Molecular biosignatures in planetary analogue salts: implications for transport of organics in sulfate-rich brines beyond Earth

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Supplementary Information

The Supplementary Information includes:
- ➢ Methodology
- ➢ Figures S-1 to S-4
- ➢ Supplementary Tables S-1 to S-3
- ➢ Supplementary Information References

Methodology

1. Lipid analysis

Molecular biosignatures were extracted at the BECS organic geochemistry lab at the University of Glasgow, Glasgow, UK, following the BECS lipid biomarker extraction protocol (Toney *et al.,* 2010) with freeze-dried salt sample material, not powdered. Due to the low concentration of organic matter in the salts, between 3.8 and 22.7 g of sample were used. All glass vials used for this procedure were combusted at 450 °C for 8 hr prior to analysis. The total lipid extract (TLE) was separated from salt matrices using a Dionex Accelerated Solvent Extractor (ASE) 350 with a mixture of dichloromethane and methanol (9:1 DCM;MeOH). Solvent was evaporated from the TLE using nitrogen in a Turbovap and the weight of TLE was recorded. The TLE was then separated into neutral and acid fractions using solid phase extraction (SPE). SPE was performed using silica columns comprised of a glass wool stopper in a Pasteur pipette with, ~4cm dry aminopropyl silica gel, topped with combusted sand. Columns were washed with 3 bed volumes of 1:1 DCM_isopropyl alcohol (ISO) before the TLE was loaded onto the column using 1:1 DCM:ISO solution. Subsequently the Total Neutral Fraction (TNF) was eluted with 4 mL1:1 DCM:ISO and collected into an 8 mL vial. Following, the Total Acid Fraction (TAF) was eluted with 4 mLof ether with 4% acetic acid, and TAF collected in an 8 mL vial. The TNF was further separated using a second silica SPE column. TNF was separated on 35-70 µm particle size silica powder. Columns were washed with 3 bed volume of hexane before TNF was loaded on to the column. Columns were

eluted with hexane to obtain the non-polar, aliphatic hydrocarbon fraction containing *n*-alkanes. Branched fatty acids I-15 and a-15 were not observed as anything lower than C16 had evaporated by the time the branched fatty acid analysis was performed.

n-Alkanes were analysed on an Agilent 7890B gas chromatography flame ionisation detector (GC-FID). The GC-FID was fitted with an Agilent Rtx-1 column (60 m length, 250 µm i.d., 0.25 µm film thickness). Hydrogen was used as the carrier gas at a 1.2ml/min constant flow rate. The method used splitless injection (1µl) and the oven temperature was programmed from 60 °C (held for 2 mins) to 120 °C at a rate of 30 °C/min, then ramped to 330 °C at a rate of 5 °C/min and held for 15 minutes. An external standard mix of C_{16}, C_{18}, C_{19}, C_{23}, C_{25}, C_{26}, C_{28}, C_{30}, C_{32}, C_{37} was measured every 10 samples and used to identify retention times of *n*-alkanes in sediment samples. The *n*-alkanes were quantified using an external calibration of the standard mix ranging from 2.5- 10 µg/ml. Each chain length identified in samples was calibrated to its chain length in the standard mix or if not present in the standard mix, the closest chain length was chosen (e.g., C_{27} calibrated using C_{28} in the standard mix).

A sample of derivatized fatty acids (as FAMEs) were run on an Agilent 7890B Series GC with 5977A GC-EI mass spectrometer with helium as a carrier gas and GC method was the same as the GC-FID outlined above. Fatty acid presence was confirmed using the ion chromatograms of the corresponding homologue's fatty acid methyl ester (FAME) ion chromatogram. Straight chain and branched FAMEs are characterised by the presence of m/z 74 and 84. Unsaturated FAMEs had similar ion chromatograms but with a suppressed m/z 74. Identity of compounds was also confirmed by comparison to known ion chromatograms reported in Sanchez Garcia *et al.*, (2018).

Stable carbon isotopes were analysed for samples presenting enough concentration of *n*-fatty acids (AB-1, AB-4, AB-5, AB-6, RB-1, RB-2, RB-2sub, RB-3, RB-4, D-1, D-5) and *n*-alkanes (RB-3, RB4, D-6, D-7.1, D-7sub, D-8). The samples were analysed on an Agilent 7890B GC-FID connected to an Isoprime 100 Mass Spectrometer (Elementar). The GC-FID was fitted with an Agilent Rtx-1 column (60 m length, 250 µm internal diameter, 0.25 µm film thickness). Hydrogen was used as the carrier gas at a 1.2ml/min constant flow rate. The GC method used splitless injection (1µl) and the oven temperature was programmed from 60 °C (held for 2 mins) to 120 °C at a rate of 30 °C/min, then ramped to 325 °C at a rate of 5 °C/min and held for 16 minutes. Samples were measured in duplicate and $\delta^{13}C$ values were converted to the V-PBD scale by linear regression to an in-house standard of known $\delta^{13}C$. Standards were measured in duplicate every 10 samples and in triplicate at the start and end of each batch. The in-house standard was characterised using Indiana B5 containing C_{16} to C_{30} alkanes with an isotopic range between –26.15 and –40.9 ‰. Instrument precision on duplicate analysis of standards gave a standard deviation of <1. Samples that gave higher than 3‰ excluded from

results as concentrations were not high enough to obtain reasonable precision. The mean and 1 standard deviation are reported in results.

