Supplementary Information

Hypersaline sapropels act as hotspots for microbial dark matter

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Supplementary methods

Analytical techniques
The extraction and estimation of dissolved CH₄ concentrations at the sediment-water interface were determined at the sampling site, using the head-space method on an instrumental package (West System, Pontedera, Italy) equipped with semiconductor (range 0-2000 ppmv; lower detection limit of 1 ppmv; resolution 1 ppmv), catalytic (range 2000 ppmv – 3 % v/v) and thermal conductivity (range 3 % - 100 % v/v) detectors.

The organic matter content (% OM) of sapropel samples was determined by using the loss-on-ignition (LOI) and wet oxidation techniques. LOI determination was executed as described by Ball¹, with the assumption that the total organic carbon content (% TOC) is 50 % of the OM. The dichromate oxidation followed by titration was performed as described by Walkley and Black², following a % TOC correction estimation by a factor of 1.16 (equivalent to ca. 86 % organic carbon recovery).

As most of the organic nitrogen in aquatic sediments seems to be deposited under the form of amide bonds³, the total alkaline extractable protein content was estimated in the sapropels by the Lowry method⁴ modified by Rice⁵. Ammonium nitrogen was quantified by colorimetry using a Lambda 25 spectrophotometer (Perkin Elmer, Beaconsfield, UK). The contents of total nitrogen (TN) as bound nitrogen (including free ammonia, ammonium, nitrite, nitrate and organic nitrogen, but not dissolved nitrogen gas) were assessed by thermal oxidation, conversion of nitrogen oxides to NO, followed by NO oxidation with ozone and subsequent chemiluminescence detection using the Multi N/C 2100 S Analyzer (Analytik Jena, Jena, Germany). Organic nitrogen (ON) in the form of dissolved organic nitrogen was calculated by subtracting ammonium nitrogen, nitrate and nitrite nitrogen from the total nitrogen. Total carbon (TC), total dissolved carbon (TDC) and dissolved organic carbon (DOC - calculated as difference between total dissolved carbon and dissolved inorganic carbon measured upon sample acidification) were quantified by combustion and NDIR detection of CO₂ released using the multi N/C 2100 S Analyzer. Chloride (Cl⁻), carbonate (as CaCO₃) and bicarbonate (HCO₃⁻) anions were measured by titrimetric methods. Phosphate (PO₄³⁻), sulfate (SO₄²⁻), nitrate (NO₃⁻), and nitrite (NO₂⁻) were measured by ion chromatography on a 761 Compact IC (Metrohm, Herisau, Switzerland). The concentration of sulfides was determined by methylene blue method after fixation of samples with 2 % (v/v) Zn-acetate⁶. Concentrations of major metals (Mg, Ca, Na, K, Fe, Mn) were measured by inductively coupled plasma atomic emission spectrometry.
(ICP-AES) using OPTIMA 5300 DV spectrometer (Perkin Elmer, Norwalk, USA) after homogenization of sediments and digestion in concentrated \textit{aqua regia}. Relative expanded measurement uncertainty (U_{rel}) was calculated according to ISO-GUM using a coverage factor (k) of 2 (k = 2), equivalent to a confidence of approximately 95\%. Uc ranged from 4.5 to 13\% depending on the compound as follows: 8\% for TC, HCO$_3$; Fe, Mn, Mg and Ca; 9.5\% for PO$_4^{3-}$; 9.8\% for Na$^+$; 10\% for TN, TDC, CO$_3^{2-}$, SO$_4^{2-}$, HS$^-$ and K; 11\% for NO$_3^-$ and ON; 13\% for NO$_2^-$, NH$_4^+$ and Cl$^-$. 

For X-ray fluorescence analysis (XRF), sapropel samples were dried to constant weight at 60 °C and grinded to pass a < 200 µm sieve. Major and trace elements (Cl, Fe, Ca, K, Ti, S, Mn, Cu, Zn, etc.) were determined by using a dispersive X-ray fluorescence spectrometer (Innov-X Alpha 6500, The Netherlands) equipped with X-ray tube, W anode and Si PiN diode detector. XRF spectra were recorded at Kα line on samples placed in small plastic cups covered by Mylar X-ray window film. The energy-dispersive X-ray (EDX) spectroscopic analyses of dried sapropel samples were performed over an area of 250 x 200 µm$^2$, at 20 keV beam energy using a FEI Quanta 3D FEG dual beam SEM equipped with an energy dispersive X-ray detector.

The mineral composition of bulk sediment samples was assessed by X-ray diffraction (XRD), using a Bruker D8 Advance diffractometer with CoKα$_1$ with $\lambda = 1.78897$, Fe filter and a one-dimensional detector using corundum (NIST SRM1976a) as internal standard. The data were collected on a 5 - 64º 2θ interval, at a 0.02º 2θ, with the measuring step of 0.2 seconds. The clay fraction was analyzed on oriented samples (pipetted from suspension in distilled water), which were further treated with ethylene glycol. Data were collected on the same interval as mentioned above, with a measuring step of 0.5 seconds. The identification of mineral phases was performed with the Diffrac.Eva 2.1 software from Bruker AXS, using the PDF2 (2012) database.

