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Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry

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Abstract

Rationale: The hydrogen isotopic composition of lipids ($\delta^2\text{H}_{\text{lipid}}$) is widely used in food science and as a proxy for past hydrological conditions. Determining the $\delta^2\text{H}$ values of large,

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well-preserved triacylglycerides and other microbial lipids, such as glycerol dialkyl glycerol tetraether (GDGT) lipids, is thus of widespread interest but has so far not been possible due to their low volatility which prohibits analysis by traditional gas chromatography pyrolysis isotope ratio mass spectrometry (GC/P/IRMS).

Methods: We determined the $\delta^2\text{H}$ values of large, polar molecules and applied high temperature gas chromatography (HTGC) methods on a modified GC-/P/IRMS system. The system used a high temperature 7 m GC column, and a glass Y-splitter for low thermal mass. Methods were validated using authentic standards of large, functionalised molecules (triacylglycerides, TG), purified standards of GDGTs, and compared to $\delta^2\text{H}$ values determined by elemental analyser pyrolysis isotope ratio mass spectrometry (HTEA/IRMS); and subsequently applied to the analysis of GDGTs in a sample from a methane seep and a Welsh peat.

Results: $\delta^2\text{H}$ values of TGs agreed within error between HTGC/P/IRMS and HTEA/IRMS, with HTGC/P/IRMS showing larger errors. Archaeal lipid GDGTs with up to three cyclisations could be analysed: $\delta^2\text{H}$ values were not significantly different between methods with standard deviations of 5 to 6 ‰. When environmental samples were analysed, $\delta^2\text{H}$ values of isoGDGTs were 50 ‰ more negative than those of terrestrial brGDGTs.

Conclusions: Our results indicate that the high temperature GC/P/IRMS (HTGC/P/IRMS) method developed here is appropriate to determine the $\delta^2\text{H}$ values of TGs, GDGTs with up to two cyclisations, and potentially other high molecular weight compounds. The methodology will widen the current analytical window for biomarker and food light stable isotope analyses. Moreover, our initial measurements suggest that bacterial and archaeal GDGT $\delta^2\text{H}$ values can record environmental and ecological conditions.

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Introduction

The stable hydrogen isotopic composition ($\delta^2\text{H}$ values) of water varies systematically across the globe^{1–3}. The $\delta^2\text{H}$ values of biological molecules, in turn, are dependent on the $\delta^2\text{H}$ of the H_2O available to the producing organism (source water), overprinted by biochemical processes. The $\delta^2\text{H}$ values of bulk organic matter and individual compounds are used across a range of disciplines, e.g., in ecology and biology to trace animal migration patterns and food webs^{4,5}, in forensic science to identify geographical origins of victims or suspects⁶, and in food science to determine the provenance of products such as honey⁷, milk⁸, and meat⁹. The determination of $\delta^2\text{H}$ values has also resulted in substantial discoveries in archaeology, such as the earliest horse milking¹⁰, or manuring practices¹¹, and has improved our understanding of past environments and precipitation regimes^{12–14}.

The $\delta^2\text{H}$ values of individual lipid biomarkers are particularly useful in paleoenvironmental studies. In particular, the correlation of lipid $\delta^2\text{H}$ with source water $\delta^2\text{H}$ has been widely documented^{12,15,16}, such that leaf waxes are now widely used to reconstruct past hydrological conditions^{12,16–18}. Long-chain *n*-alkanes and other alkanes are often used in this endeavour because they are—due to their relatively high pK_a (~ 50)—less susceptible to hydrogen exchange than functionalized compound classes commonly found in soils and sediments. However, a wide range of sedimentary lipids have been analysed for their stable hydrogen isotopic composition, including *n*-alkanes, fatty acids, alkenones, and, to a lesser extent, sterols and hopanols^{19–23}.

The routine and rapid compound-specific $\delta^2\text{H}$ value determination of biomarkers (as opposed to labour intensive approaches requiring compound isolation and purification) requires the application of gas chromatography, coupled to an on-line reactor containing active graphite, converting individual organic compounds into graphite, CO and H_2 ^{21,24–27}. The produced gas is introduced into an isotope ratio mass spectrometer monitoring m/z 2

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(^1H - ^1H) and 3 (^1H - ^2H). This setup requires analytes to be GC-amenable²⁸, limiting analyses to compounds of a molecular weight and polarity low enough to elute at a typical maximum capillary column operating temperature of 320 °C. Therefore, only very few larger compounds (eluting later than a C_{36} *n*-alkane on an apolar stationary phase) have had their $\delta^2\text{H}$ values successfully determined. Existing measurements were achieved by implementing long isothermal holds at 320 °C but only with highly purified and ^2H -labelled compounds²⁹, due to the low GC resolution and $\delta^2\text{H}$ precision associated with this methodology.

However, the $\delta^2\text{H}$ values of large and/or polar compounds can be of significant interest. For example, the origin of vegetable oils and milk products can be constrained^{30–32} with greater specificity when isotopic fingerprinting is based on individual fatty acids instead of bulk organics^{33,34}. Moreover, determining the $\delta^2\text{H}$ values of intact triacylglycerides (TG, Suppl. Fig. 1A), instead of hydrolysed and derivatised fatty acids, could have many benefits such as eliminating derivatisation biases and increased specificity. TG are routinely characterised in food forensics by high temperature gas chromatography (HTGC^{35–37}), but their ^2H signatures are yet to be exploited. Another potential application arises from very long-chain *n*-alkanes that are major constituents of crude oil; their $\delta^2\text{H}$ values could be used to assess source rock potential^{17,18,38,39}, or for correlating different oils and source rocks^{38,40}.

A third suite of applications centres on glycerol dialkyl glycerol tetraether lipids (GDGTs, Suppl. Fig. 1BC), derived from both Archaea and Bacteria and of wide interest in geochemistry. These membrane lipids are frequently used in proxies for paleotemperature and other environmental variables⁴¹. In many sedimentary archives, GDGTs are of mixed origins (e.g. ^{42,43}), and their $\delta^2\text{H}$ values could thus be used to distinguish terrigenous from in situ-produced GDGTs, for example in marine sediments. This would substantially improve the application of these GDGT-based proxies. Moreover, in single-source environments, the hydrogen isotopic composition of GDGTs could serve as a paleohydrological proxy, enabling reconstruction of salinity, elevation, or precipitation. More recently, it has been shown that

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$\delta^2\text{H}$ values of bacterial lipids document the metabolic state of the source organisms, potentially representing another application in biogeochemical investigations ⁴⁴, and this method will allow to extend such investigations to archaea.

In order to determine the stable isotopic composition of some of these large molecules, they are often subjected to chemical degradation, and only fragments (mostly aliphatic moieties) that are more GC-amenable than the parent molecule are analysed by GC/IRMS. For TGs, this involves acid methanolysis ⁴⁵. For GDGTs, this involves ether cleavage, followed by reduction ^{46–51}, often including laborious preparative HPLC steps for cleaning and preconcentration ⁵². Aside from being labour intensive, such procedures under acidic conditions could result in hydrogen exchange.

However, recently, HTGC methods for more direct analysis of these compounds have been developed; identification and quantification of GDGTs has been achieved employing HTGC coupled to time-of-flight mass spectrometry (HTGC/TOFMS) and flame ionisation detection (HTGC/FID ^{53,54}). Here, we develop these methods further and demonstrate $\delta^2\text{H}$ analysis of polar and high molecular weight compounds by HTGC coupled to pyrolysis isotope ratio mass spectrometry (HTGC/P/IRMS). We compare the values of purchased, authentic standards (TGs), and purified standards (GDGTs) determined by elemental analyser pyrolysis isotope ratio mass spectrometry (HTEA/IRMS) with the values determined by HTGC/P/IRMS. We then report the $\delta^2\text{H}$ values of GDGTs in a number of environmental samples.