1. Evolved Gas Analysis (EGA)

Evolved Gas Analysis (EGA) was conducted to constrain sample mineralogy and investigate potential associations between organic compounds and salt phases. EGA-MS was carried out with a Setaram LabSys-Evo Thermo-gravimeter/Differential Scanning Calorimeter interfaced to a Pfieffer OmniStar quadrupole mass spectrometer. Five milligrams of freeze-dried sample was loaded into the EGA-MS pyrolysis oven and heated from 70 to 1400 °C, with a ramp rate of 35 °C/minute. A He carrier gas was used at a flow rate of 5 mL/min, and a pressure of ~25 millibar was maintained inside the oven during the pyrolysis run. Any gases evolved during heating were carried to the MS where they were identified by the mass-to-charge ratio (m/z) of the molecule or one of its MS fragments. Here we focused on SO_2 (m/z 64), CO_2 (m/z 44), O_2 (m/z 32), alkane fragments (e.g., m/z 39, 41) and m/z 15 which may result from methane or CH3 fragments of a larger organic molecule (studied using the signal at m/z 15; m/z 16 was not used directly because of several mass interferences at m/z 16 (e.g., O MS fragments of SO_2 and CO_2) but less mass interferences occur at the m/z 15 MS fragment of methane (CH_3^-)) or CH3 fragments of a larger organic molecule).

