**Supplement 1. Methods Expanded**

**SM1.1 Water sampling and analyses**

**SM1.1.1 Physicochemical parameter measurements**

A YSI 6600 V2-2 water quality monitoring and profiling probe was used to determine, prior to water sampling, a few key physicochemical parameters of the bottom water column. These parameters included dissolved molecular oxygen ($O_2$) concentrations, Eh, pH, temperature, and conductivity. Sampling of the bottom waters was conducted in November 2019. The probing resolution was 1 m above and below the $O_2$ minimum zone and 0.5 m at the chemocline. Water samples were collected directly from the lake using a Ruttner sampler with a capacity of 1.7 L. Flushing/rinsing of the sampling device with $dH_2O$ was performed between samples.

**SM1.1.2. Dissolved inorganic carbon and methane determinations**

Aliquots of the lake water collected were immediately transferred from the sampler to 12 mL exetainer septum capped vials (Labco) prefilled with He(g) and 1mL NaCl oversaturated solution (40%) for $CH_4$, or 1 mL 85% phosphoric acid for $\Sigma CO_2$. On board, the vials were filled with ~11 mL water samples (*i.e.,* no head space) 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.

**SM1.1.3. Environmental DNA**

For each sampling depth, an aliquot of 1 L was transferred to clean polyethylene (PET) bottles using a Sterifil® Aseptic System loaded with sterile cellulose nitrate Whatman® Microplus-21 ST filters (0.45 µm cutoff, 47 mm diameter). These filters were stored in a liquid N container using sterile 2 mL CryoTube vials (Thermo Scientific). After each sample collection, the filtration system was rinsed 3 times with ddH2O, and a new filter was carefully placed onto the system. Samples for rRNA gene analyses were collected from the two redox compartments of Lake Medard: the dysoxic hypolimnion and anoxic monimolimnion.

**SM1.1.4 Cation and ion concentration analyses**

For cation and anion concentration analyses, aliquots of 15 mL were filtered using high flow polyethersulfone filters to remove particles >0.45 µm and placed in acid-cleaned, PET centrifuge tubes where they were acidified using concentrated trace metal grade $HNO_3$. For metal compositions, in the lab these water aliquots were digested with trace metal grade $HNO_3$ (8 N) and analyzed using an Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Relative standard deviations (2σ level) were better than 0.01 wt. % for Fe, and between 0.001 and 0.002 % for the rest of the analyzed elements.

### SM1.1.5 Microbiome profile

DNA was extracted according to the manufacturer's instructions from the water filters using Quick DNA Soil Microbe Kit (Zymo Research). A total of 11 water samples, each consisting of 1 L water, were pumped through the membrane filters described above with the aid of a hand pump connected to the three-times dd$H_2O$-rinsed filtration system. The filters were separated from the filtrating apparatus using a pair of sterilized tweezers (70% ethanol and Bunsen burner) and transferred into the cryo-vials described above. These were store into liquid N for transport to the lab, where the DNA extraction from the biomass collected on the filters took place.

A two-step PCR protocol targeting the small subunit 16S rRNA gene in bacteria and archaea was conducted using the universal primer combination. The samples were then 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, *i.e.*, the V3-V4a hypervariable region's forward and reverse primer sequences for bacteria and the whole 16S target region for 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 (Magoc & Salzberg, 2011) which yielded a total of 945,214 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 above 97 % of the V3-V4a hypervariable region for bacteria and full-length sequences for archaea are 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).

### SM1.1.6 $\Sigma CO_2$, $CH_4$ and VFAs analyses

A dissolved inorganic carbon ($\Sigma CO_2$) concentration profile was produced using a peak height area calibration curve obtained on a MAT253 Plus isotope ratio mass spectrometer (IRMS; Thermo Scientific). The same instrument was used for also determining isotope ratios of $\Sigma CO_2$ ($\delta^{13}C_{\Sigma CO2}$, $\delta^{18}O_{\Sigma CO2}$) 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, 50, 100, 250 µL 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·min$^{-1}$ and transferred via a Conflo IV interface to the instrument. For methane, prior to transfer to the IRMS, 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 ($1\sigma$) < 4 % for $\Sigma CO_2$ 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 (VPDB) 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 ‰ ($1\sigma$), 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 ‰.

