3 Methods

3.1 Water sampling and analyses

3.1.1 Physicochemical parameter measurements and water column sampling

A water quality monitoring and profiling probe (YSI 6600 V2-2) was used—prior to sampling—to measure conductivity, temperature, O_2 concentrations, pH, and Eh in the stratified portion of the water column of Lake Medard (from 47 to 55 m depth) in its central location (Fig. 1a, star). The probing resolution was 1 m above and below the O_2 minimum zone and 0.5 m at the redoxcline. Water column samples (n = 8 and 4 replicates) were collected in November 2019 using a Ruttner sampler with a capacity of 1.7 L. Flushing/rinsing of the sampling device with distilled water (dH_2O) was performed between samples. A total of eight samples were taken at 47, 48, 48.5, 49, 50, 52, 54 and 55 m depth. Replicate samples were taken at 47, 48.5, 50 and 54 m depth. On aliquots of our water samples, we performed (i) environmental DNA (eDNA) extraction followed by MiSeq Illumina 16S rRNA gene amplicon sequencing; (ii) mass determinations of cations (iron, manganese, potassium, sodium, magnesium and calcium); (iii) high pressure liquid chromatography for concentrations of chlorine, sulfate, nitrate, ammonium and phosphate anions, and VFA abundances; (iv) measurement of dissolved inorganic carbon and methane concentrations, and (v) isotope ratio analyses of $\delta^{13}C$ in total dissolved inorganic carbon and methane; and (vi) isotope ratio analyses of $\delta^{34}S$ and $\delta^{18}O$ values in dissolved sulfate. Details on these analyses follow.

3.1.2 Environmental DNA (eDNA)

For each eDNA sampling depth, an aliquot of 1 L was transferred to polyethylene (PET) bottles using a hand pump connected to sterile a Sterifil® Aseptic System loaded with sterile cellulose nitrate Whatman® Microplus-21 ST filters (0.45 μm cutoff, 47 mm diameter). The filters were separated from the filtrating apparatus using a pair of sterilized tweezers (70% ethanol and Bunsen burner) and transferred into sterile 2 mL CryoTube vials (Thermo Scientific). These were store into liquid N for transport to the lab, where the DNA extraction from the biomass collected on the filters took place. After each sample collection, the filtration system was rinsed 3 times with dH_2O, and a new filter was carefully placed onto the system. Samples for rRNA gene analyses were collected from the two redox compartments of the lake: i.e., the dysoxic hypolimnion and anoxic monimolimnion. The rinsing water (1 L) prior to second-last sampling (52 m) was used as a control for eDNA.

3.1.3 Microbiome profile

DNA was extracted from the water filters described above using Quick DNA Soil Microbe Kit (Zymo Research) according to the manufacturer's instructions. A total of 11 water samples (i.e., 47 to 54 m depth and replicates) were evaluated. The eDNA extracted from these samples was ≥ 6 ng as per Qubit dsDNA BR fluorometric assays (Life Technologies), and below limits of quantification (<L.Q.) for the control (i.e., nucleic acids <0.2 ng). DNA integrity was assessed by agarose gel (2%) electrophoresis.

A two-step PCR protocol targeting the small subunit 16S rRNA gene in bacteria and archaea was conducted using the universal primer combinations 341F/806R (CCTAYGGGRBGCASCAG and GGACTACNNGGGTATCTAAT) and 519F/915R (CAGCCGCCGCGGTAA and GTGCTCCCCCGCCAATTCCT), respectively. The samples were sequenced on the MiSeq Illumina platform. The 16Ss rRNA gene amplicon datasets were analyzed with a pipeline consisting of an initial step where all reads passing the standard Illumina chastity filter (PF reads) were demultiplexed according to their index sequences. This was followed by a primer clipping step, in which the 16S target forward and reverse primer sequences for bacteria and archaea were identified and clipped from the starts of the raw forward and reverse reads. Only read pairs

exhibiting forward and reverse primer overlaps were kept for merging by using FLASH 2.2.00 (Magoč and Salzberg, 2011). This yielded a total of 1,799,339 high-quality sequence reads, with an average length, after processing, of 412 bp.