Supplementary Figures
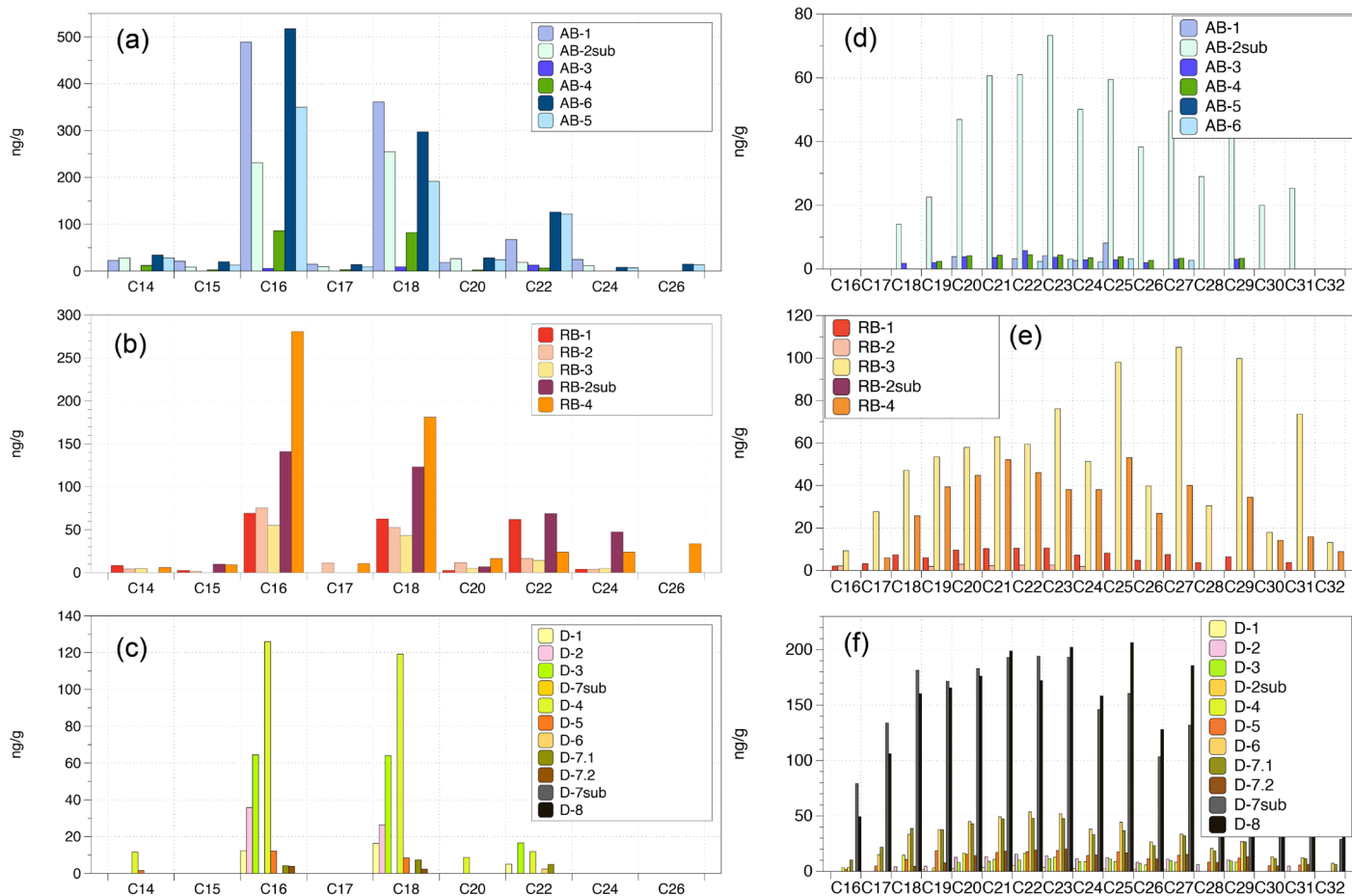
Figure S-1 (a-c) FAMES distribution for **(a)** Active Brine samples, **(b)** Relic Brine samples, and **(c)** Deposit samples. **(d-f)** Distribution of n-alkanes for **(d)** Active Brine samples, **(e)** Relic Brine samples, and **(f)** Deposit samples.