**SEM, cell counts, chlorophyll \textit{a} and total carotenoids analyses**

For SEM analyses, samples of sapropel and plant material recovered from sapropels were fixed in 4\% glutaraldehyde, filtered onto 0.25 µm polycarbonate Millipore filters (Bedford, MA, USA) and subsequently washed with 1 x PBS (pH 7.4). Henceforth, they were subjected to an alcohol dehydration series after which they were fixed with 100\% hexamethyldisilizane (Electron Microscopy Sciences, Hatfield, Pennsylvania) and air-dried. After mounting on SEM specimen holders they were sputter-coated (Agar Automatic Sputter Coater, Agar Scientific, UK) with 7-10 nm of gold and visualized with a Jeol JEM 5510LV scanning electron microscope operated at 15 kV. For total cell counts, sapropel pore water samples were fixed in 1\% glutaraldehyde
and filtered through black, gridded cellulose ester membrane filters (0.45 µm, d=47 mm). Subsequently, they were stained directly using 5 µg/mL of DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride) solution and examined by epifluorescence (BX60, Olympus Optical, Tokyo, Japan). Chlorophyll $a$ and total carotenoids concentrations were determined as described by Wetzel and Likens.$^7$

**Fungal community analyses**

The collected sapropel samples were subsampled (i.e. 3 subsamples from each sapropel sample) and small pices of decaying plant material (leaves) were recovered from Ursu and Fara Fund and processed for DNA extraction using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research) in accordance with the manufacturer’s instructions. The PCR reactions were carried out in triplicates using the primers pair FR1-GC/FF390 as described by Vainio and Hantula.$^8$

The purified PCR-amplified DNA products were compared (700 ng) by DGGE analysis on 8 % (w/v) polyacrylamide gels (containing 37.5:1 of acrylamide to bisacrylamide) with a linear denaturing gradient ranging from 40 % to 65 % (the 100 % denaturant contains 40 % v/v formamide and 7 M urea). Gels electrophoresis were run for 18 h at a constant temperature (58 °C) and voltage (50 V) in 1% Tris-acetate-EDTA buffer, using the DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). Subsequently, the gels were stained with a 1 x SYBR Gold® solution (Invitrogen, Carlsbad, CA) for 40 minutes and visualized using the UVP Imaging and Analysis System and LabWorks software (UVP Inc.). The most intense DGGE bands were excised and incubated overnight in ultrapure water at 4 °C. 3 µl of the DNA-containing water was used in PCR reactions and the purified products were cloned using a commercial system (InsTAclone™ PCR cloning kit, Thermo Scientific) according to the manufacturer’s instructions. Plasmids were purified using the GeneJet Plasmid Miniprep kit (Thermo Scientific) and sequenced using the Sanger method at a commercial company (Macrogen Europe). The fungal community DGGE fingerprints were analyzed with the software package BioNumerics 6.5 (Applied Maths, Ghent, Belgium) according to the provider’s instructions.

**References**

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Table S1. Semiquantitative analysis of elements in dried sapropel samples from Ursu and Fara Fund lakes by X-ray fluorescence (XRF) and scanning-electron microscopy/energy-dispersive X-ray (SEM/EDX). Values are average of three measurements in XRF and five different scanning of distinct spot areas in SEM/EDX.

| Element | Ursu Lake sediment | Fara Fund Lake sediment |
|---------|--------------------|-------------------------|
|         | XRF | EDX | XRF | EDX |
| **Major elements (>1 %)** | | | | |
| C   | NA  | 6.8 | NA  | 5.3 |
| O   | NA  | 37.8| NA  | 35.2|
| Si  | NA  | 21.8| NA  | 28.4|
| Na  | NA  | 5.3 | NA  | 3.7 |
| Al  | NA  | 6.2 | NA  | 7.3 |
| Cl  | 4.4 | 6.6 | 2.9 | 4.3 |
| Fe  | 3.2 | 2.3 | 2.2 | 2.1 |
| Ca  | 1.9 | 1.2 | 1.0 | 0.8 |
| K   | 1.1 | 1.2 | 1.8 | 1.3 |
| **Minor elements (0.1-1 %)** | | | | |
| Mg  | NA  | 0.8 | NA  | 0.6 |
| Ti  | 0.44| 0.3 | 0.45| 0.5 |
| S   | 0.13| 0.6 | 0.10| 0.5 |
| Mn  | 0.15| 0.2 | 0.04| 0.1 |
| **Trace elements (<0.1 %)** | | | | |
| Ba  | 0.022| ND  | 0.019| ND  |
| Zr  | 0.014| ND  | 0.020| ND  |
| Sr  | 0.013| ND  | 0.014| ND  |
| Cu  | 0.010| Traces | 0.010| Traces |
| Zn  | 0.011| ND  | 0.006| ND  |
| Rb  | 0.007| ND  | 0.005| ND  |
| Co  | 0.005| Traces | <0.002| Traces |
| Br  | 0.001| Traces | <0.0007| Traces |
| Pb  | 0.002| Traces | 0.002| Traces |

Elements below detection limits of XRF

Ni, Cr, As, Se, Mo*, Ag, Cd, Sn, Sb, I, Au, Hg

* Traces of Mo were observed by EDX analysis.