VFAs (Volatile fatty acids) were measured on a Dionex IC25 IC + Eluent Generator EG40; suppression of the $Cl^-$ ion was implemented. To optimize and calibrate the method, and determine the limits of detections, we used stock standard mixtures of IC grade formate, oxalate, acetate, lactate, pyruvate, and butyrate standards for preparing working saline stocks solutions. Detection limits were better than 60 ppb for lactate and oxalate, and 200 ppb for byturate, formate, and acetate.

**SM1.1.7 Dissolved sulfur analyses**

For measuring dissolved acid-volatile sulfur (AVS) in the monimolimnion (i.*e.*, $HS^-$, $H_2S$ 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 5 M NaOH was added. In the lab, 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. In the lab, 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 a pre-weighed membrane filter, rinsed thoroughly using deionized water, stored in a pre-weighed plastic petri dish, and dried, using a sulfate-free desiccant, in a desiccator. The dry $BaSO_4$ powder was quantify gravimetrically, scraped into clean vials, 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 IRMS system (Manchester, UK) in continuous flow mode. The $SO_4$ isotope data are expressed in in the $\delta$-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 Vienna Standard Mean Ocean Water (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.

## SM1.2 Sediment samples

### SM1.2.1 Sediment sampling

Replicate sediment cores (~16 cm depth) were collected with a messenger-activated gravity corer attached to 20 cm-long polycarbonate tubes (5 cm in diameter). The cores were immediately sealed with butyl rubber stoppers, preserving about 3 cm of anoxic bottom 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. In the lab, the sediment 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 sliced sediments subsampled were freeze-dried using liquid N and then stored at -18 ºC.

### SM1.2.2 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 (*i.e.*, after Poulton & 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 (up to 75 %), and an unknown, yet typically negligible fraction of pyrite (Rickard & 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 during the ICP-MS measurements described above

*SM1.2.3 Total S, and C, O and S isotope analyses of solid phases*

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 ($\rho$= 2.95). The gypsum was then dissolved in $ddH_2O$ to extract sulfate. The sediments were also annealed with Eschka mixture in an oxidizing atmosphere to evaluate the S isotope signature of organic-bound sulfur. In both cases, the free sulfate obtained was precipitated as $BaSO_4$ as described above. 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); the mass spectroscopic measurement of the evolved $SO_2$ were conducted on a Finnigan MAT 251 IRMS dedicated to S isotope determinations. The results are expressed in delta notation and reported in V-CDT. The accuracy of the measurements was checked by also measuring international standards. The reproducibility of the analyses was better than 0.2 ‰.

The same instrument used to evaluate the $\delta^{34}S$ isotope ratios of dissolved sulfate in the waters at the Biogeosciences Laboratory of the Universite de Bourgogne in Dijon, France (section SM1.1.7, above), was used to evaluate the $\delta^{34}S$ of the pyrite finely dispersed in the upper anoxic sediments. Prior to analyses, a AVS/CRS wet chemical extraction scheme as 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 IRMS. 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 Vienna Canyon Diablo Troilite standard. Analytical reproducibility was better than 0.8 ‰ (3σ) based on replicates for standard materials and samples.

The isotope ratios of siderite were evaluated, after removal of organic carbon with $H_2O_2$, by implementing the methods described in Rosenbaum and Sheppard (1986) and measured using a Delta V mass spectrometer (Thermo Fisher Scientific) coupled with a Fisons EA-1108 elemental analyzer. The same instrument was used for measuring the $\delta^{13}C_{DOC}$. 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 C isotope measurements of DOC was better than ±0.12 ‰ for DOC and better than ± 0.1 ‰ for both carbon and oxygen isotopes of siderite. For siderite, the accuracy of the measurement was checked by analyses of the international standard (IAEA) NBS 18 ($\delta^{13}C$= -5.014 ‰, $\delta^{18}O$= -23.2 ‰) and two in-house standards, and with a long-term reproducibility for all standards is better than 0,05 ‰ for $\delta^{13}C$ and 0,1 for $\delta^{18}O$.