Experimental

Standards and environmental samples

Triacylglyceride [TG; trimyristin (TG 42:0), tripalmitin (TG 48:0), and tristearin (TG 54:0)] and *n*-alkane standards were purchased from Sigma Aldrich (Gillingham, UK). isoGDGT-2 and isoGDGT-3 standards were purified from biomass of *Sulfolobus solfataricus* (DSM 1616),

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which was grown in two batches (2 L each) of modified Allen medium⁵⁵ using water with a $\delta^2\text{H}$ value of -55.0 ± 0.2 ‰. Each batch was inoculated with 20 mL of a late log-phase culture, incubated aerobically at 76 °C with agitation at 200 RPM, and harvested in mid-log phase at an optical density of 0.442 (600 nm). Cells were collected by centrifugation at 4 °C, frozen in liquid nitrogen, and freeze-dried. 0.5 g of the freeze-dried cell pellet was subjected to acid hydrolysis in 5 mL of 1.5 N methanolic HCl (10 % H₂O made from 37% HCl) for 3 hours at 70°C, and lipids were extracted by ultrasonication in dichloromethane:methanol (1:1; v/v) as previously described⁵⁶. The total lipid extract (TLE) was dried under a stream of N₂, dissolved in 1 mL of *n*-hexane: isopropanol (97:3; v/v), and filtered through a 0.45 µm PTFE filter.

To produce purified standards for both HTEA/IRMS, and for GC/P/IRMS, individual isoprenoidal GDGTs containing 2 and 3 cyclopentyl moieties (isoGDGT-2 and isoGDGT-3) were isolated by preparative normal phase (NP) high-performance liquid chromatography (HPLC). To this end, aliquots (25 µL) of the filtered TLE were injected onto an Agilent 1100 HPLC system fitted with an Econosphere NH₂ column (250 × 10 mm, 10 µm; Grace/Alltech). GDGTs were eluted isocratically with a solvent mixture of 1.35 % isopropanol (IPA) in *n*-hexane at a flow rate of 1 mL min⁻¹ for 45 min, and the column was cleaned with 16 % IPA for 12 min and re-equilibrated to initial conditions for 13 min after every run. GDGTs were recovered by time-based fraction collection, according to the elution times determined by atmospheric pressure chemical ionisation-mass spectrometry (APCIMS) using an Agilent 1100 MSD quadrupole mass spectrometer (Agilent Technologies, Cheshire, UK)⁵⁷. The collected fractions were analysed by flow injection analysis-APCIMS on the same instrument, and subsequently pooled by compound. The purity of each isolated GDGT was >97 % as assessed by NP and reverse phase HPLC/APCIMS analysis of the combined fractions⁵⁸, scanning the range *m/z* of 350–1350.

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Environmental samples analysed by GC/P/IRMS included a sediment sample from a marine methane seep, and a sample from a Welsh peat⁵³. In order to improve gas chromatographic performance, GDGTs were purified prior to HTGC/P/IRMS. The Welsh peat extract was passed over a column containing 130-270 mesh silica (pore size 60 Å 288608, Sigma Aldrich, Gillingham, UK) conditioned in methanol, using two column volumes of each hexane, ethylacetate/hexane 1:9 (v/v), 25:75, 50:50, pure ethylacetate, and methanol. Concentrations of GDGTs in the fractions were confirmed by adding triglyceride quantification standards and analysis by HTGC/FID⁵³. All fractions containing GDGTs (Suppl. Fig. 2) were combined to avoid any isotope fractionation which may have occurred during column chromatography.

²H analysis by HTEA/IRMS

The $^2\text{H}/^1\text{H}$ ratios of the triacylglycerides (TGs) and C_{50} and C_{60} *n*-alkanes were analysed via HTEA/IRMS at Elementar UK Ltd (EUK; Stockport, UK) and University of Colorado (CUB; Boulder, USA). CUB also analysed GDGTs. CUB performed HTEA/IRMS on a Flash HT Plus elemental analyser at 1450 °C with zero blank autosampler coupled to a Delta V Plus IRMS via ConFlo-IV Interface (both Thermo Fisher Scientific, Waltham, MA, USA). At EUK, HTEA/IRMS measurements were performed using a Geovision, which comprised a vario PYRO cube elemental analyser coupled to an isoprime vision IRMS (both EUK). Both laboratories measured samples using glassy carbon reactors in oxygen-free environments, and performed multipoint calibrations using reference materials provided by Arndt Schimmelmann (Indiana University, Bloomington, IN, USA) to normalise the measured $\delta^2\text{H}$ values against the international reference Vienna Standard Mean Ocean Water (VSMOW). CUB calibrated using 5 α -androstane #3 (-293.2 ± 1.0 ‰), eicosanoic acid methyl ester #Z1 / USGS 70 (-183.9 ± 1.4 ‰), and eicosanoic acid methyl ester #Z2 / USGS 71 (-4.9 ± 1.0 ‰), and EUK calibrated using tetracosane #1: -53.0 ± 1.6 ‰, pentacosane #4: -263.6 ± 2.2 ‰ and heptacosane #3: -172.80 ± 1.6 ‰, and a standard provided by the International Atomic Energy

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Agency, Vienna (IAEA CH-7: -100.2 ± 1.0 ‰). Across both labs, the standard deviation (SD) of triplicate sample analyses was typically $< \pm 0.75$ ‰.

Because the oxygen-bound H atoms of the GDGTs' hydroxyl moieties are easily exchanged, the ^2H content at these positions may have been altered during solvent extraction/evaporation. We therefore vapour-equilibrated the dried GDGT fractions with local deionised water (-121.8 ± 1.3 ‰) before analysis (24 h at 25 °C). GDGT fractions were then dissolved in ethyl acetate at $\sim 10 \mu\text{g } \mu\text{L}^{-1}$ and 10 μL aliquots were pipetted into combusted (450 °C, 10 h) silver capsules (4x6 mm), which were pre-loaded with small discs ($d = 4$ mm) of combusted glass fibre filters (Whatman GF/F) as a solvent adsorbent. The solvent was then completely evaporated in a closed chamber continuously purged with N_2 (30 min at $\sim 30 \text{ mL min}^{-1}$). Analysis by HTEA/IRMS was then conducted as described above.

To test for the efficiency of the vapour equilibration, a synthetic diglycerol-trialkyl-tetraether (C_{46} -GTGT; Patwardhan and Thompson, 1999) was exposed to vapour of both ^2H -enriched water (7 atom % ^2H) and local deionised water (24 h at 25 °C). Exposure to ^2H -enriched water vapour increased the ^2H content of the molecule by 0.1 atom % (from 0.014 to 0.113 atom % relative to total H), corresponding to a ^2H content of ~ 5 atom % at the OH positions after exposure (assuming all exchange is localised to the hydroxyl moieties).

Exposure to natural water vapor, however, did not lead to a change in $\delta^2\text{H}$ within analytical precision of the measurement. The induced ^2H content at the OH positions decreased again to a ^2H content of ~ 2 atom % at the OH-positions after a 12 h exposure to ambient lab air.

Together this indicates that OH-bound H of diglycerol tetraethers is readily exchanged with ambient water vapor, and any ^2H enrichment resulting from the evaporation of OH-containing solvents (e.g. methanol) was likely diminished either by spontaneous re-equilibration with ambient air, or by the latest through 24 h exposure to natural water vapor in a desiccator as described above.

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$\delta^2\text{H}$ value determination by high-temperature HTGC/P/IRMS

Before analysis by HTGC/P/IRMS, fractions containing GDGTs and the sample from the Black Sea methane seep were dissolved in 50 μL pyridine and derivatised to trimethylsilylethers with 50 μL 99% N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), 1% trimethylchlorosilane (TMCS), for one hour at 70 °C. The $\delta^2\text{H}$ value of the TMS moieties used to derivatise the hydroxyl-groups ($\delta^2\text{H}_{\text{TMS}}$) was determined by derivatisation of sodium palmitate of a known $\delta^2\text{H}$ ($\delta^2\text{H}_P$, -239.10 ‰), and analysis by GC/IRMS to yield the values of derivatised palmitate $\delta^2\text{H}_{\text{TMSP}}$, as -82.35 ‰ according to Eqn. 1. The use of δ -values in this specific case is possible and recommended (natural abundance ranges), when larger differences are present, D/H ratios must be used.

$$\delta^2\text{H}_{\text{TMS}} = \frac{\delta^2\text{H}_{\text{TMSP}} \cdot 40 - \delta^2\text{H}_P \cdot 31}{9} \quad (\text{Eqn 1})$$