Abbreviations: NA – not analyzed; ND – not detected.
Table S2. Total read counts and alpha diversity estimates for the sapropels from Ursu and Fara Fund lakes

|                                | Ursu Lake | Fara Fund Lake |
|--------------------------------|-----------|----------------|
| Total read counts             | 84,125    | 63,102         |
| Number of OTUs per sample     | 2,121     | 1,806          |
| Good's coverage               | 0.992     | 0.985          |
| Phylogenetic diversity (PD)   | 113.731   | 93.981         |
| Simpson (S)                   | 0.996     | 0.995          |
| Shannon (H)                   | 9.444     | 9.053          |
Table S3. Nearest BLAST hits of the archaeal and bacterial OTUs classified within the uncultivated and candidate bacterial and archaeal phyla. Operational taxonomic units are organized by decreasing abundance of each phylum as well as decreasing abundance of each OTU within the respective phylum.

### A. Ursu Lake

| OTU*   | Classification | Sequence number | Nearest match (% identity) | Source location of the match | GenBank ID     |
|--------|----------------|----------------|-----------------------------|-------------------------------|----------------|
| 192    | Bacteria       | 118            | Clone MEDEE_16SB_65_MIF (98 %) | Hypersaline Lake Medee, Eastern Mediterranean | JX446064.1     |
| 388    | Bacteria       | 88             | Clone MS-A229 (87 %)         | Calcareous sandy sediment, Spain | FJ94902.1      |
| 465    | Bacteria       | 88             | Clone MEDEE_16SB_542_MIF (98 %) | Hypersaline Lake Medee, Eastern Mediterranean | JX446043.1     |
| 421    | Bacteria       | 87             | Clone C149 (99 %)            | Soil, China                   | KJ817694.1     |
| 411    | Bacteria       | 84             | Clone B0618R002_D01 (96 %)   | Rice paddy soil, Japan         | ABE58668.1     |
| 190    | Bacteria       | 82             | Clone GUAY_50enr_Bac50 (94 %) | Hydrothermal sediments, Guaymas Basin, Pacific Ocean | FR682656.1     |
| 284    | Bacteria       | 80             | Clone: p816_b_5.05 (91 %)    | Trough                        | AB305603.1     |
| 573    | Bacteria       | 75             | Clone GUAY_37enr_Bac5s (89 %) | Hydrothermal sediments, Guaymas Basin, Pacific Ocean | FR682626.1     |
| 436    | Bacteria       | 71             | Clone Shimokita-2-70 (89 %)  | Deep-sea surface sediment, Shimokita, Pacific Ocean | AB734635.1     |
| 538    | Bacteria       | 63             | Clone TKTMmv-a-B4(5 (88 %)   | Hydrothermal vent, Okinawa, Japan | ABE61531.1     |
| 691    | Bacteria       | 63             | Clone MEDEE_16SB_542_MIF (96 %) | Sediment, Okinawa Trough, Japan | AB825411.1     |
| 4027   | Bacteria       | 56             | Clone MEDEE_16SB_542_MIF (96 %) | Hypersaline Lake Medee, Eastern Mediterranean | JX446043.1     |
| 707    | Bacteria       | 48             | Clone pYK04-20B-60 (99 %)    | Hypersaline vent, Okinawa, Japan | AB464834.1     |
| 409    | Bacteria       | 45             | Clone MAT-CH-CH-11 (97 %)    | Hypersaline microbial mat, Candelaria lagoon, Puerto Rico | EU245196.1     |
| 694    | Bacteria       | 44             | Clone N2_D1 (100 %)          | Microbial mat petola, Slovenia  | FN768681.2     |
| 934    | Bacteria       | 44             | Clone Pre-sep_090802W1 (87 %) | Hot spring, USA               | KM897417.1     |
| 976    | Bacteria       | 44             | Clone MEDEE_16SB_42_MIF (98 %) | Hypersaline Lake Medee, Eastern Mediterranean | JX446051.1     |
| 571    | Bacteria       | 43             | Clone SBYZ_1565 (94 %)       | Hypersaline mat, Guerrero Negro, Mexico | JN496253.1     |
| 85     | OP1            | 878            | Clone MEDEE_16SB_42_MIF (98 %) | Hypersaline sediment, Death Valley, USA | AB533929.1     |
| 124    | OP1            | 143            | Bacterium Medee_0001 (100 %) | Brine, Medee Lake, Eastern Mediterranean | JX456400.1     |