Sequence features (herein described as representative operational taxonomic units, OTU) were clustered using QIIME2 (VSEARCH cluster-features-de-novo option; Rognes et al., 2016). To assign taxonomic information to each OTU, we performed DC−MEGABLAST alignments of cluster-representative sequences regarding the NCBI sequence database (Release 2019−10−10). A taxonomic assignment for each OTU was then transferred from the set of best-matching reference sequences (lowest common taxonomic unit of all best hits). Hereby, a sequence identity of >70% across at ≥ 80% of the representative sequence was a minimal requirement when considering reference sequences. We assigned significant tentative correspondence of OTUs to reference species provided that an identity threshold ≥ 97 % of the V3−V4 hypervariable region for bacteria and V4-V5 for archaea were meet. Further processing of OTUs and taxonomic assignments (75.8% of the sequences after chimera detection and filtering; Edgar et al., 2011) and read abundance estimation for all detected OTUs was performed using the QIIME2 software package (version 1.9.1, http://qiime.org/). Abundances of bacterial and archaeal taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates (Angly, 2014). The metagenomic data for this study (total 1,799,339 read base pairs, lengths ≥ 402 bp) were deposited in the European Nucleotide Archive (ENA) at EMBL−EBI under accession number PRJEB47217.

3.1.4 Cation concentration analyses

For cation concentration analyses, aliquots of 15 mL were filtered using sterile high flow, 28 mm diameter, polyethersulfone (PES) filters to remove particles >0.45 µm and then placed in acid-cleaned, PET centrifuge tubes. The aliquots were acidified using concentrated trace metal grade HNO_3. At the lab these water aliquots were digested with trace metal grade HNO_3 (8 N) and analyzed at the Pôle Spectrométrie Océan at IUEM in Brest, France. A Thermo Element2 high resolution inductively coupled plasma mass spectrometer set on solution mode was used. The data were calibrated against multi-element standards at concentrations of 0.5, 5, and 50 ppb that were measured repeatedly throughout the session. Multi-element solutions were measured at the beginning, end, and twice in the middle of the sequence and the 5 $\mu g \cdot L^{-1}$ standard was further repeated after every five samples throughout the sequence. Additionally, 5 ppb indium (In) was added directly to the 2% HNO_3 diluant employed to prepare all solutions and was used to monitor signal stability and correct for instrumental drift across the session. Each sample and standard were bracketed by a rinse composed of the same diluant (i.e., the 2% HNO_3 with In) for which data was also acquired to determine the method detection limit. Relative standard deviations (2σ level) were better than 0.01 wt. % for Fe and Mn, and between 0.001 and 0.002 % for the rest of the analyzed elements, e.g., K, Na, Mg, Ca which concentrations were used for aqueous mineral equilibrium modeling (Appendix A, Supplement 2).

3.1.5 Ions, ammonia, and VFAs concentration analyses

Alkalinity (i.e., the capacity of water to neutralize free hydrogen ions, H^+) was measured as HCO_3^- through acidometric titration of filtered water samples. Titrations were conducted on board immediately upon sample collection by using 1.16 N sulfuric acid cartridges on a digital titrator (Hach). Ions, ammonia and VFAs concentrations were measured in filtered, unacidified water sample aliquots via high pressure liquid chromatography (HP-LC) at BC-CAS, České Budějovice. For analyses a ICS5000 + Eluent Generator (Dionex) with conductivity detection application and suppression was used. Separations were made on Dionex IonPac AS11-HC-4 µm (anions, VFAs) and IonPac CS16-4 µm (ammonium), both in 2x250 mm size. The flow rate was 0.36 mL/min; run time was 65 min (anions, VFAs) and 17 min for ammonium. Potassium hydroxide was the eluent for inorganic anions and monovalent organic acids; methanesulfonic acid was the eluent for ammonium ion detection/quantification. A combined stock calibration standard solution featuring environmentally relevant anions ratios was used for determining concentrations and was prepared from corresponding analytical-reagent grade salts. . To optimize and calibrate the method for VFA analyses and determine the limits of detection, we used stock mixtures of IC grade formate, oxalate, acetate, lactate, pyruvate, and butyrate standards for preparing our working saline stocks solutions.

Detection limits were better than 60 ppb for lactate and oxalate, and 200 ppb for pyruvate, formate, and acetate. Recoveries, based on these standards, exceed 80 % for all anions and VFAs reported

3.1.6 Dissolved (in)organic carbon and methane

Aliquots of the lake water collected were immediately transferred from the sampler to pre-cleaned (i.e., three-times rinsed with ddH_2O and oven-dried at 550 ºC) 12 mL glass exetainer septum capped vials (Labco), pre-filled with He(g) and 1mL NaCl oversaturated solution (40%) for CH_4, or 1 mL 85% phosphoric acid for ΣCO_2. On board, the vials were filled with ~11 mL water samples using a syringe connected to 15 cm PES tube that was introduced from below into the sampler for preventing any diffusion of atmospheric gases into the exetainer vials.