Values of derivatised GDGTs $\delta^2\text{H}_{\text{meas}}$ were corrected by mass balance to give $\delta^2\text{H}_{\text{GDGT}}$ with n representing the number of non-exchangeable hydrogens of the compounds and k the number of TMS groups added (1 for archaeol, 2 for GDGTs and hydroxyarchaeol; Eqn. 2).

$$\delta^2\text{H}_{\text{GDGT}} = \frac{\delta^2\text{H}_{\text{meas}}(n + k \cdot 9)}{n} - \frac{k \cdot 9 \cdot \delta^2\text{H}_{\text{TMS}}}{n} \quad (\text{Eqn 2})$$

This was combined into Eqn. 3.

$$\delta^2\text{H}_{\text{GDGT}} = \frac{\delta^2\text{H}_{\text{meas}}(n + k \cdot 9)}{n} - \frac{k \cdot 40 \cdot \delta^2\text{H}_{\text{TMSP}}}{n} + \frac{k \cdot 31 \cdot \delta^2\text{H}_P}{n} \quad (\text{Eqn 3})$$

Errors of $\delta^2\text{H}_{\text{meas}}$ were determined according to error propagation laws:

$$\sigma_{\delta^2\text{H}_{\text{GDGT}}}^2 = \sigma_{\delta^2\text{H}_{\text{meas}}}^2 \cdot \left(\frac{n + k \cdot 9}{n}\right)^2 + \sigma_{\delta^2\text{H}_{\text{TMSP}}}^2 \cdot \left(\frac{k \cdot 40}{n}\right)^2 + \sigma_{\delta^2\text{H}_P}^2 \cdot \left(\frac{k \cdot 31}{n}\right)^2 \quad (\text{Eqn 4})$$

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Samples were screened by HTGC/FID as described by Lengger et al.⁵³ before they were analysed by an Elementar isoprime visION HTGC/P/IRMS (Elementar UK Ltd., Cheadle, UK). The instrument comprised an Agilent 7890B GC fitted with an on-column injector, linked to a GC5 interface (maintained at 380 °C) and a hollow ceramic reactor, in which a stripped transfer line (Zebron Z-Guard high temperature guard column, 0.25 mm ID, Phenomenex Ltd., Aschaffenburg, Germany) was inserted carrying analytes from the GC, enabling pyrolysis at 1450 °C. A PTV injector was not available on this instrument, but was observed to inhibit elution of GDGTs in separate investigations (data not shown). Ferrules used to connect the ceramic furnace and GC-column, as well as the sample line He used as an additional carrier in the HTGC/P/IRMS system, were 100% graphite. Ion beams at m/z 2 and 3 were monitored via an isoprime visION mass spectrometer. The H_3^+ factor was determined daily or at least every 4 runs. Compounds were injected in ethylacetate (1 μL) and separated on a Zebron ZB-5HT analytical column (7 m \times 0.25 mm \times 0.25 μm , Phenomenex Ltd., Aschaffenburg, Germany) with high-temperature resistant polyimide coating, which was fitted to a transfer line (Zebron Z-Guard Hi-Temp guard column 0.25 mm ID, Phenomenex Ltd., Aschaffenburg, Germany) that was inserted directly into the reactor (with the reactor-facing side thermally stripped of polyimide coating), and an exhaust to allow diversion of the solvent peak to waste via a glass Y-splitter, in which columns were fixed with high temperature resin (Phenomenex Ltd., Aschaffenburg, Germany). He was used as a carrier gas at a flow rate of 2.2 mL min^{-1} , and the oven was programmed as follows: 1 min hold at 70 °C, increase by 10 °C min^{-1} to 350 °C, followed by an increase at 3 °C min^{-1} to 400 °C (10 min hold). Results were calibrated using a mixture of *n*-alkanes (B3, A. Schimmelmann, Indiana University, Bloomington, IN, USA) according to Sessions et al.^{21,60}, which was injected at least every four analyses (RMS detailed in Tab. S1), and analysed using a He flow of 1 mL min^{-1} , with a different temperature program (injection at 50 °C held for 1 min followed by an increase of 10 °C min^{-1} to 300 °C and a 10 min hold). Resultant calibrated $\delta^2\text{H}$

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values were calculated based on the derived linear regression. Root mean standard errors of normalised values of the *n*-alkane mixture were typically between 4 and 6 ‰, and never exceeded 10 ‰. Data was processed using ionOS stable isotope data processing software (Elementar UK Ltd., UK), using an automated multi-point linearisation based on the certified values of the 15 individual *n*-alkanes comprising the B3 standard.

The fractionation factor $\epsilon_{\text{H}_2\text{O}/\text{GDGT}}$ was determined from the $\delta^2\text{H}_{\text{H}_2\text{O}}$ and the $\delta^2\text{H}_{\text{GDGT}}$ (Eqn. 5).

$$\epsilon_{\text{GDGT}/\text{H}_2\text{O}} = \left(\frac{\delta_{\text{GDGT}} + 1}{\delta_{\text{H}_2\text{O}} + 1} - 1 \right)$$

(Eqn. 5)

Results and discussion

Chromatographic method

The modifications of the GC/P/IRMS instrumentation enabled operating temperatures of up to 400 °C. Utilisation of a 7-m column and on-column injection (as previously discussed⁵³) enabled elution of isoGDGTs up to GDGT-3, as well as acceptable values for the B3 standard. The HTGC/P/IRMS setup required a polyimide-coated column rather than the metal column commonly employed in HTGC-methodologies, as this allowed flow diversion via a glass Y-splitter in which the column was secured using high temperature resin (no other modifications to the standard Elementar flow diversion system were made). The glass Y-splitter ensured minimal thermal mass. The small ID of the ceramic reactor and insertion of the transfer line close to the pyrolysis site, and of contact with any metal surfaces (glass Y-splitter instead of metal valve, silicon transfer to pyrolysis site in ceramic reactor), have likely contributed to avoid the peak broadening and fronting often observed in GC/P/IRMS. Furthermore, the pneumatically operated heart-cut valve enabling diversion of the solvent

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away from the furnace reactor was moved to a location outside of the GC-oven in order to avoid potential leaks associated with the high temperatures. Extended (> 10 min) high temperature (> 400 °C) isothermals such as used successfully with metal columns to analyse isoGDGTs by HTGC/FID and HTGC-TOFMS⁵³, could not be employed to elute isoGDGTs in analogous HTGC/P/IRMS analyses due to the comparatively lower stability of the polyimide-coated columns at these temperatures.

The unusual HTGC configuration, with a short 7 m column, high flow, and on-column injector, was tested by analysing a mixture of 15 *n*-alkanes: the so-called Indiana B-standard mix routinely used for standardisation of GC/P/IRMS results. Baseline separation of individual *n*-alkane peaks and acceptable root mean square errors were achieved with this method (Fig. 1A): this standard was subsequently used for quality control and isotope calibration. Root mean square error (RMSE) and linearisation equations for all analyses of the standards are given in Supplementary Fig. 3 and Table 1, with linearisation applied to the samples based on the most contemporary analysis of the standard. RMSE for all accepted analyses were always below 10 ‰: whenever 10 was exceeded, inlet maintenance or column changes were performed. An *n*-alkane standard containing higher molecular weight compounds (up to C₆₀, Fig. 1B), a mixture of triacylglycerides (Fig. 1C), a seep sample containing GDGT-0, -1, -2, and -3, and the two GDGT standards (GDGT-2 and -3) (Fig. 1D) were analysed and chromatograms were similar to previous results employing HTGC/FID and a 7 m column⁵³. The brGDGTs eluted earlier than isoGDGTs (cf. ⁵³).