| 102    | OP1            | 87             | Clone G06U47 (99 %)          | Evaporitic crust, Guerrero Negro, Mexico | EF106490.1     |
| 417    | OP1            | 46             | Clone GeoB15101-6-85-Bac93 (96 %) | Urania Basin brine, Eastern Mediterranean | KM278921.1     |
| 179    | OP3            | 626            | Clone MEDEE_16SB_09_UIF (98 %) | Water, Hypersaline Lake Medee, Eastern Mediterranean | JX446112.1     |
| 676    | OP3            | 62             | Clone p816_b_5.05 (87 %)     | Hydrothermal sediments, Southern Okinawa Trough, Japan | AB305603.1     |
| 654    | OP3            | 54             | Clone E6aB08 (100 %)         | Hypersaline endoevaporitic microbial mat, Elat, Israel | DQ103867.1     |
| 728    | OP3            | 50             | Clone 113B509 (93 %)         | Sediment - Cheren mud volcano, Eastern Mediterranean | EF875713.1     |
| 880    | OP3            | 43             | Clone WLS4-5 (98 %)          | Wuliangshai Lake, China        | KC456412.1     |
| 970    | OP3            | 43             | Clone B41.20 (95 %)          | Sediments, Bohai Sea           | KF856854.1     |
| 278    | WS3            | 318            | Clone SBZP_6337 (100 %)      | Hypersaline mat, Guerrero Negro, Mexico | JN539471.1     |
| 137    | WS3            | 206            | Clone HA9-SRB-CD3 (97 %)     | Sediment, hydrothermal vent, Bransfield Strait, Antarctica | FM868270.1     |
| 288    | WS3            | 170            | Clone SBZI_6025 (99 %)       | Hypersaline mat, Guerrero Negro, Mexico | JN526162.1     |
| 535    | WS3            | 68             | Clone PM-150-Bac-24 (96 %)   | Sediment, cold seep, Pakistan Margin | JQ925060.1     |
| 754    | WS3            | 46             | Clone V1F127B (97 %)         | Iron oxide sediments, Volcano 1, Tonga Arc | FJ905749.1     |
| 1043   | WS3            | 44             | Clone SBZC_5732 (100 %)      | Hypersaline mat, Guerrero Negro, Mexico | JN509263.1     |
| 49     | WWE1           | 508            | Clone 104B375 (92 %)         | Sediment, Cheren mud volcano, Eastern Mediterranean | EF887304.1     |
| OTU*  | Classification | Sequence number | Nearest match (% identity) | Source location of the match | GenBank ID   |
|-------|----------------|----------------|----------------------------|------------------------------|-------------|
| 446   | Parvarchaeota  | 133            | Clone Exp331_INH_50U_57     | Ocean drilling core sediment, Okinawa, Japan | AB825844.1  |
| 190   | Bacteria       | 319            | Clone GUAY_50enr_Bac50       | Hydrothermal sediments, Guaymas Basin, Pacific Ocean | FR682656.1  |
| 112   | Bacteria       | 256            | Clone GeoB15101-6-B5-Bact-49| Urania Basin brine lake, Eastern Mediterranean | KM278882.1  |
| 335   | Bacteria       | 86             | Clone GeoB15101-6-B5-Bact-49| Urania Basin brine lake, Eastern Mediterranean | KM278882.1  |
| 284   | Bacteria       | 83             | Clone p816_b_5_05(91 %)     | Trough                        | AB305603.1  |
| 388   | Bacteria       | 70             | Clone MS-A229(87 %)         | Calcareous sandy sediment, Spain | FJ949402.1  |
| 1957  | Bacteria       | 70             | Clone LGNSa2_BC_022(95 %)   | Hypersaline mat, Guerrero Negro, Mexico | EU687381.1  |
| 646   | Bacteria       | 61             | Clone Napoli-4B-82(91 %)    | Napoli mud volcano hypersaline marine sediment | AY952796.1  |
| 409   | Bacteria       | 57             | Clone MAT-CR-H3-H11(97 %)   | Hypersaline microbial mat, Candelaria lagoon, Puerto Rico | EU245196.1  |
| 495   | Bacteria       | 51             | Clone 90b(92 %)             | Anaerobic reactor fed from the chemical industry | FJ462088.1  |
| 545   | Bacteria       | 51             | Clone AS07-7-39(88 %)       | Biofilm from subsurface sulfidic cave sediment | KM410351.1  |
| 889   | Bacteria       | 51             | Clone TVG12-B32(93 %)       | Deep-sea hydrothermal vent sediments, Pacific Ocean | KM071721.1  |