A dissolved inorganic carbon (ΣCO_2) concentration profile was produced using a peak area calibration curve obtained on a MAT253 Plus isotope ratio mass spectrometer (IR-MS; Thermo Scientific). The same instrument was used for also determining isotope ratios of ΣCO_2 ($\delta^{13}C_{\Sigma CO2}$, $\delta^{18}O_{\Sigma CO2}$) and methane ($\delta^{13}C_{CH4}$), and for a rough estimation of the CH_4 concentrations at the monimolimnion. In brief, CO_2 (or CH_4) is purged from the headspace of the exetainer vials, then the gas passes through a Nafion water trap and into a sample loop PoraPlot-Q column (0.32 mm ID) cooled in liquid N; with He as the carrier gas. The sample gases are then separated via a Carboxen PLOT 1010 (0.53 mm ID; Supelco) held at 90°C with a flow rate of 2.2 $mL \cdot min^{-1}$ and transferred via a Conflo IV interface to the instrument. For methane, prior to transfer to the IR-MS, the sample is transferred via a multi-channel device to a nickel oxide conversion reactor tube with copper oxide as catalyst (1,000°C). $\delta^{13}C$ values obtained relative to CO_2 working gas are then corrected for blanks and linearity and normalized to laboratory working standards calibrated against CO_2 evolved from the international standard IAEA-603.

The concentration measurements have an error (1σ) < 4 % for ΣCO_2 and < 25 % for CH_4. Isotope data are expressed in delta notation, $\delta = R_{sample}/R_{standard} - 1$, where R is the mole ratio of $^{13}C/^{12}C$ or $^{18}O/^{16}O$ and reported in units per mil (‰). The $\delta^{13}C$ data are reported *vs*. the Vienna Pee Dee Belemnite (V-PDB) standard. The $\delta^{18}O$ data are reported *vs.* the international Vienna Standard Mean Ocean Water (V-SMOW) standard. The reproducibility of the $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CH4}$ measurements was better than ±0.05 ‰ and ±0.3 ‰ (1σ), respectively, based on replicates for reported values of the standard materials and the samples. Reproducibility of $\delta^{18}O_{\Sigma CO2}$ measurements is better than 0.4 ‰. DOC was analyzed in untreated samples by catalytic combustion at 680 °C (Shimadzu 5000A, Japan) with a detection limit of ~0.05 $mg \cdot L^{-1}$.

3.1.7 Dissolved sulfur analyses

For measuring dissolved acid-volatile sulfur (AVS) in the monimolimnion (i.e., HS^-, intermediate sulfur species, H_2S and the aqueous FeS clusters; Rickard & Morse, 2005), 500 mL aliquots of water samples collected at the 52-54 m depth interval were transferred to PET sample bottles pre-filled with 2 mL of 1 M Zn acetate, then 50 mL of 5 M NaOH were added. The combined concentrations of AVS bound into the ZnS precipitates were spectrophotometrically determined in an acidified solution of phenylenediamine and ferric chloride by using a Specord 210UV/Vis (Analitik, Jena). Detection limit of the method is ≥ 0.25 µM.

As for cation analyses, the 1L aliquot of the filtered water samples were intended for sulfate S and O isotope analyses. These samples were acidified to a pH ~3 with 6N reagent grade HCl. Also, to oxidize and degas dissolved organic matter, we added 6 ml of hydrogen peroxide (H_2O_2) 6 % and heated the samples (90 °C) until clear (i.e., 1 to 3 h). Dissolved sulfate was then precipitated as purified barite ($BaSO_4$) by using a saturated $BaCl_2$ solution. Accordingly, after heating, ~5 ml of 10 % $BaCl_2$ was added to the water samples that were then allowed to cool down overnight. An additional 1mL of $BaCl_2$ solution was added the next day to ensure that all possible $BaSO_4$ precipitated. The precipitates were then collected on pre-weighed membrane filters, rinsed thoroughly using deionized water, stored in plastic petri dishes, and dried in a desiccator using a sulfate-free desiccant, the dry $BaSO_4$ powder was scraped into clean vials, weighted, and stored until shipped to the Biogéosciences Laboratory, Dijon, France, for solid phase isotope analysis.