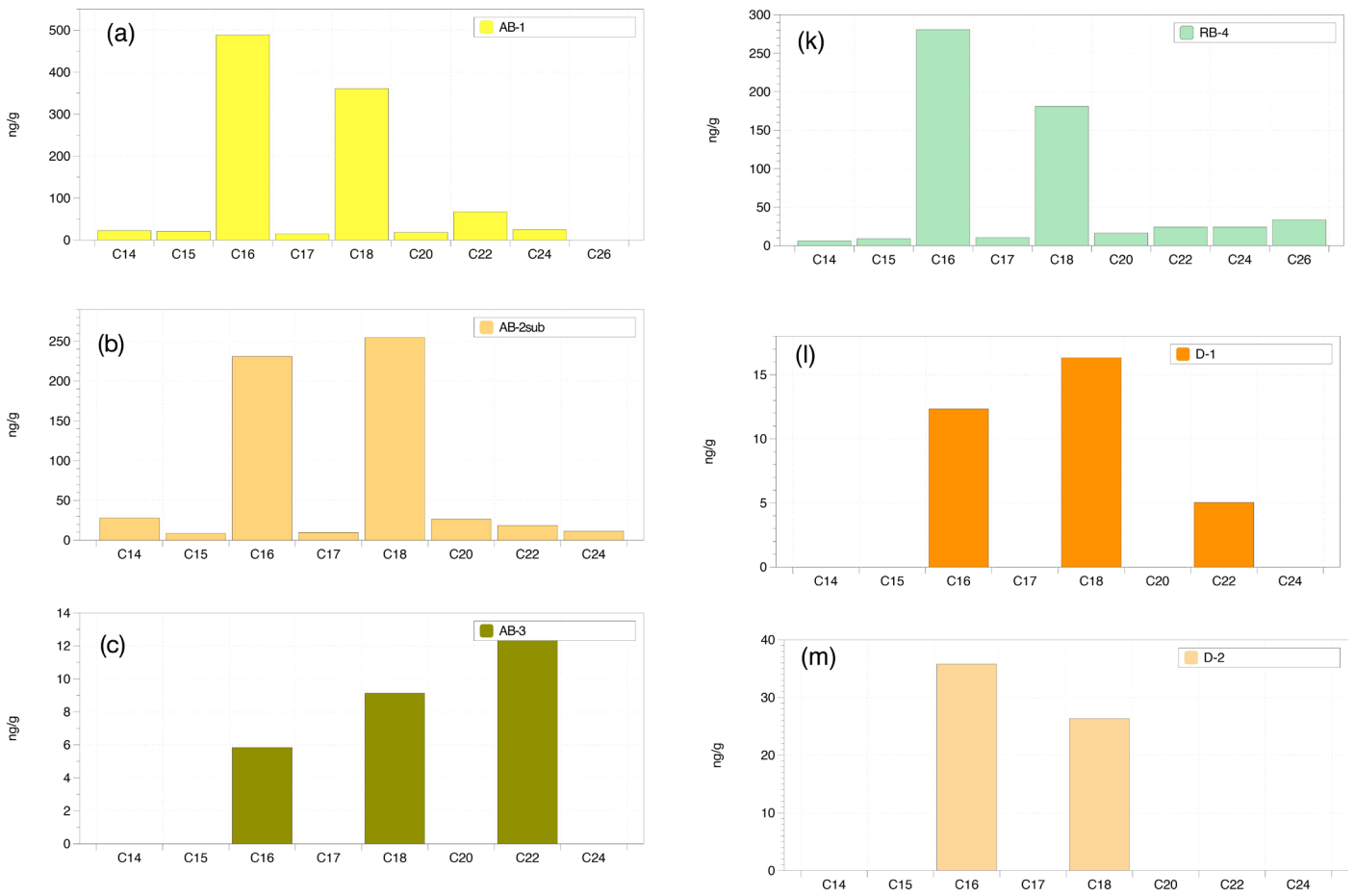
Figure S-2 (across 3 pages) Individual fatty acid distribution for all different samples. Samples D-4 and D-7sub didn't present any alkanes. On the other hand, D-8 presented only C18:1 unsaturated, which are not counted for these graphs.