### SM1.2.4 Textural features

For scanning electron microscopy (SEM) we either used a Mira 3GMU scanning electron microscope (TESCAN, Brno, Czech Republic) combined, for semi-quantitative chemical petrography, with a NordlysNano electron back-scattering diffraction (EBSD) system, or a FEI Magellan 400 SEM (.

## References to SM1


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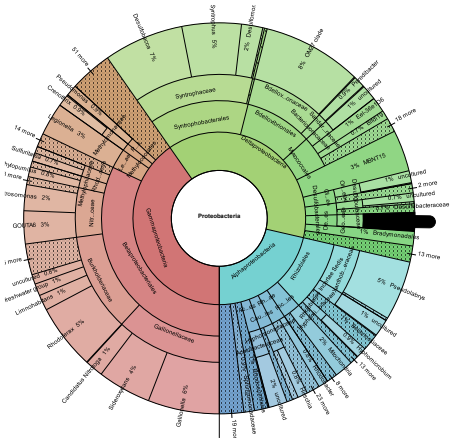
**Supplement 2. link to the supplement Krona chart**




**Supplement 3. Mineral Equilibrium Model: Database: PHREEQC.DAT.**

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  Phase               SI** log IAP   log K(279 K,   1 atm)

  Al(OH)3(a)        -0.56     11.57    12.12  Al(OH)3
  Alunite            2.13      3.24     1.11  KAl3(SO4)2(OH)6
  Anhydrite         -1.00     -5.08    -4.08  CaSO4
  Aragonite         -0.07     -8.31    -8.24  CaCO3
  Calcite            0.09     -8.31    -8.40  CaCO3
  CO2(g)            -2.73     -3.94    -1.21  CO2
  Dolomite           0.66    -15.96   -16.62  CaMg(CO3)2
  Fe(OH)3(a)        -2.53      2.36     4.89  Fe(OH)3
  FeS(ppt)           1.49     -2.43    -3.92  FeS
  Gibbsite           2.32     11.57     9.25  Al(OH)3
  Goethite           2.64      2.36    -0.28  FeOOH
  Gypsum            -0.48     -5.08    -4.60  CaSO4:2H2O
  H2(g)             -8.37    -11.40    -3.04  H2
  H2O(g)            -2.03     -0.00     2.03  H2O
  H2S(g)            -6.43    -14.47    -8.05  H2S
  Halite            -6.15     -4.60     1.55  NaCl
  Hausmannite      -22.84     43.21    66.05  Mn3O4
  Hematite           7.19      4.72    -2.47  Fe2O3
  Jarosite-K       -16.73    -24.38    -7.65  KFe3(SO4)2(OH)6
  Mackinawite        2.22     -2.43    -4.65  FeS
  Manganite         -9.55     15.79    25.34  MnOOH
  Melanterite       -3.24     -5.71    -2.47  FeSO4:7H2O
  NH3(g)            -8.42     -6.20     2.22  NH3
  O2(g)            -73.40    -76.12    -2.73  O2
  Pyrite            10.48     -8.56   -19.04  FeS2
  Pyrochroite       -3.58     11.62    15.20  Mn(OH)2
  Pyrolusite       -24.66     19.97    44.63  MnO2:H2O
  Rhodochrosite      1.70     -9.36   -11.06  MnCO3
  Siderite           1.83     -8.94   -10.77  FeCO3
  Sulfur            -4.25      1.11     5.36  S
  Sylvite           -6.83     -6.03     0.80  KCl

**For a gas, SI = log10(fugacity). Fugacity = pressure * phi / 1 atm.
  For ideal gases, phi = 1.
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