| 849   | Bacteria       | 48             | Clone NNTCHY2917(92 %)      | Anaerobic UASB reactor fed with domestic sewage | KM071721.1  |
| 571   | Bacteria       | 43             | Clone SBY2Z_1565(94 %)      | Hypersaline mat, Guerrero Negro, Mexico | JN496253.1  |
| 165   | Bacteria       | 41             | Clone LGNSa2_BC_022(94 %)   | Hypersaline mat, Guerrero Negro, Mexico | EU687381.1  |
| 9     | Bacteria       | 41             | Clone LGNSa2_BC_022(100 %)  | Hypersaline mat, Guerrero Negro, Mexico | EU687381.1  |
| 293   | Bacteria       | 37             | Clone TOSC-55(88 %)         | Sediment                      | LN650518.1  |
| 518   | Parvarchaeota  | 35             | Clone OGAP_AB16_38cm_06(91 %)| West Pacific abyssal plain sediments | AB827103.1  |
| 227   | Parvarchaeota  | 35             | Clone OGAP_AB16_38cm_27(91 %)| West Pacific abyssal plain sediments | AB827103.1  |
| 618   | Parvarchaeota  | 35             | Clone Naples(95 %)          | Evaporite crust, Guerrero Negro, Mexico | EF106720.1  |
| 679   | Parvarchaeota  | 35             | Clone A42(94 %)             | Moderate saline soil, China    | EU328116.1  |
| 652   | Parvarchaeota  | 35             | Clone Exp331_INH_65U_06(92 %)| Ocean drilling core sediment, Okinawa, Japan | AB825870.1  |
| OTU | Family | OTU Description | Location | Sequence Accession |
|-----|--------|-----------------|----------|------------------|
| 3125 | Parvarchaeota | Clone P53A (98 %) | Evaporitic crust, Guerrero Negro, Mexico | EF106700.1 |
| 906  | Parvarchaeota | Clone GNA02D12 (98 %) | Hypersaline mat, Guerrero Negro, Mexico | EU731484.1 |
| 1371 | Parvarchaeota | Clone Y17A (97 %) | Hypersaline mat, Guerrero Negro, Mexico | EF106701.1 |
| 3987 | Parvarchaeota | Clone TVG1-A103 (90 %) | Deep-sea hydrothermal vent sediments, East Pacific Ocean | KJ564030.1 |
| 90   | Parvarchaeota | Clone GN21D13B (99 %) | Hypersaline mat, Guerrero Negro, Mexico | EU731482.1 |
| 142  | SAR406    | Clone Kasin-B3-C09 (92 %) | Hypersaline sediment, Kalmykian Steppe, Russia | HE604724.1 |
| 301  | SAR406    | Clone ST-12K17 (98 %) | Brine-seawater interface, Shaban Deep, Red Sea | AJ347757.1 |
| 1911 | SAR406    | Clone ST-12K17 (95 %) | Brine-seawater, Shaban Deep, Red Sea | AJ347757.1 |
| 1191 | SAR406    | Clone E10Dbac (99 %) | Great Salt Lake sediment, USA | DQ386205.1 |
| 179  | OP3       | Clone MEDEE_16SB_09_UIF (98 %) | Water, Hypersaline Lake Medee, Eastern Mediterranean | JX446112.1 |
| 487  | OP3       | Clone SCS_HX36_154 (96 %) | Surface sediment, China Sea | HM598263.1 |
| 821  | OP3       | Clone Clip 7 (95 %) | Stratified lagoon, Clipperton Island | HQ691945.1 |
| 137  | WS3       | Clone HA9-SRB-CD3 (97 %) | Sediment from hydrothermal vent, Bransfield Strait, Antarctica | FM868270.1 |
| 278  | WS3       | Clone SBZP_6337 (100 %) | Hypersaline mat, Guerrero Negro, Mexico | JN599471.1 |
| 250  | Caldithrix | Clone SBYZ_5190 (98 %) | Hypersaline mat, Guerrero Negro, Mexico | JN493439.1 |
| 3721 | Caldithrix | Clone SBYZ_5190 (98 %) | Hypersaline mat, Guerrero Negro, Mexico | JN493439.1 |
| 520  | Caldithrix | Clone SBYZ_5190 (95 %) | Hypersaline mat, Guerrero Negro, Mexico | JN493439.1 |
| 454  | OD1       | Clone Kasin-B1-G09 (90 %) | Hypersaline sediment, Kalmykian Steppe, Russia | HE604802.1 |
| 1189 | OD1       | Clone HA-EUB-T9-47 (95 %) | Sediment from hydrothermal vent, Bransfield Strait, Antarctica | FM867974.1 |
| 850  | OD1       | Clone HA-EUB-T9-47 (89 %) | Sediment from hydrothermal vent, Bransfield Strait, Antarctica | FM867974.1 |
| 510  | OD1       | Clone Kasin-B2-D09 (99 %) | Hypersaline sediment, Kalmykian Steppe, Russia | HE604672.1 |
| 2433 | OD1       | Clone Kasin-B2-D09 (96 %) | Hypersaline sediment, Kalmykian Steppe, Russia | HE604672.1 |
| 307  | WWE1      | Clone 104B378 (98 %) | Sediment from hydrothermal vent, Bransfield Strait, Antarctica | EF687307.1 |
| 49   | WWE1      | Clone MAT-CR-H3-B03 (91 %) | Hypersaline microbial mat, Candelaria lagoon, Puerto Rico | EU245152.1 |