Figure S-2 continued
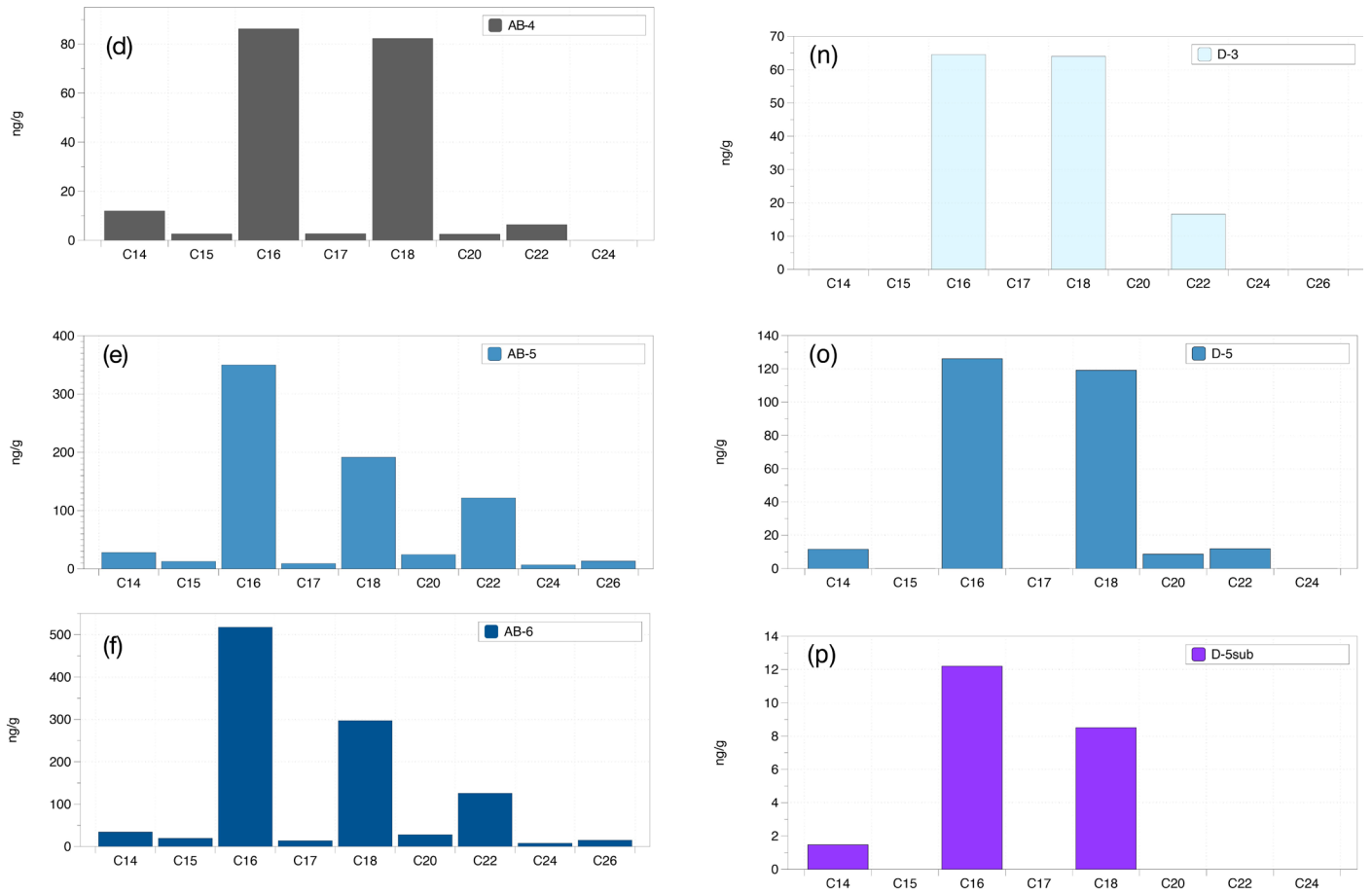
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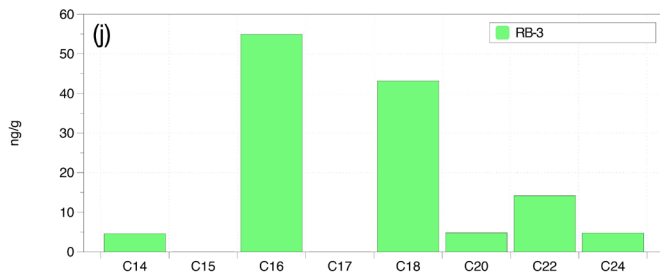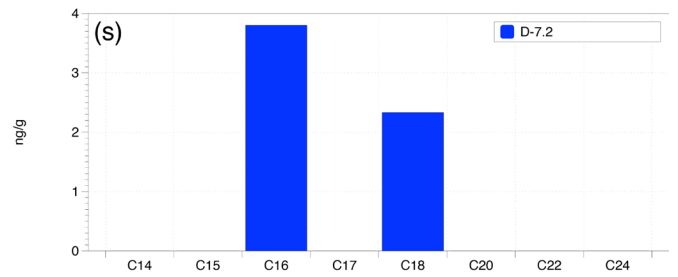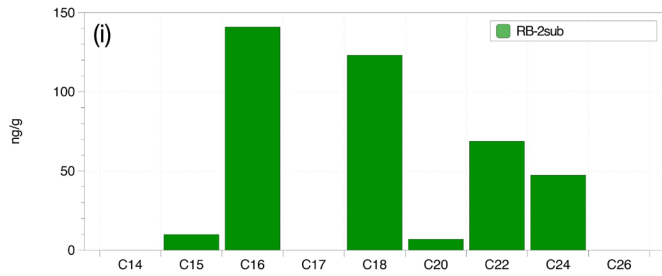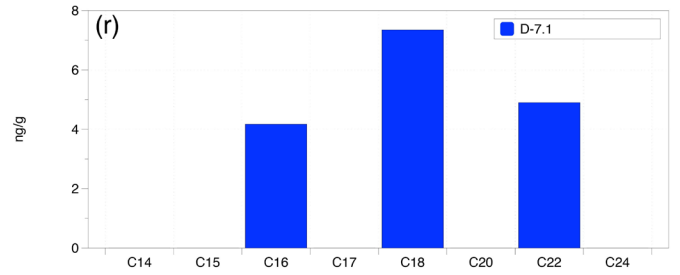