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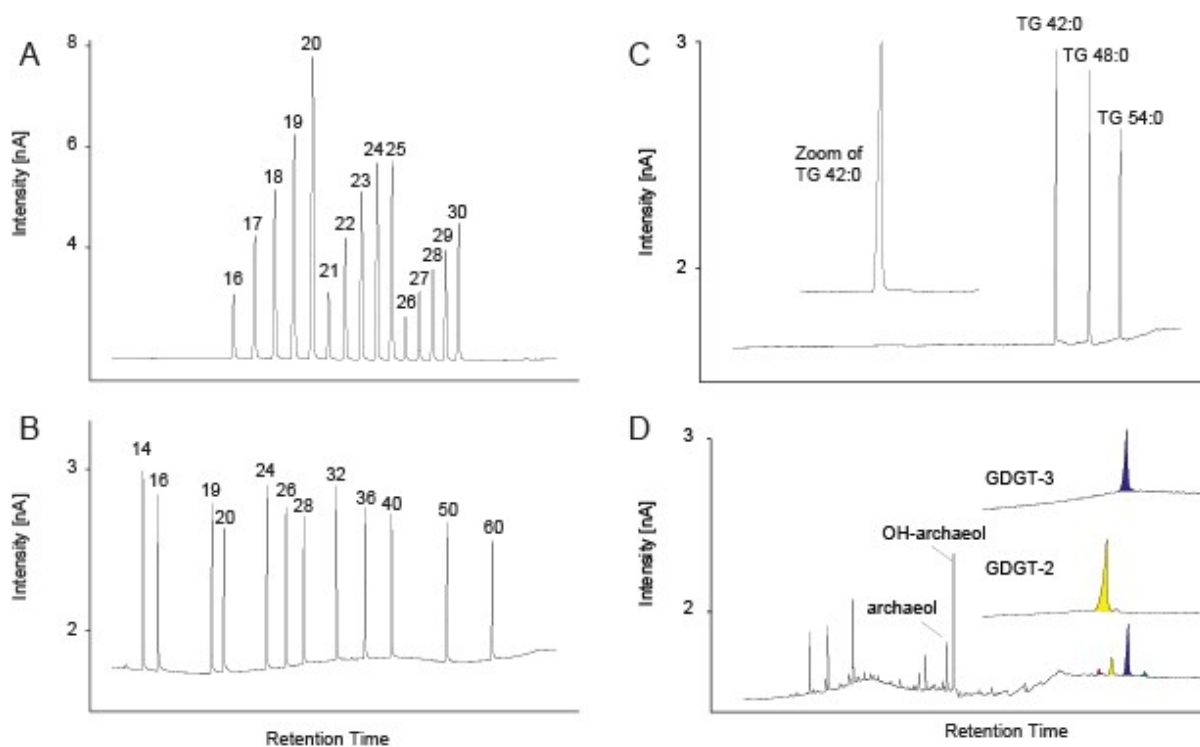


Figure 1. GC/P/IRMS chromatograms under HT conditions; different temperature ramps were applied to the different mixtures. Shown is A, a mixture of *n*-alkanes up to *n*-C₃₀ with known $\delta^2\text{H}$ values (Indiana B3-standard); B, a mixture of long-chain *n*-alkanes up to *n*-C₆₀; C, triacylglycerides; and D, a sample from a Black Sea methane seep with GDGT-2 and GDGT-3 standards shown as inserts; note that the small second peak in GDGT-2 was a contaminant introduced during analysis that did not affect the measurement

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Accuracy and precision of $\delta^2\text{H}$ values of high molecular weight compounds

Triacylglyceride (TG) reference compounds and purified GDGT standards were used to test the methodology for accuracy by determining the $\delta^2\text{H}$ values of these compounds by HTGC/IRMS at GC temperatures of up to 400 °C as well as by EA-analysis. The prepared isoGDGT-2 and isoGDGT-3 standards were analysed by one laboratory (CU Boulder), while the purchased standards were examined by HTEA/IRMS in two different laboratories (CU Boulder and Elementar UK Ltd). The average $\delta^2\text{H}$ values determined for the TGs were within 5 ‰ for all analyses (Tab. 1, Fig. 2). HTGC-analysed samples generally yielded $\delta^2\text{H}$ values between the values determined by the EA analyses. Standard deviations were smaller for the EA methods (< 2 ‰) than for the HTGC method (9-18 ‰, which represents 2-3× the typical precision of $\delta^2\text{H}$ value determinations by GC/P/IRMS⁶¹, and is thus a larger error than expected). Often, precision of GC/P/IRMS measurements is determined using the same concentration, while here, injection concentrations varied. This likely contributed to the high standard deviation, and we investigate this further below. It is expected that further application of this technique – and routine analysis of TGs, as compounds of particular interest to the food industry – will lead to improvements in analytical precision as methods are improved by optimising solvents, injection temperatures, and concentrations. The $\delta^2\text{H}$ values determined for the high molecular weight *n*-alkanes with 50 and 60 carbon atoms (Table 1) were more variable among all methods and laboratories. This was surprising, and possibly a result of insufficient mixing of these large waxy compounds before distribution to other laboratories.

The $\delta^2\text{H}$ values of purified GDGTs obtained by HTEA/IRMS and HTGC/P/IRMS (Tab. 1) were not significantly different for GDGT-2 at a high confidence level (Welsh's t-test, $df = 2$, $t = 1.32$, $p = 0.32$). However, for GDGT-3, which eluted later, the $\delta^2\text{H}$ value derived by HTGC/IRMS was 9 ‰ higher than the value determined by HTEA/IRMS ($df = 2$, $t = 3.32$, $p = 0.080$). A high baseline could be a possible cause for this discrepancy. However, ionOS

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software applies an automated correction. Both GDGTs eluted on an isothermal baseline when samples were injected (Fig. 1D). Another cause could be fractionation due to chromatographic separation, adsorption to cold spots, or thermal decomposition. Another possibility is minor contamination of GDGT-3, resulting in a flawed HTEA/IRMS measurement but not affecting HTGC/P/IRMS measurements; however, this would be surprising as GDGT-2 and GDGT-3 were isolated from the same organism and the HTEA/IRMS results match expectations of similar $\delta^2\text{H}$ values. The standard deviation of 5 – 6 ‰ achieved for purified GDGTs using the HTGC/P/IRMS system is similar to the precision of lower molecular weight compounds on a conventional GC/P/IRMS instrument ⁶¹.

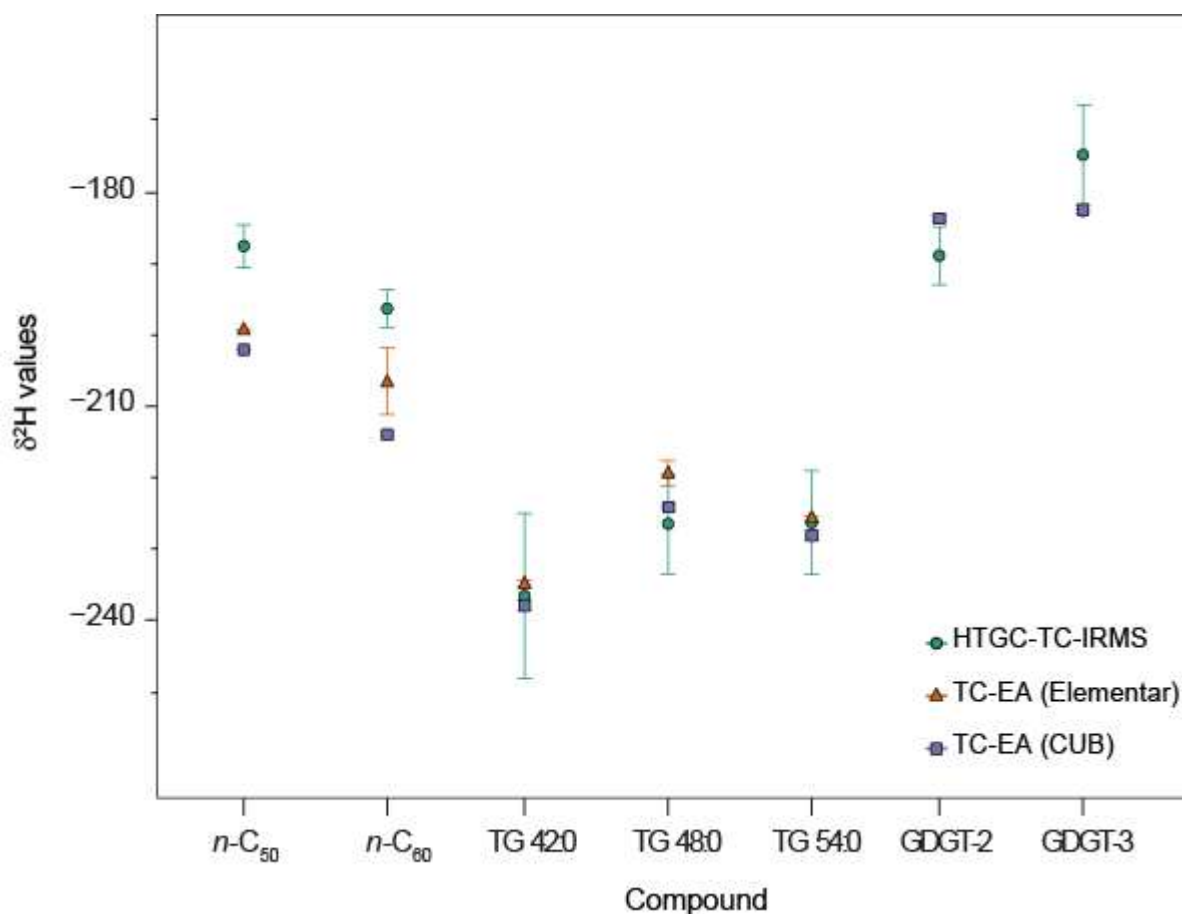


Figure 2. $\delta^2\text{H}$ values of purchased triacylglyceride standards and isolated GDGTs determined by HTEA/IRMS compared with values determined by HTGC/P/IRMS; values and standard errors are given in Table 1.

