter (Berberich et al., 2020). However, at both the DS and PS in Lake Geneva, the sediment layers of maximum rates of CO_2 reduction did not coincide with higher TOC contents. Instead, combined rate and isotopic measurements revealed that methanogenesis rates via CO_2 reduction corresponded to less ^{13}C-depleted bulk organic carbon (Fig. 1 and 4). This suggests that the source and microbial decomposition of organic matter, rather than its quantity, primarily influenced the methanogenic activity via the CO_2-reduction pathway.

Methane production rates in lake sediments are generally low across various latitudes but can increase significantly with the addition of fresh organic carbon (Bartosiewicz et al., 2024; Schwarz et al., 2008; West et al., 2012). Indeed, high lipid contents in phytoplankton biomass have been shown to enhance methane production in both engineered systems and lake sediments (West et al., 2015; Zhao et al., 2014). Additionally, existing evidence suggest that terrestrial OC can stimulate methane ebullition in reservoirs (DelSontro et al., 2011; Sobek et al., 2012). Our findings from rate measurement and organic carbon composition agree with earlier studies, indicating that both aquatic and terrestrial OC can significantly contribute to methane production (Berberich et al., 2020; Grasset et al., 2018). It has also been suggested that the availability of easily degradable organic matter controls both methanogenic pathways

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and methanogenic archaeal communities (Liu et al., 2017). However, the influence of organic matter source or composition on the relative importance of methane production pathways and their corresponding rates remains poorly constrained and has rarely been explored. Within the zone of high hydrogenotrophic activity at PS, TOC became less depleted in ^{13}C, a trend also observed, though less pronounced, in the deltaic sediments. The $\delta^{13}C$ shift of TOC within the most active zone at PS likely resulted from the selective loss of isotopically light OM fractions (Lehmann et al., 2002b; Meyers, 1994), or the changes in organic matter sources, as suggested by variations in both short-chain and long-chain fatty acids indicative of autochthonous origins.

Contrary to previous studies demonstrating that algal deposition stimulated the activity of acetoclastic methanogens (Schulz and Conrad, 1995; Schwarz et al., 2008), our results revealed higher acetate concentrations in tandem with higher acetoclastic methanogenesis occurring in surface sediments of deltaic site, where high C/N ratios indicated terrestrial inputs. This suggests that allochthonous OC may play a role in acetate production and acetoclastic methanogenesis. However, the apparently much lower overall acetoclastic rates compared CO_2 reduction were likely due to the active acetate consumption via syntrophic oxidation (Conrad et al., 2020). At PS, autochthonous organic matter was the dominant OC source to the sediments, and the CH_4 produced likely resulted from the decomposition of buried algal detritus (Fig. 6). This interpretation is also supported by the 2-fold higher brassicasterol, the 3-fold higher phytol, and 3-4-fold higher long chain fatty acids concentrations (with elevated $\delta^{13}C$ values) at PS compared to DS. These biomarkers, in combination with lower C/N ratios, are indicative of algal material as the dominant organic contributor to early diagenetic methane production.

While the dominant sedimentary organic matter source (i.e., the partitioning between autochthonous versus allochthonous OM inputs to the sediments) varied between the two studied sites, the similar overall methane production rates imply that methanogenesis occurred ubiquitously in these lacustrine sediments, independent of the OC sources, their apparent susceptibility to organic matter degradation, and/or their overall diagenetic state. This finding aligns with a recent study across the river/deltaic-pelagic continuum in a reservoir system in southwest Ohio (Berberich et al., 2020), and holds important implications for understanding the lacustrine methane cycle. On the one hand, eutrophication in lakes and other aquatic systems often increases the supply of autochthonous organic carbon, thereby enhancing methanogenesis and leading to

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increased methane emissions (Davidson et al., 2018). On the other hand, the potential of terrestrial organic matter to contribute to lacustrine methane production can be significantly impacted by anthropogenic activities, such as dam construction (Li et al., 2023), or as a result of deforestation and soil erosion, which alter sediment inputs into the lake (Bélanger et al., 2017). Difference in the source and lability of organic carbon in the lacustrine organic matter pool may affect sediment methane production in distinct ways (Grasset et al., 2018; Stibal et al., 2012). Our study demonstrates that diverse OC sources and varying diagenetic states in both profundal and deltaic sediments support substantial rates of methane formation through CO_2 reduction. However, identifying the exact fractions of organic matter that were degraded and fueled methane production remains challenging. While acetoclastic methanogenesis appears to be relevant to the break-down and decomposition of terrestrial organic matter that favors acetate production and accumulation in deltaic sediments, the sources of organic carbon driving the more dominant CO_2-reducing methanogenesis in the studied sediments remains unclear. The key environmental factors controlling the organic matter degradation in methanogenic sediments remain unresolved and require further investigation. Understanding these mechanisms is essential for predicting how changes in organic carbon inputs and sediment dynamics may influence methane production and emissions from lacustrine environments.

5. Conclusions

Our study demonstrates that both profundal and deltaic sediments of Lake Geneva are significant sources of methane, despite clear differences in origins and compositions of organic matter. The profundal site is dominated by aquatic OM, while the deltaic site features a more variable mix of OM sources, including substantial terrestrial contributions at certain depths. Methane production at both sites is overwhelmingly driven by CO_2 reduction, which accounted for over 95% of total methane production and was most probably mediated by *Methanoregula*. While phylogenetic data suggest a link between methanogen communities and methanogenetic pathways, the broader microbial community dynamics, particularly those involved in H_2 and acetate production, remain insufficiently understood.

At the profundal site, methane production is mainly associated with the decomposition of aquatic organic matter, as terrestrial OC is less abundant. At the

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deltaic site, acetoclastic methanogenesis appears linked to the higher terrestrial organic matter inputs. Nevertheless, the overall dominance of methanogenesis via CO_2 reduction at both sites suggests that depositional regime, as well as OM source and composition are not the primary determinants of the prevailing methane producing pathway. Instead, factors such as the metabolic interactions within microbial communities (e.g., with syntrophic partner organisms), particularly the balance of H_2 and acetate production, may play a larger role. Future research should focus on unraveling these interactions to better predict if, and how, changes in OM inputs and sediment dynamics may impact methane emissions from lacustrine environments.

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Data availability statement

Raw reads of the 16S rRNA sequencing data are available on NCBI GenBank with BioProject ID PRJNA736863, under accession number SRR14794332- SRR14794339.

Author contributions

CJS, GS and MAL conceived the research. CJS acquired funding for this study. GS performed field work, lab experiments and data analyses. JT assisted with Pyrolysis-GC data analysis. CG and MAL measured porewater volatile fatty acids and analyzed the data. JZ and MFL helped with rate measurements and molecular analyses. GS wrote the original draft of the manuscript. All authors revised and approved the submitted version.

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Competing interests

At least one of the (co-)authors is a member of the editorial board of Biogeosciences.

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