* Only OTUs with abundances above 0.05% are included.
Table S4. Taxonomic composition of sediments from Ursu and Fara Fund lakes (A) and the extended phylum level diversity (B).

A.

### Phylum-level taxonomy*

| Phylum     | Abundance (%) | Lake | Phylum     | Abundance (%) | Lake |
|------------|---------------|------|------------|---------------|------|
| OD1        | 1.1           | Ursu | Proteobacteria | 32.4          | Ursu |
| WWE1       | 1.2           | Ursu | SAR406     | 1.0           | Fara Fund |
| Caldithrix | 1.3           | Ursu | Acidobacteria | 1.7           | Fara Fund |
| WS3        | 1.4           | Ursu | Euryarchaeota | 1.8           | Fara Fund |
| OP1        | 1.5           | Ursu | Gemmatimonadetes | 1.8          | Fara Fund |
| OP3        | 1.7           | Ursu | Planctomycetes | 1.9           | Fara Fund |
| Planctomycetes | 2.4       | Ursu | Parvarchaeota | 2.2           | Fara Fund |
| Actinobacteria | 2.6        | Ursu | Firmicutes  | 2.4           | Fara Fund |
| Crenarchaeota | 2.7        | Ursu | Spirochaetes | 3.1           | Fara Fund |
| Parvarchaeta | 3.6         | Ursu | Crenarchaeota | 5.7           | Fara Fund |
| Euryarchaeota | 4.4        | Ursu | Actinobacteria | 7.4           | Fara Fund |
| Spirochaetes | 4.8          | Ursu | Chloroflexi | 7.8           | Fara Fund |
| Chloroflexi | 8.7           | Ursu | Bacteroidetes | 10.6          | Fara Fund |
| Bacteroidetes | 11.6        | Ursu | Proteobacteria | 39.3          | Fara Fund |

### Class-level taxonomy*

| Class          | Abundance (%) | Lake | Class          | Abundance (%) | Lake |
|----------------|---------------|------|----------------|---------------|------|
| Rhodothermi    | 1.3           | Ursu | MBGB           | 1.6           | Fara Fund |
| Phycisphaerae  | 1.6           | Ursu | Acidimicrobia  | 1.7           | Fara Fund |
| Saprospirae    | 2.0           | Ursu | Actinobacteria | 1.7           | Fara Fund |
| MBGB           | 2.2           | Ursu | Clostridia     | 1.8           | Fara Fund |
| Actinobacteria | 2.2           | Ursu | Parvarchaeta   | 2.2           | Fara Fund |
| Thermoplasmata | 3.2           | Ursu | Bacteroidia    | 2.5           | Fara Fund |
| Parvarchaeta   | 3.5           | Ursu | Ellin6529      | 2.8           | Fara Fund |
| Spirochaetes   | 4.0           | Ursu | Spirochaetes   | 3.0           | Fara Fund |
| Bacteroidia    | 5.0           | Ursu | Thermoleophilia| 3.1           | Fara Fund |
| Alphaproteobacteria | 6.4       | Ursu | Anaerolinea    | 3.1           | Fara Fund |
| Anaerolinea    | 7.9           | Ursu | Thaumarchaeota | 3.4           | Fara Fund |
| Gammaproteobacteria | 8.1      | Ursu | Betaproteobacteria | 4.2           | Fara Fund |
| Deltaproteobacteria | 16.6     | Ursu | Gammaproteobacteria | 6.8          | Fara Fund |
| Rhodothermi    | 1.0           | Fara Fund | Alphaproteobacteria | 12.1         | Fara Fund |
| Phycisphaerae  | 1.1           | Fara Fund | Deltaproteobacteria | 16.1         | Fara Fund |
| Saprospirae    | 1.1           | Fara Fund |  |  |  |

### Order-level taxonomy*

| Order          | Abundance (%) | Lake | Order          | Abundance (%) | Lake |
|----------------|---------------|------|----------------|---------------|------|
| MSBL9          | 1.0           | Ursu | GCA004         | 1.2           | Fara Fund |
| Rhodothermales | 1.3           | Ursu | Myxococcales   | 1.3           | Fara Fund |
| Thiotrichales  | 1.4           | Ursu | Burkholderiales| 1.3           | Fara Fund |
| Saprospirales  | 2.0           | Ursu | MND1           | 1.4           | Fara Fund |
| Chromatiales   | 2.1           | Ursu | OPB11          | 1.7           | Fara Fund |
| Actinomycetales| 2.1           | Ursu | Actinomycetales| 1.7           | Fara Fund |
| Desulfarculales| 2.5           | Ursu | Acidimicrobia  | 1.7           | Fara Fund |
| Desulfovibrionales | 2.6         | Ursu | Rhodobacteriales| 1.7          | Fara Fund |