Each purified $BaSO_4$ sample was analyzed for $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$. Samples were measured on an Elementar Vario PYRO cube elemental analyzer in-line with an IsoPrime 100 IR-MS in continuous flow mode. The SO_4 isotope data are expressed in in the δ-notation, $\delta \equiv R_{sample}/R_{standard} - 1$, where R is the mole ratio reported in units per mil (‰) relative to the Vienna Canyon Diablo Troilite (V-CDT) and V-SMOW standards for $^{34}S/^{32}S$ and $^{18}O/^{16}O$, respectively. Analytical errors are better than ± 0.4 ‰ (2σ) based on replicate analyses of the international barite standard NBS-127, which was used for data correction via standard-sample-standard bracketing. International standards IAEA-S-1, IAEA-S-2 and IAEA-S-3 were used for calibration, with a reproducibility better than 0.3 ‰ (1σ) based on replicates for reported values of the standard materials and samples.

3.2 Sediment samples

We also sampled the upper anoxic sediment column to a depth of ~8 cm. The mineralogy of these fine-grained sediments (silt to clay in size). was qualitatively and semi-quantitatively assessed via X-ray diffraction (XRD). The $\delta^{34}S$ and $\delta^{18}O$ of gypsum ($CaSO_4 \cdot 2H_2O$), $\delta^{13}C$ of siderite ($FeCO_3$), and $\delta^{34}S$ isotope values of pyrite (FeS_2) from these sediments were also measured and reported as described above using the delta notation, $\delta = R_{sample}/R_{standard} - 1$, where R is the mole ratio. Scanning electron microscopy aided by electron dispersive spectrometry (SEM-EDS) was used for textural analyses focused on the S- and/or Fe-bearing phases. In addition, a sequential extraction scheme (after Poulton et al., 2004; Goldberg et al., 2012) was conducted to characterize the sedimentary partitioning of reactive Fe and Mn fractions. Further details follow.

3.2.1 Sampling

Replicate sediment cores (~16 cm in length) were collected with a messenger-activated gravity corer attached to 20 cm-long polycarbonate tubes (5 cm in diameter). The cores were immediately sealed upon retrieval with butyl rubber stoppers, preserving about 3 cm of anoxic lake water. The head water showed no signs of oxidation (i.e., no reddish hue observed) upon transport—within ~6 h from collection—to the lab. The sediment pile was extruded and sectioned each 2 cm. Surfaces of the silty clayey sediment in contact with the core liner were scrapped to remove potential contamination from the lake water and to minimize smearing effects. The sediment subsamples were rapidly frozen using liquid N and then stored at −18 ºC until freeze-dried. We interrogated the upper part of the sediment pile to a depth of 8 cm (i.e., 4 replicate samples).

3.2.2 Mineralogy

The mineralogy of the sediment was determined, semi-quantitatively, via X-ray diffraction (XRD). Powder XRD data were collected on a D8 Advance powder diffractometer (Bruker) with a Lynx Eye XE detector, under a Bragg-Brentano geometry and Cu K_1 radiation (λ=1.5405 Å). Collection in the 2Θ range 4−80° was performed using 0.015° step-size increments and 0.8 s collection time per step size. Qualitative phase analyses were performed by comparison with diffraction patterns from the PDF-2 database. A semi-quantitative phase analysis was performed by the Rietveld refinement method (Post & Bish, 1989), as implemented in the computer code Topas 5 (Bruker). The crystal structure of the mineral phases used for refinement were obtained from the Inorganic Crystal Structure Database (ICSD) database. During Rietveld refinement, only the scale factors, unit-cell parameters, and size of coherent-diffracting domains were refined. A correction for preferred orientation was applied for selected mineral phases (i.e., K-feldspar, mica, gypsum).

The abundance of sedimentary Fe- and Mn-bearing phases was established by applying a sequential extraction scheme aiming to quantify the contribution of the operationally defined reactive pool capable of reacting after reductive dissolution with sulfide (after Poulton and Canfield, 2005). The wet chemical extraction scheme was applied to liberate (i) the fraction of total acid volatile sulfur (AVS) in the sediment, which might consist of mackinawite, a portion of greigite, and an usually unknown, yet typically negligible fraction of pyrite (Rickard and Morse, 2005); and (ii) chromium reducible sulfur (CRS), consisting primarily in pyrite but also in the sediment intermediate sulfur compounds (Canfield et al., 1986). AVS was extracted with cold concentrated HCl for 2 h. Then, the resulting hydrogen sulfide concentration (i.e., between 0.004 and

0.036 wt. %) was precipitated as Ag_2S by using a 0.3 M $AgNO_3$ solution. Subsequently, CRS was liberated using a hot and acidic 1.0 M $CrCl_2$ solution (Canfield et al., 1986). The resulting H_2S was trapped as Ag_2S. Mass balance after gravimetric quantification was used to calculate the amount of AVS and CRS. Concentration analyses of Fe and Mn dissolved in each of these extracts were conducted via ICP-MS measurements (Xseries II, Thermo Scientific) at the Department of Environmental Geosciences, Czech University of Life Sciences, Prague.