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Table 1. $\delta^2\text{H}$ values determined by HTEA/IRMS and HTGC/P/IRMS.

	HTEA/IRMS (Elementar)			HTEA/IRMS (CU Boulder)			HTGC/IRMS		
	Mean	St. dev.	N	Mean	St. dev.	N	Mean	St. dev.	N
	[‰ V-SMOW]			[‰ V-SMOW]			[‰ V-SMOW]		
GDGT-2	-	-	-	-181.6	0.4	3	-186	4	3
GDGT-3	-	-	-	-182.6	0.2	3	-173	7	3
C₄₂-TG 42:0	-235.0	0.5	4	-238.0	0.7	3	-232	9	9
C₄₈-TG 48:0	-219	2	3	-224.1	0.3	3	-223	18	7
C₅₄-TG 54:0	-225.9	0.4	4	-228.2	0.7	3	-223	12	7
<i>n</i>-C₆₀ alk	-206	5	3	-214.0	0.4	3	-196	3	3
<i>n</i>-C₅₀ alk	-199.29	0.02	4	-202.05	0.06	3	-188	3	3

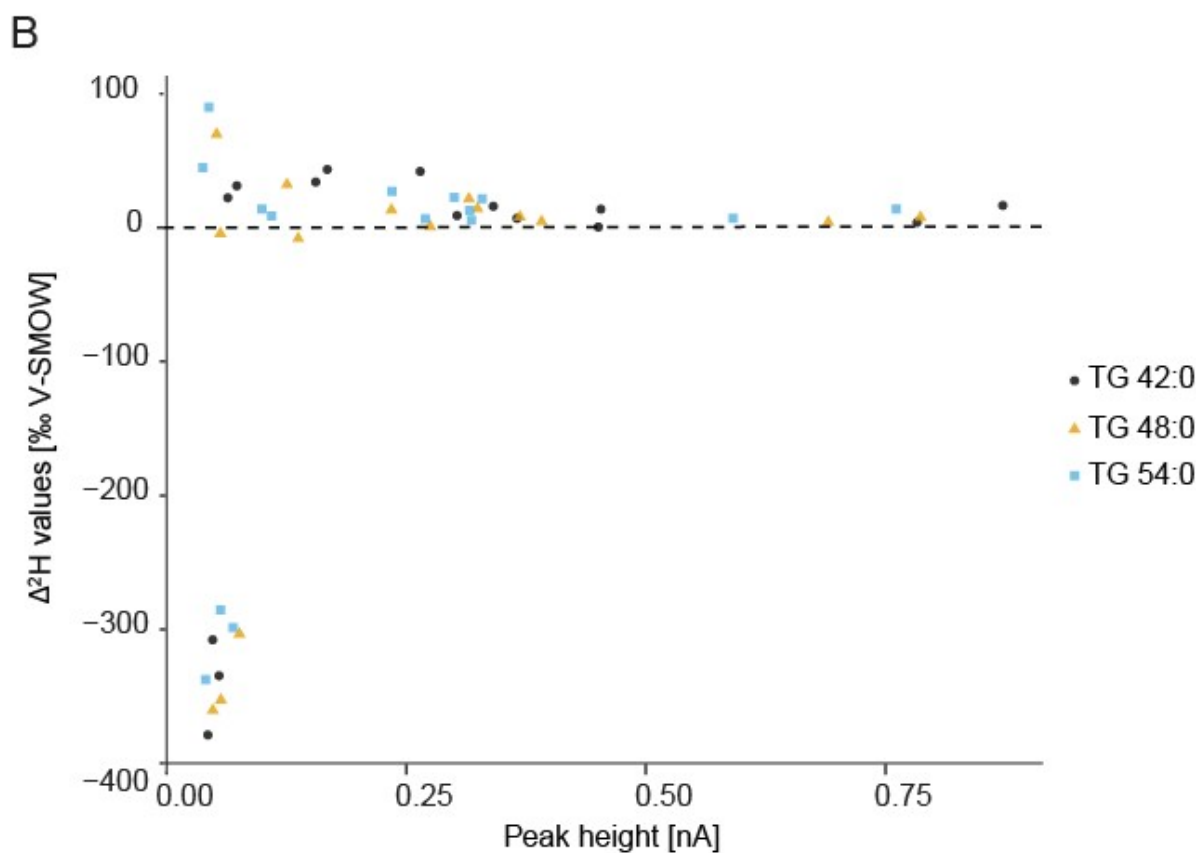
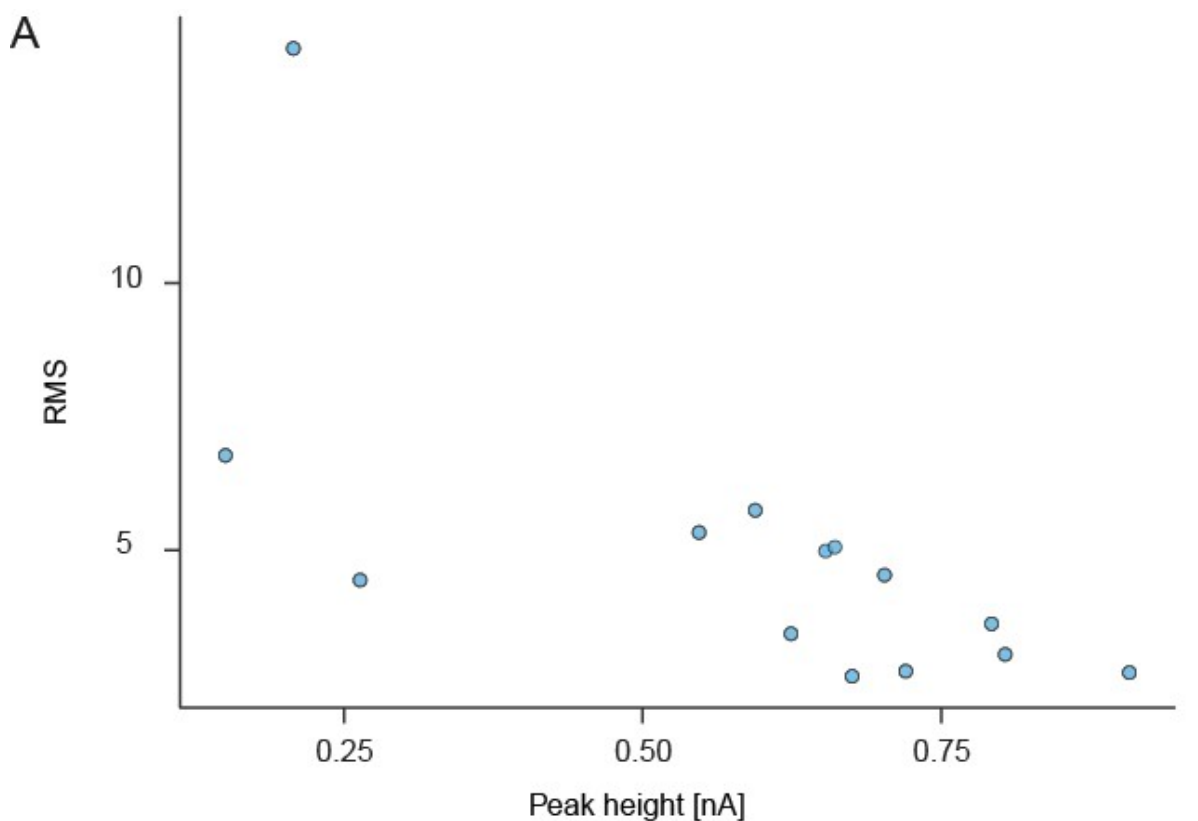
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Response vs accuracy