*Note: Abundances are given as percentages of the total community, and lakes are indicated as Ursu or Fara Fund.
### Family-level taxonomy

| Family               | Abundance (%) | Lake    | Family               | Abundance (%) | Lake    |
|----------------------|---------------|---------|----------------------|---------------|---------|
| Balneolaceae         | 1.3           | Ursu    | Comamonadaceae       | 1.3           | Fara Fund |
| Piscirickettsiaceae  | 1.4           | Ursu    | Rhodobacteraceae     | 1.6           | Fara Fund |
| Saprospiraceae       | 2.0           | Ursu    | Desulfarculaceae     | 1.9           | Fara Fund |
| Desulfobacteriaceae  | 2.5           | Ursu    | Sphingomonadaceae    | 1.9           | Fara Fund |
| Rhodobacteraceae     | 3.1           | Ursu    | Sinobacteraceae      | 2.7           | Fara Fund |
| Spirochaetaceae      | 4.0           | Ursu    | Spirochaetaceae      | 3.0           | Fara Fund |
| Desulfovibrioaceae   | 8.3           | Fara Fund | Nitrososphaeraceae   | 3.4           | Fara Fund |
| Halanaerobiaceae     | 1.0           | Fara Fund | Hyphomicrobiaceae    | 4.4           | Fara Fund |
| Desulfohalobiaceae   | 1.0           | Fara Fund |                     |               |         |

### Genus-level taxonomy

| Genus                | Abundance (%) | Lake    | Genus                | Abundance (%) | Lake    |
|----------------------|---------------|---------|----------------------|---------------|---------|
| KSA1                 | 1.2           | Ursu    | Spirochaeta          | 1.8           | Fara Fund |
| Desulfotignum        | 1.4           | Ursu    | Rhodoplanae          | 2.8           | Fara Fund |
| Desulfovermiculus    | 1.7           | Ursu    | Candidatus           | 3.4           | Fara Fund |
| Spirochaeta          | 1.8           | Ursu    |                     |               |         |

*Only taxa with abundances ≥ 1% are presented.

### Domain

#### Archaea

| Genus                      | No of OTUs | No of reads | % of total reads |
|----------------------------|------------|-------------|------------------|
| Parvarchaeota              | 231        | 2988        | 3.55             |
| Crenarchaeota              | 33         | 2293        | 2.73             |
| Eurarchaeota               | 107        | 3675        | 4.37             |
| Caldithrix                 | 22         | 1081        | 1.28             |
| Thermi                     | 5          | 780         | 0.93             |
| AC1                        | 8          | 199         | 0.24             |
| Acidobacteria              | 69         | 316         | 0.38             |
| Actinobacteria             | 198        | 2198        | 2.61             |
| Armatimonadetes            | 2          | 9           | 0.01             |
| Bacteroidetes              | 248        | 9750        | 11.59            |
| BH80-139                   | 1          | 0           | 0.00             |
| BRC1                       | 18         | 132         | 0.16             |
| Chlamydia                 | 19         | 100         | 0.12             |
| Chlorobi                   | 18         | 517         | 0.61             |
| Chloroflexi                | 171        | 7346        | 8.73             |
| Cyanobacteria              | 8          | 356         | 0.42             |
| Deferrribacteres           | 3          | 48          | 0.06             |
| Elusimicrobia              | 9          | 62          | 0.07             |
| FCPU426                    | 1          | 10          | 0.01             |
| FBP                        | 1          | 0           | 0.00             |