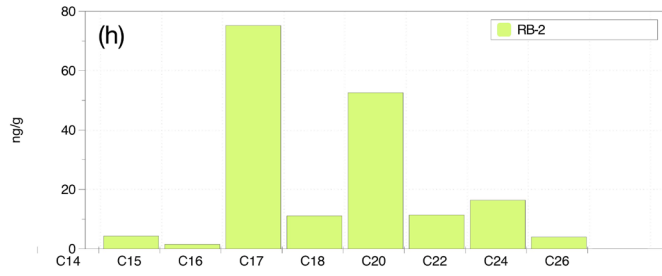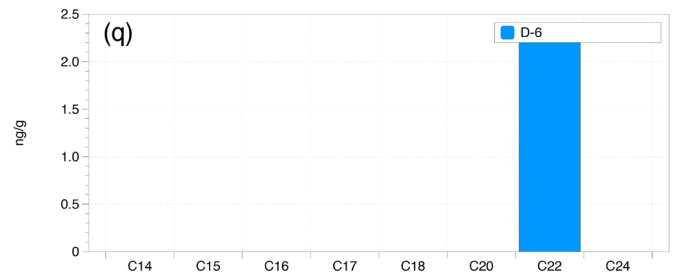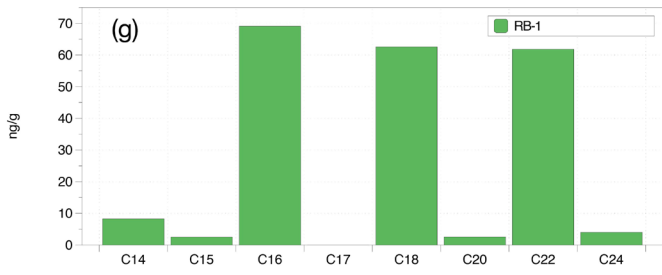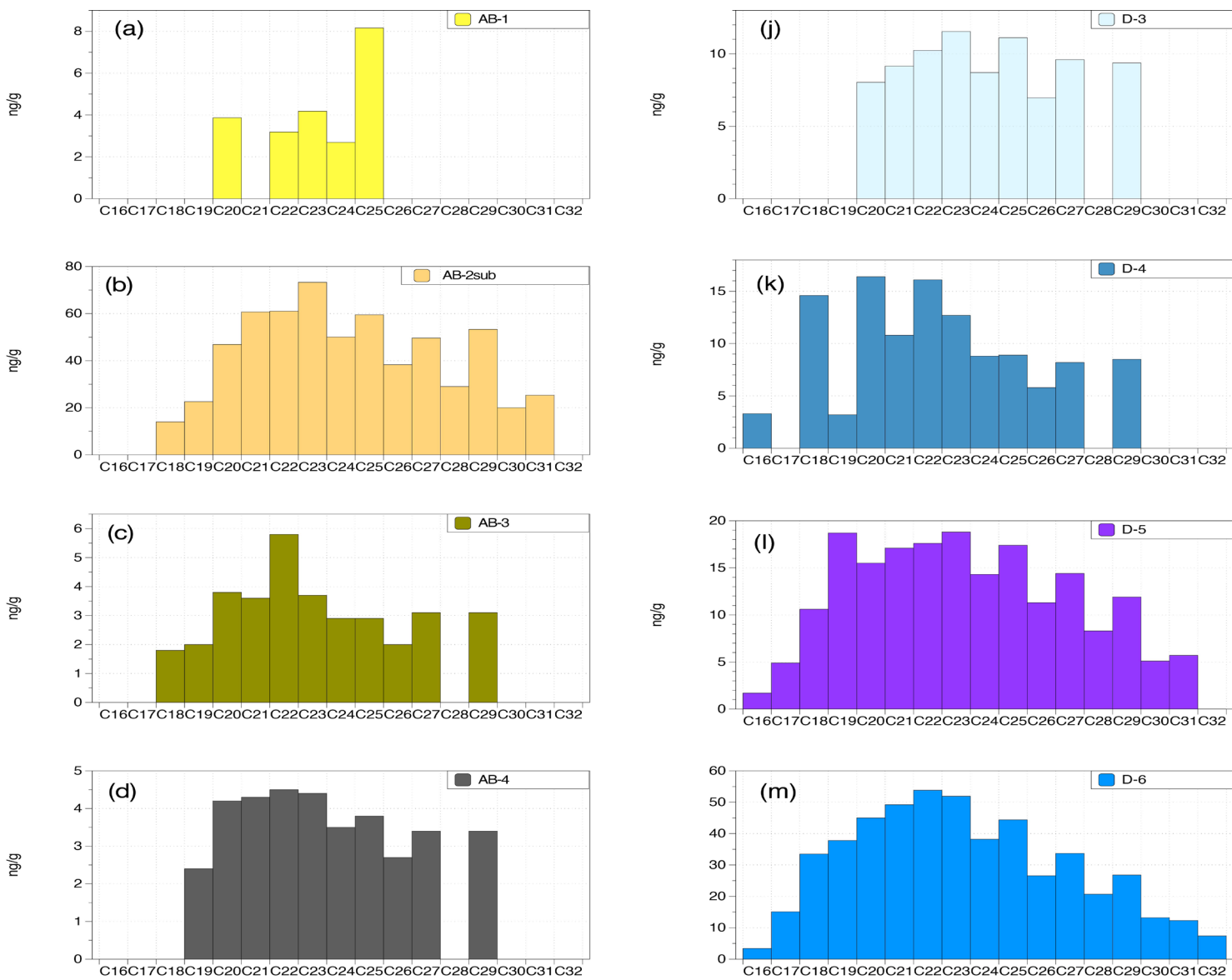
Figure S-3 (across 3 pages) Individual n-alkanes distribution for all samples.