Whilst GDGTs are ubiquitous, they are typically only present at ppm to ppb concentrations in environmental samples such as sediments and soils. In addition, many high molecular weight compounds are not very soluble in solvents suitable for GC/IRMS, and on-column injection only allows small amounts of sample to be used. Therefore, only small amounts of GDGT (ng) were injected for each HTGC/P/IRMS analysis. To assess accuracy in relationship to signal intensity, different concentrations of the TG standard were tested and compared to peak heights (Fig. 3). This yielded a response of 0.07 – 0.08 nA per ng H per compound for m/z 2 (equivalent to 70-80 mV on an IRMS with a 10^9 Ohm resistor on the operational amplifier for the m/z 2 faraday cup). Below ~0.25 nA peak height, values begin to deviate substantially (by ~20 ‰) from the values measured by HTEA/IRMS, with differences of up to 400 ‰ when peak heights were around 0.1 nA. We thus excluded peak heights < 0.25 nA, corresponding to less than 3.5 ng H injected on column, from any further analysis. Typical H amounts required to achieve 3-5 ‰ precision were ~10 ng, translating to m/z 2 peak heights of 0.7 – 0.8 nA.

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Figure 3. Measured $\delta^2\text{H}$ values compared with peak heights. A, RMSE of the B3 mixture compared with peak heights of the minimum peak height in the mixture. B, Difference between $\delta^2\text{H}$ values of TGs determined by HTGC/P/IRMS from values determined by HTEA/IRMS, plotted vs peak height

GDGTs in environmental samples and $\epsilon_{\text{H}_2\text{O}/\text{isoGDGT}}$

A sample from a Mediterranean cold seep⁵³ was analysed, and $\delta^2\text{H}$ values for archaeol, hydroxyarchaeol, GDGT-1, and GDGT-2 were determined to be -245 ± 7 , -253 ± 13 , -216 ± 15 , and -225 ± 14 ‰, respectively (n=3; Fig. 1D, Fig. 4). These values show a limited range, as expected for ether lipids derived from a common archaeal source, and are similar to published $\delta^2\text{H}$ values of the biphytanes of GDGTs in *Sulfolobus sp.* determined after ether cleavage (-229 to -257 ‰⁴⁶). However, the values are not identical: the diphytanyl glycerol diether lipids archaeol and hydroxyarchaeol were ^2H -depleted relative to GDGTs. Though the difference between the di- and tetraethers is small, and similar to what is commonly observed between different fatty acids from the same organism⁶², it could potentially reflect different archaeal origins, given that ANME-2 group Archaea appear to preferentially produce GDGTs in cold seep settings (e.g., 63). This would be particularly true if the differing source Archaea exhibit different metabolisms (see below). A sample from a Mediterranean cold seep⁵³ was analysed, and $\delta^2\text{H}$ values for archaeol, hydroxyarchaeol, GDGT-1, and GDGT-2 were determined to be -245 ± 7 , -253 ± 13 , -216 ± 15 , and -225 ± 14 , respectively (n=3; Fig. 1D, Fig. 4). These values show a limited range, as expected for ether lipids derived from a common archaeal source, and are similar to published $\delta^2\text{H}$ values of the biphytanes of GDGTs in *Sulfolobus sp.* determined after ether cleavage (-229 to -257 ‰)⁴⁶. However, the values are not identical, with the diphytanyl glycerol diether lipids archaeol and hydroxyarchaeol being ^2H -depleted relative to GDGTs. The difference is small, and similar to what is commonly observed between different fatty acids from the same organism⁶², however, it could potentially reflect different archaeal origins, given that ANME-2 group Archaea appear to preferentially produce GDGTs in cold seep settings (e.g. 63). This would be particularly true if the differing source Archaea exhibit different metabolisms (see below).

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The $\epsilon_{\text{H}_2\text{O}/\text{GDGT}}$ for the *Sulfolobus* cultures used to purify the standards was determined as -134 ‰ and was not as large as previously reported $\epsilon_{\text{H}_2\text{O}/\text{GDGT}}$ (-213‰ to -161‰⁴⁶). The application of this fractionation factor to the environmental iso-GDGTs would nonetheless result in an unrealistic $\delta^2\text{H}$ value for the seawater of -93 ‰, suggesting that metabolism, salinity, temperature, and other factors contribute strongly to the extent of fractionation.

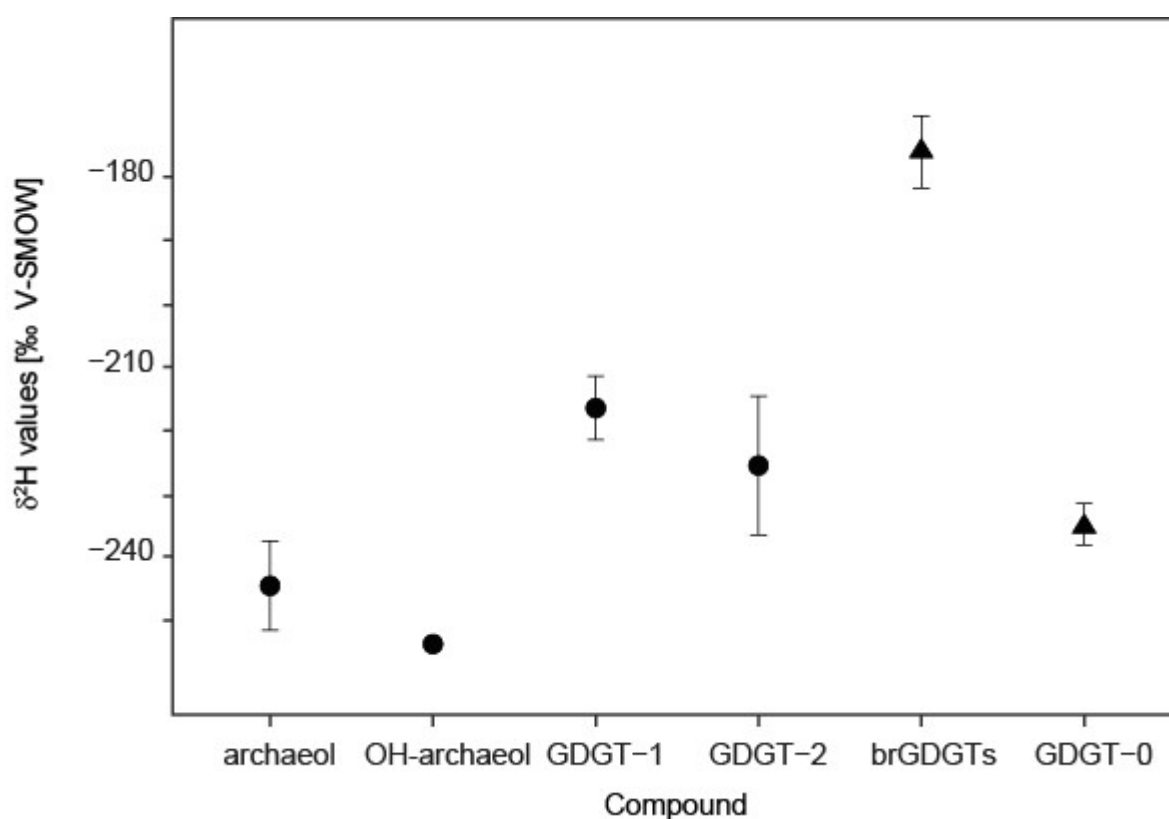


Figure 4. $\delta^2\text{H}$ values of ether lipids determined from environmental samples. brGDGTs and GDGT-0 were extracted from a peat (triangles) and all other compounds derived from a methane seep (circles). Error bars represent standard deviations.