#### Bacteria

| Domain | Phylum | No of OTUs | No of reads | % of total reads |
|--------|--------|------------|-------------|------------------|
| Archaea| Parvarchaeota | 231 | 2988 | 3.55 |
| Archaea| Crenarchaeota | 33 | 2293 | 2.73 |
| Archaea| Eurarchaeota | 107 | 3675 | 4.37 |
| Bacteria| Caldithrix | 22 | 1081 | 1.28 |
| Bacteria| Thermi | 5 | 780 | 0.93 |
| Bacteria| AC1 | 8 | 199 | 0.24 |
| Bacteria| Acidobacteria | 69 | 316 | 0.38 |
| Bacteria| Actinobacteria | 198 | 2198 | 2.61 |
| Bacteria| Armatimonadetes | 2 | 9 | 0.01 |
| Bacteria| Bacteroidetes | 248 | 9750 | 11.59 |
| Bacteria| BH80-139 | 1 | 0 | 0.00 |
| Bacteria| BRC1 | 18 | 132 | 0.16 |
| Bacteria| Chlamydia | 19 | 100 | 0.12 |
| Bacteria| Chlorobi | 18 | 517 | 0.61 |
| Bacteria| Chloroflexi | 171 | 7346 | 8.73 |
| Bacteria| Cyanobacteria | 8 | 356 | 0.42 |
| Bacteria| Deferrribacteres | 3 | 48 | 0.06 |
| Bacteria| Elusimicrobia | 9 | 62 | 0.07 |
| Bacteria| FCPU426 | 1 | 10 | 0.01 |
| Bacteria| FBP | 1 | 0 | 0.00 |
| Bacteria     | Fibrobacteres | 6   | 75  | 19  | 0.09  | 0.03 |
|--------------|---------------|-----|-----|-----|-------|------|
| Bacteria     | Firmicutes    | 96  | 486 | 1505| 0.58  | 2.39 |
| Bacteria     | Fusobacteria  | 4   | 8   | 18  | 0.01  | 0.03 |
| Bacteria     | GAL15         | 1   | 0   | 3   | 0.00  | 0.00 |
| Bacteria     | Gemmatimonadetes | 42  | 641 | 1116| 0.76  | 1.77 |
| Bacteria     | GN02          | 9   | 96  | 18  | 0.11  | 0.03 |
| Bacteria     | GN04          | 17  | 356 | 13  | 0.42  | 0.02 |
| Bacteria     | H-178         | 3   | 176 | 0   | 0.21  | 0.00 |
| Bacteria     | Hyd24-12      | 5   | 221 | 72  | 0.26  | 0.11 |
| Bacteria     | KSB3          | 6   | 276 | 11  | 0.33  | 0.02 |
| Bacteria     | LCP-89        | 5   | 82  | 26  | 0.10  | 0.04 |
| Bacteria     | Lentisphaerae | 24  | 724 | 73  | 0.86  | 0.12 |
| Bacteria     | Nitrospirae   | 8   | 202 | 0   | 0.00  | 0.32 |
| Bacteria     | NKB19         | 17  | 202 | 15  | 0.24  | 0.02 |
| Bacteria     | OD1           | 79  | 908 | 536 | 1.08  | 0.85 |
| Bacteria     | OP1           | 17  | 1286| 167 | 1.53  | 0.26 |
| Bacteria     | OP11          | 8   | 143 | 17  | 0.17  | 0.03 |
| Bacteria     | OP3           | 51  | 1402| 623 | 1.67  | 0.99 |
| Bacteria     | OP8           | 9   | 673 | 88  | 0.80  | 0.14 |
| Bacteria     | OP9           | 3   | 251 | 491 | 0.30  | 0.78 |
| Bacteria     | PAUC34f       | 3   | 58  | 0   | 0.07  | 0.00 |
| Bacteria     | Planctomycetes| 227 | 2017| 1195| 2.40  | 1.89 |
| Bacteria     | Proteobacteria| 858 | 27270| 24794| 32.42 | 39.29 |
| Bacteria     | SAR406        | 12  | 251 | 644 | 0.30  | 1.02 |
| Bacteria     | SBR1093       | 1   | 120 | 0   | 0.14  | 0.00 |
| Bacteria     | Spirochaetes  | 95  | 4074| 1967| 4.84  | 3.12 |
| Bacteria     | SR1           | 3   | 35  | 3   | 0.04  | 0.00 |
| Bacteria     | Synergistetes | 4   | 53  | 24  | 0.06  | 0.04 |
| Bacteria     | TA06          | 1   | 5   | 0   | 0.01  | 0.00 |
| Bacteria     | Tenericutes   | 7   | 45  | 45  | 0.05  | 0.07 |
| Bacteria     | Thermotogae   | 4   | 738 | 254 | 0.88  | 0.40 |
| Bacteria     | TM6           | 26  | 187 | 98  | 0.22  | 0.16 |
| Bacteria     | TM7           | 9   | 34  | 31  | 0.04  | 0.05 |
| Bacteria     | Verrucomicrobia| 60  | 593 | 538 | 0.70  | 0.85 |
| Bacteria     | WS1           | 11  | 133 | 75  | 0.16  | 0.12 |
| Bacteria     | WS2           | 3   | 27  | 0   | 0.03  | 0.00 |
| Bacteria     | WS3           | 41  | 1158| 552 | 1.38  | 0.87 |
| Bacteria     | WWE1          | 17  | 1020| 222 | 1.21  | 0.35 |
| Bacteria     | ZB3           | 2   | 35  | 0   | 0.04  | 0.00 |
Supplementary figures

Figure S1

(A) Mineral composition of Ursu (U) and Fara Fund (FF) sapropels from three distinct sediment samples. (Q - quartz, P – plagioclase feldspar, O – orthoclase feldspar, M – muscovite, Ch – chlorite, A – amphibole, C – calcite, D – dolomite, H – halite). (B) X-ray diffractions on the <2µm size fraction separated from the sapropels (gray – oriented sample; black – sample treated with ethylene glycol): M1 – montmorillonite, M – muscovite, K – Kaolinite, Ch – chlorite; small amounts of quartz (Q) and plagioclase feldspar (P) are also present in the samples.
Epifluorescence microscopy images from Ursu and Fara Fund sapropels. (A) epifluorescence DAPI image of prokaryotic morphotypes (indicated by arrows) in Fara Fund Lake sapropel. (B) superimposed image of Ursu Lake sapropel showing the presence of chlorophyll-containing microorganisms (indicated by arrows). (C) and (D) show the presence of filamentous photosynthetic bacteria in Ursu Lake sapropel (indicated by arrows).
Figure S3

Habitat distribution of the Ursu and Fara Fund sapropels' OTUs (>0.1% abundance) closest blastn hits, as found in the NCBI's nucleotide database.
Figure S4

SEM images of fungal morphotypes (indicated by arrows) present in the sapropel samples (A and B) and on the surface of plant material recovered from sapropels (C and D).
Figure S5

(A) Dendrogram constructed using the fungal community fingerprints generated from twelve environmental samples. (B) Fungal phylogenetic tree created using the V7-V8 SSU rDNA hypervariable regions. Legend: L-lane; B-band.