Figure S-3 continued
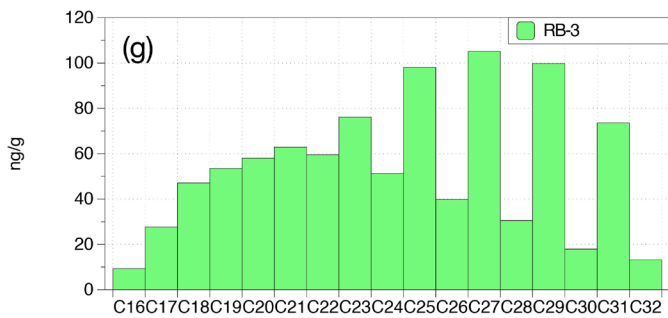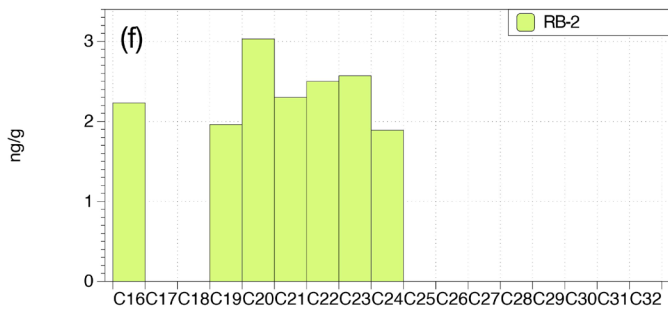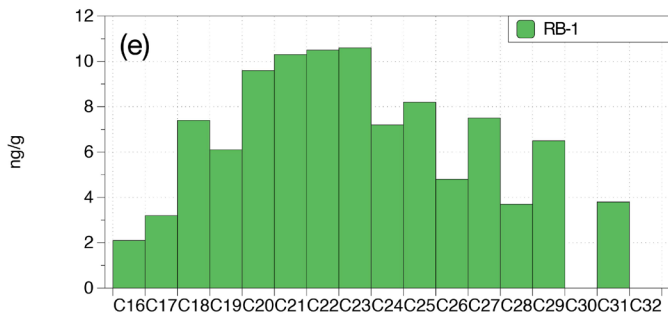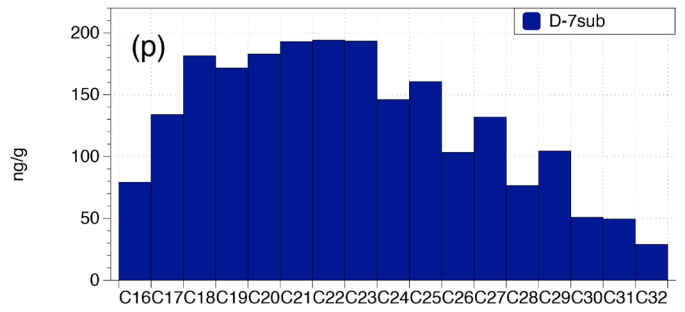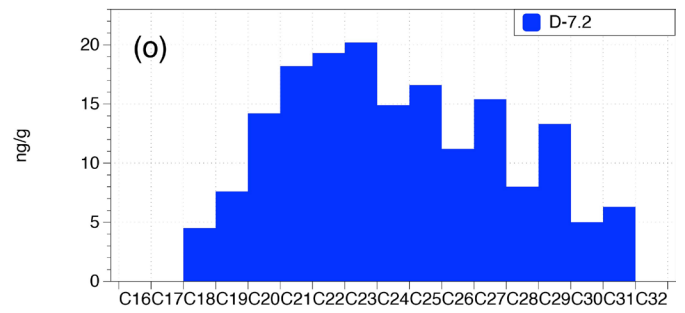
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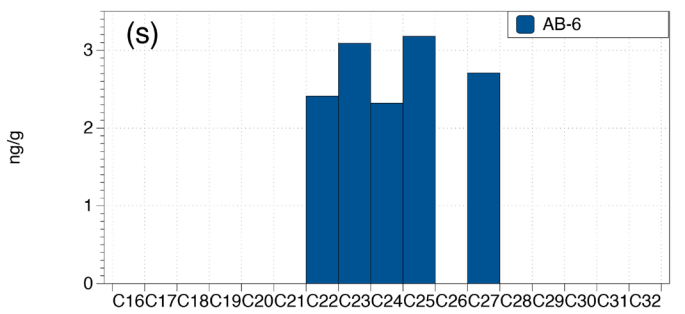
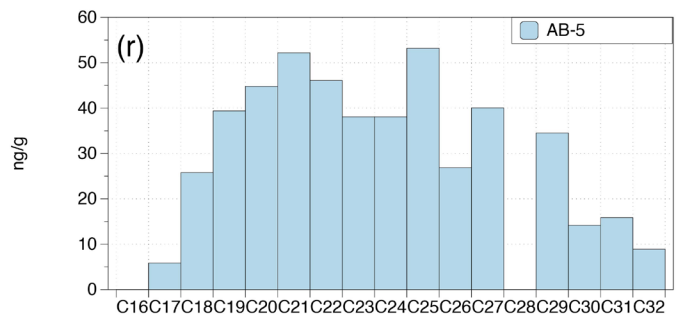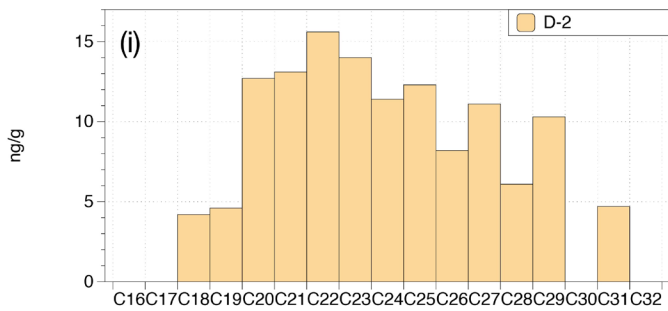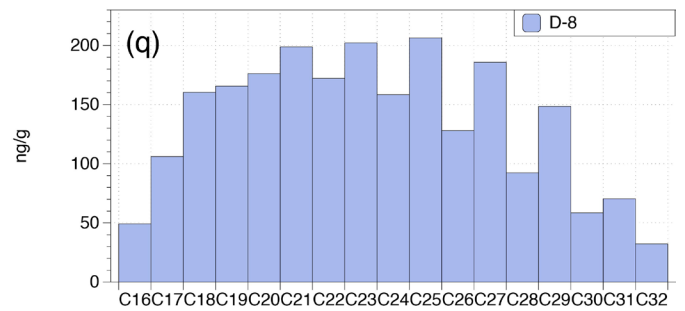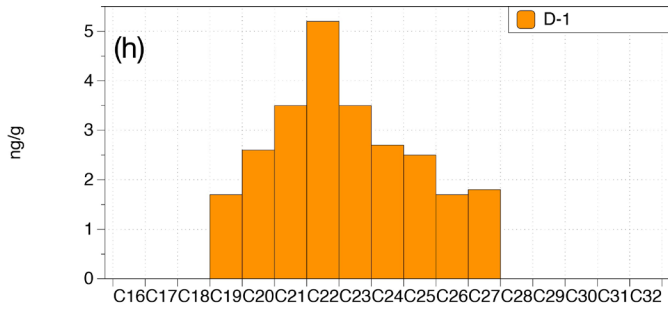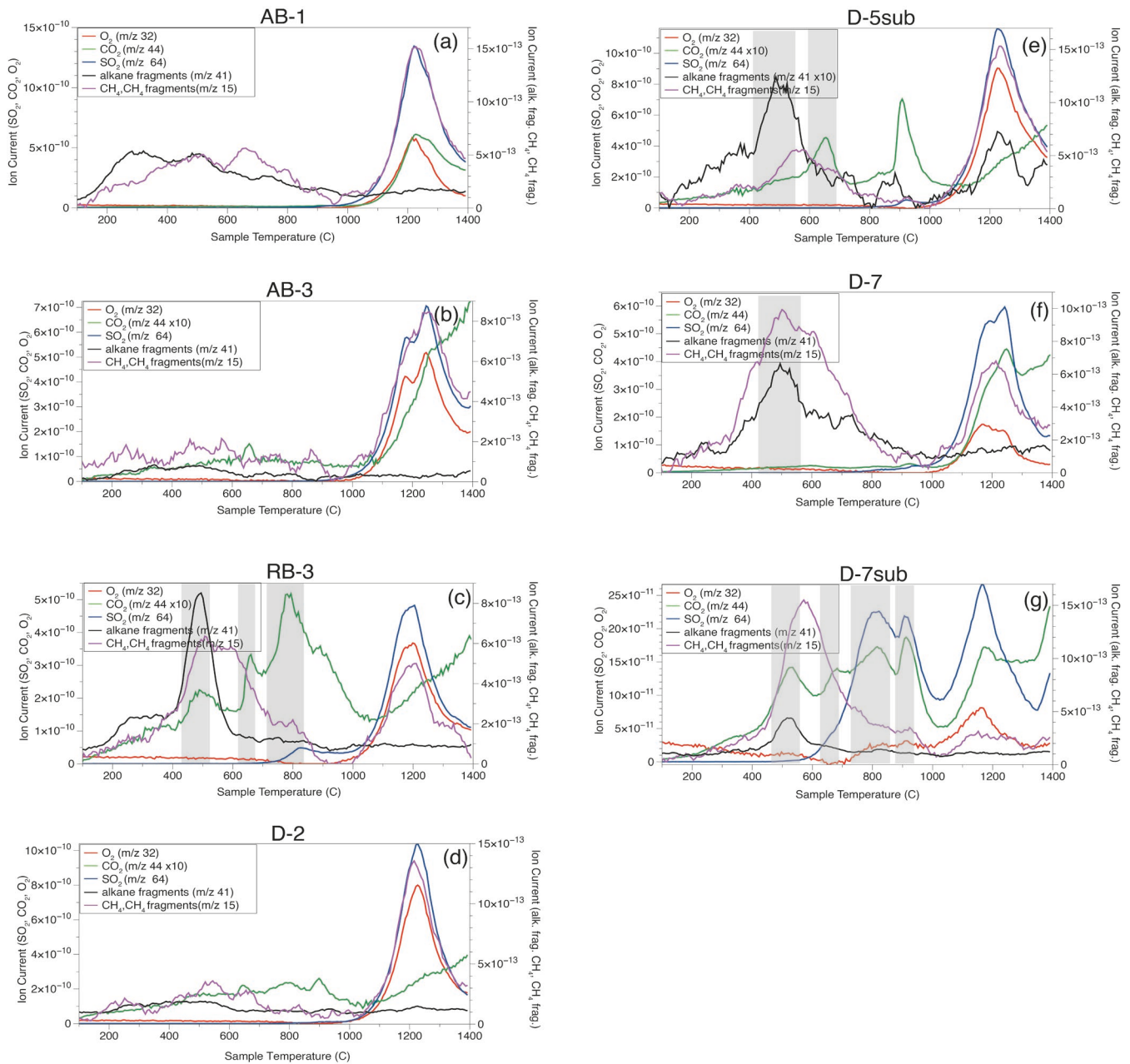**Figure S-4** Evolved Gas Analyses (EGA) results for 7 samples. Active Brine (AB) samples **(a, b),** Relic Brine sample **(c),** Deposit samples **(d, e, f, g).** Grey areas for interesting peaks discussed through main text.

Supplementary Tables

Table S-1 FAMES Normalised to sample mass (ng/g of sample)

Table S-2 n-alkanes normalised to sample mass (ng/g of sample)

Table S-3 Compound specific C isotope results

Tables S-1 to S-3 are available for download (.xlsx) from the online version of this article at http://doi.org/10.7185/geochemlet.2434.

Supplementary Information References

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Toney, J.L., Huang, Y., Fritz, S.C., Baker, P.A., Grimm, E., Nyren, P. (2010) Climatic and environmental controls on the occurrence and distributions of long chain alkenones in lakes of the interior United States. *Geochimica Cosmochimica Acta* 74, 1563-1578. https://doi.org/10.1016/j.gca.2009.11.021