Values of $\delta^2\text{H}$ of GDGT-0 from the peat (Suppl. Fig. 4) were similar to the isoGDGTs in the seep sample (-235 ± 3 ‰, $n = 2$), whereas values for brGDGTs (integrated as one peak) were relatively enriched in ^2H (-176 ± 6 ‰, $n = 6$). It is possible that the ^2H -enrichment of brGDGTs relative to co-occurring isoGDGTs could be due to fractionation associated with the biosynthetic pathways for isoprenoidal (isoGDGTs) vs. *n*-acyl lipids (brGDGTs), in which

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isoprenoidal lipids (which undergo successive hydrogenation) exhibit more ^2H -depleted signatures^{21,64}. However, recently, it has also been shown that the energy and metabolism pathways of source organisms are highly correlated with $\delta^2\text{H}$ values of their lipids^{44,65,66}; it is also thought that NADPH/NADH ratios and transhydrogenases play an important role, particularly in anaerobic organisms^{67–70}. In general, heterotrophic bacteria consuming TCA-cycle intermediates exhibit $\delta^2\text{H}$ values similar to or more positive than the source water, heterotrophs assimilating carbohydrates are depleted relative to source water, and photoautotrophic and chemoautotrophic bacteria show the greatest ^2H -depletion⁴⁴. While archaeal metabolisms were not examined in this work in detail, some of our results are consistent with the idea that chemoautotrophic archaea are the presumed producers of isoGDGTs in both settings, and heterotrophic bacteria are thought to be the producers of brGDGTs⁷¹.

The differences between the peat and seep samples for isoGDGTs are unexpected: As the $\delta^2\text{H}$ of the peat water is likely around -52‰ ¹ – a ^2H content that is depleted compared to seawater – we expected isoGDGTs from peat to also be depleted in ^2H relative to GDGTs from marine environments. However, isoGDGTs from peat are up to 10 to 20 ‰ more ^2H -enriched from peat, invoking a difference in metabolic state between the anaerobic methanogens in peat, and the anaerobic methane oxidising communities in the seep. It could also indicate syntrophy, which has been shown to affect ^2H values of lipids⁶⁸. These findings speak to the potential of isoGDGT $\delta^2\text{H}$ analyses in probing microbial ecology and metabolic state, while brGDGTs, which are presumably of heterotrophic bacterial origin in peat settings, could prove useful as proxies for source water $\delta^2\text{H}$ and hydrology.

The novel HTGC/P/IRMS method enables the determination of the $\delta^2\text{H}$ values of compounds with a high molecular weight, including TG and GDGTs, hereby extending the range of analytes for $\delta^2\text{H}$ value determination. Accuracy and precision are as small as 3 ‰ in some cases and comparable to HTEA/IRMS. Our initial measurements suggest that

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bacterial and archaeal GDGT $\delta^2\text{H}$ values are likely related to both environmental parameters, and the metabolic and ecological function of the source organisms. Future applications include but are not limited to food forensics, archaeology, oil-source rock correlations, microbial ecology and paleoclimate.

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Rapid Communications in Mass Spectrometry 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>

References

1. Bowen GJ, Revenaugh J. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resour Res.* 2003;39(10). doi:10.1029/2003WR002086
2. Craig H. Isotopic Variations in Meteoric Waters. *Science.* 1961;133(3465):1702-1703. doi:10.1126/science.133.3465.1702
3. West JB, Bowen GJ, Dawson TE, Tu KP, eds. *Isoscapes*. Springer Netherlands; 2010. doi:10.1007/978-90-481-3354-3
4. Hobson KA, Wassenaar LI. *Tracking Animal Migration with Stable Isotopes*. Academic Press; 2008.
5. Soto DX, Wassenaar LI, Hobson KA. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Funct Ecol.* 2013;27(2):535-543. doi:10.1111/1365-2435.12054
6. Fraser I, Meier-Augenstein W. Stable ^2H isotope analysis of modern-day human hair and nails can aid forensic human identification. *Rapid Commun Mass Spectrom.* 2007;21(20):3279-3285. doi:10.1002/rcm.3209
7. Schellenberg A, Chmielus S, Schlicht C, et al. Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chem.* 2010;121(3):770-777. doi:10.1016/j.foodchem.2009.12.082
8. Chesson LA, Valenzuela LO, O'Grady SP, Cerling TE, Ehleringer JR. Hydrogen and Oxygen Stable Isotope Ratios of Milk in the United States. *J Agric Food Chem.* 2010;58(4):2358-2363. doi:10.1021/jf904151c

Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry.

Rapid Communications in Mass Spectrometry 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>

9. Chesson LA, Podlesak DW, Erkkila BR, Cerling TE, Ehleringer JR. Isotopic consequences of consumer food choice: Hydrogen and oxygen stable isotope ratios in foods from fast food restaurants versus supermarkets. *Food Chem.* 2010;119(3):1250-1256. doi:10.1016/j.foodchem.2009.07.046
10. Outram AK, Stear NA, Bendrey R, et al. The Earliest Horse Harnessing and Milking. *Science.* 2009;323(5919):1332-1335. doi:10.1126/science.1168594
11. Reynard LM, Hedges REM. Stable hydrogen isotopes of bone collagen in palaeodietary and palaeoenvironmental reconstruction. *J Archaeol Sci.* 2008;35(7):1934-1942. doi:10.1016/j.jas.2007.12.004
12. Sachse D, Billault I, Bowen GJ, et al. Molecular Paleohydrology: Interpreting the Hydrogen-Isotopic Composition of Lipid Biomarkers from Photosynthesizing Organisms. *Annu Rev Earth Planet Sci.* 2012;40(1):221-249. doi:10.1146/annurev-earth-042711-105535
13. Schefuß E, Schouten S, Schneider RR. Climatic controls on central African hydrology during the past 20,000 years. *Nature.* 2005;437(7061):1003-1006. doi:10.1038/nature03945
14. Tierney JE, Russell JM, Huang Y, Damsté JSS, Hopmans EC, Cohen AS. Northern Hemisphere Controls on Tropical Southeast African Climate During the Past 60,000 Years. *Science.* 2008;322(5899):252-255. doi:10.1126/science.1160485
15. Estep MF, Hoering TC. Biogeochemistry of the stable hydrogen isotopes. *Geochim Cosmochim Acta.* 1980;44(8):1197-1206. doi:10.1016/0016-7037(80)90073-3
16. da Silveira Lobo Sternberg L. D/H ratios of environmental water recorded by D/H ratios of plant lipids. *Nature.* 1988;333(6168):59-61. doi:10.1038/333059a0

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
17. Sessions AL. Review: Factors Controlling the Deuterium Contents of Sedimentary Hydrocarbons. *Org Geochem*. 2016;96:43-64. doi:10.1016/j.orggeochem.2016.02.012
 18. Sessions AL, Sylva SP, Summons RE, Hayes JM. Isotopic exchange of carbon-bound hydrogen over geologic timescales. *Geochim Cosmochim Acta*. 2004;68(7):1545-1559. doi:10.1016/j.gca.2003.06.004
 19. Li C, Sessions AL, Kinnaman FS, Valentine DL. Hydrogen-isotopic variability in lipids from Santa Barbara Basin sediments. *Geochim Cosmochim Acta*. 2009;73(16):4803-4823. doi:10.1016/j.gca.2009.05.056
 20. Sauer PE, Eglinton TI, Hayes JM, Schimmelmann A, Sessions AL. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. *Geochim Cosmochim Acta*. 2001;65(2):213-222. doi:10.1016/S0016-7037(00)00520-2
 21. Sessions AL, Burgoyne TW, Schimmelmann A, Hayes JM. Fractionation of hydrogen isotopes in lipid biosynthesis. *Org Geochem*. 1999;30(9):1193-1200. doi:10.1016/S0146-6380(99)00094-7
 22. Smittenberg RH, Sachs JP. Purification of dinosterol for hydrogen isotopic analysis using high-performance liquid chromatography–mass spectrometry. *J Chromatogr A*. 2007;1169(1-2):70-76. doi:10.1016/j.chroma.2007.09.018
 23. van der Meer MTJ, Baas M, Rijpstra WIC, et al. Hydrogen isotopic compositions of long-chain alkenones record freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition. *Earth Planet Sci Lett*. 2007;262(3-4):594-600. doi:10.1016/j.epsl.2007.08.014