3.2.3 Sedimentary geochemistry and stable S and C and O isotope analyses

Aliquots of the sediment samples were analyzed for total S (S_{tot}) concentration using a CS analyzer (ELTRA GmbH). The detection limit was 0.01 wt. % for S_{tot}. The relative errors using the reference material (CRM 7001) was ± 2 % for S_{tot}.

Total S for $\delta^{34}S$ determination was extracted in the form of $BaSO_4$ from the sediments. To evaluate the S isotope ratio of gypsum ($\delta^{34}S_{gy}$), first the heavy mineral fraction of the samples, which includes pyrite, was excluded by using 1,1,2,2-tetrabromethane (ρ= 2.95). The gypsum was then dissolved in ddH_2O to extract sulfate. The free sulfate obtained was precipitated as $BaSO_4$ as described above (Sect. 3.1.7). The $BaSO_4$ was then converted to SO_2 by direct decomposition mixed with V_2O_5 and SiO_2 powder and combusted at 1000 °C under vacuum (10^{-2}-10^{-3} mbar); mass spectroscopic measurements of the evolved SO_2 were conducted on a Finnigan MAT 251 IR-MS dedicated to S isotope determinations. The results are expressed in delta notation and reported against the V-CDT and V-SMOW standards. The accuracy of the measurements was checked by also measuring international standards; reproducibility was better than 0.2 ‰.

The same IR-MS used to evaluate the $\delta^{34}S$ isotope ratios of dissolved sulfate in the waters at the Biogéosciences Laboratory, Dijon, France, was used to evaluate the $\delta^{34}S$ of the pyrite in the upper anoxic sediments. Prior to analyses, an AVS/CRS wet chemical extraction scheme alike the one described above was applied. The resulting H_2S was trapped as Ag_2S. Mass balance after gravimetric quantification was used to calculate the amount of AVS and CRS. After centrifugation, the Ag_2S precipitate was washed several times with ddH_2O and oven-dried at 50 °C for 48 h. The pyrite $\delta^{34}S$ measurements were performed on SO_2 molecules via combustion of ~500 mg of silver sulfide homogeneously mixed with an equal amount of WO_3 using a Vario PYRO cube (Elementar GmbH) connected online via an open split device to the IR-MS. International standards (IAEA-S-1, IAEA-S-2, IAEA-S-3) were used for calibration. Isotope results are reported in the standard delta notation against the V-CDT standard. Analytical reproducibility was better than 0.8 ‰ based on replicates for standard materials and samples.

The isotope ratios of carbonate in the sediment fraction were evaluated—after removal of organic carbon with H_2O_2, by implementing the method described by Rosenbaum and Sheppard (1986). These were measured using a mass spectrometer (Delta V, Thermo Fisher Scientific) coupled with a Fisons EA-1108 elemental analyzer at the Czech Geological Survey, Prague. The same instrument was used for measuring the sediment $\delta^{13}C_{org}$. For this purpose, the samples where finely milled, place in tin (Sn) capsules, and oxidized to CO_2 at 1040°C in the elemental analyzer. The reproducibility of the isotope measurements for organic C was better than ±0.12 ‰, and better than ± 0.1 ‰ for both carbon and oxygen isotopes of siderite. For siderite, the accuracy of the measurement was monitored by analyses of the IAEA NBS-18 ($\delta^{13}C$= −5.014 ‰, $\delta^{18}O$= −23.2 ‰) and two in-house standards; the long-term reproducibility is better than 0.05 ‰ for $\delta^{13}C$ and 0.1 ‰ for $\delta^{18}O$.

3.2.4 Textural features

For SEM of the sediments, we either used a TESCAN Mira 3GMU scanning electron microscope combined with a NordlysNano electron back-scattering diffraction (EBSD) system for semi-quantitative chemical petrography, or a FEI Magellan 400 for higher resolution imaging in secondary electron mode.

REFERENCES CITED ARE PROVIDED IN MAITEXT