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
24. Burgoyne TW, Hayes JM. Quantitative Production of H_2 by Pyrolysis of Gas Chromatographic Effluents. *Anal Chem*. 1998;70(24):5136-5141.
doi:10.1021/ac980248v
 25. Hilkert AW, Douthitt CB, Schlüter HJ, Brand WA. Isotope ratio monitoring gas chromatography/Mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom*. 1999;13(13):1226-1230.
doi:10.1002/(SICI)1097-0231(19990715)13:13<1226::AID-RCM575>3.0.CO;2-9
 26. Scrimgeour CM, Begley IS, Thomason ML. Measurement of deuterium incorporation into fatty acids by gas chromatography/isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom*. 1999;13(4):271-274. doi:10.1002/(SICI)1097-0231(19990228)13:4<271::AID-RCM468>3.0.CO;2-6
 27. Tobias HJ, Brenna JT. On-Line Pyrolysis as a Limitless Reduction Source for High-Precision Isotopic Analysis of Organic-Derived Hydrogen. *Anal Chem*. 1997;69(16):3148-3152. doi:10.1021/ac970332v
 28. Kaal E, Janssen H-G. Extending the molecular application range of gas chromatography. *J Chromatogr A*. 2008;1184(1-2):43-60.
doi:10.1016/j.chroma.2007.11.114
 29. Bodlenner A, Liu W, Hirsch G, et al. C_{35} Hopanoid Side Chain Biosynthesis: Reduction of Ribosylhopane into Bacteriohopanetetrol by a Cell-Free System from *Methylobacterium organophilum*. *ChemBioChem*. 2015;16(12):1764-1770.
doi:10.1002/cbic.201500021
 30. Muhammad SA, Seow E-K, Omar AM, et al. Variation of delta H-2, delta O-18 & delta C-13 in crude palm oil from different regions in Malaysia: Potential of stable isotope

Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry.

Rapid Communications in Mass Spectrometry 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>

signatures as a key traceability parameter. *Sci Justice*. 2018;58(1):59-66.

doi:10.1016/j.scijus.2017.05.008

31. Richter EK, Spangenberg JE, Kreuzer M, Leiber F. Characterization of Rapeseed (Brassica napus) Oils by Bulk C, O, H, and Fatty Acid C Stable Isotope Analyses. *J Agric Food Chem*. 2010;58(13):8048-8055. doi:10.1021/jf101128f
32. Spangenberg JE. Bulk C, H, O, and fatty acid C stable isotope analyses for purity assessment of vegetable oils from the southern and northern hemispheres. *Rapid Commun Mass Spectrom*. 2016;30(23):2447-2461. doi:10.1002/rcm.7734
33. Ehtesham E, Hayman AR, McComb KA, Van Hale R, Frew RD. Correlation of Geographical Location with Stable Isotope Values of Hydrogen and Carbon of Fatty Acids from New Zealand Milk and Bulk Milk Powder. *J Agric Food Chem*. 2013;61(37):8914-8923. doi:10.1021/jf4024883
34. Paolini M, Bontempo L, Camin F. Compound-specific delta C-13 and delta H-2 analysis of olive oil fatty acids. *Talanta*. 2017;174:38-43. doi:10.1016/j.talanta.2017.05.080
35. Buchgraber M, Ulberth F, Anklam E. Cluster analysis for the systematic grouping of genuine cocoa butter and cocoa butter equivalent samples based on triglyceride patterns. *J Agric Food Chem*. 2004;52(12):3855-3860. doi:10.1021/jf035153v
36. Fontecha J, Mayo I, Toledano G, Juárez M. Use of changes in triacylglycerols during ripening of cheeses with high lipolysis levels for detection of milk fat authenticity. *Int Dairy J*. 2006;16(12):1498-1504. doi:10.1016/j.idairyj.2005.12.005
37. Ruiz-Samblas C, Gonzalez-Casado A, Cuadros-Rodriguez L. Triacylglycerols Determination by High-temperature Gas Chromatography in the Analysis of Vegetable

Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry.

Rapid Communications in Mass Spectrometry 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>

Oils and Foods: A Review of the Past 10 Years. *Crit Rev Food Sci Nutr*.

2015;55(11):1618-1631. doi:10.1080/10408398.2012.713045

38. Pedentchouk N, Turich C. Carbon and hydrogen isotopic compositions of n-alkanes as a tool in petroleum exploration. *Geol Soc Lond Spec Publ*. 2018;468(1):105-125.
doi:10.1144/SP468.1
39. Radke J, Bechtel A, Gaupp R, et al. Correlation between hydrogen isotope ratios of lipid biomarkers and sediment maturity. *Geochim Cosmochim Acta*. 2005;69(23):5517-5530. doi:10.1016/j.gca.2005.07.014
40. Li M, Huang Y, Obermajer M, Jiang C, Snowdon LR, Fowler MG. Hydrogen isotopic compositions of individual alkanes as a new approach to petroleum correlation: case studies from the Western Canada Sedimentary Basin. *Org Geochem*. 2001;32(12):1387-1399. doi:10.1016/S0146-6380(01)00116-4
41. Schouten S, Hopmans EC, Sinninghe Damsté JS. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. *Org Geochem*. 2013;54:19-61.
doi:10.1016/j.orggeochem.2012.09.006
42. De Jonge C, Stadnitskaia A, Hopmans EC, Cherkashov G, Fedotov A, Sinninghe Damsté JS. In situ produced branched glycerol dialkyl glycerol tetraethers in suspended particulate matter from the Yenisei River, Eastern Siberia. *Geochim Cosmochim Acta*. 2014;125:476-491. doi:10.1016/j.gca.2013.10.031
43. Peterse F, Kim J-H, Schouten S, Kristensen DK, Koç N, Sinninghe Damsté JS. Constraints on the application of the MBT/CBT palaeothermometer at high latitude environments (Svalbard, Norway). *Org Geochem*. 2009;40(6):692-699.
doi:10.1016/j.orggeochem.2009.03.004

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
44. Wijker RS, Sessions AL, Fuhrer T, Phan M. $^2\text{H}/^1\text{H}$ variation in microbial lipids is controlled by NADPH metabolism. *Proc Natl Acad Sci*. 2019;116(25):12173-12182. doi:10.1073/pnas.1818372116
 45. Correa-Ascencio M, Evershed RP. High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction. *Anal Methods*. 2014;6(5):1330-1340. doi:10.1039/C3AY41678J
 46. Kaneko M, Kitajima F, Naraoka H. Stable hydrogen isotope measurement of archaeal ether-bound hydrocarbons. *Org Geochem*. 2011;42(2):166-172. doi:10.1016/j.orggeochem.2010.11.002
 47. Lengger SK, Lipsewers YA, de Haas H, Sinninghe Damsté JS, Schouten S. Lack of ^{13}C -label incorporation suggests low turnover rates of thaumarchaeal intact polar tetraether lipids in sediments from the Iceland shelf. *Biogeosciences*. 2014;11(2):201-216. doi:10.5194/bg-11-201-2014
 48. Schouten S, Hoefs MJL, Koopmans MP, Bosch H-J, Sinninghe Damsté JS. Structural characterization, occurrence and fate of archaeal ether-bound acyclic and cyclic biphytanes and corresponding diols in sediments. *Org Geochem*. 1998;29(5-7):1305-1319. doi:10.1016/S0146-6380(98)00131-4
 49. Weber Y, Damsté JSS, Zopfi J, et al. Redox-dependent niche differentiation provides evidence for multiple bacterial sources of glycerol tetraether lipids in lakes. *Proc Natl Acad Sci*. 2018;115(43):10926-10931. doi:10.1073/pnas.1805186115
 50. Weber Y, De Jonge C, Rijpstra WIC, et al. Identification and carbon isotope composition of a novel branched GDGT isomer in lake sediments: Evidence for

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
- lacustrine branched GDGT production. *Geochim Cosmochim Acta*. 2015;154:118-129.
doi:10.1016/j.gca.2015.01.032
51. Wegener G, Bausch M, Holler T, et al. Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and ^{13}C -bicarbonate: Microbial carbon assimilation in the sub-seafloor. *Environ Microbiol*. 2012;14(6):1517-1527.
doi:10.1111/j.1462-2920.2012.02739.x
52. Weijers JWH, Wiesenberg GLB, Bol R, Hopmans EC, Pancost RD. Carbon isotopic composition of branched tetraether membrane lipids in soils suggest a rapid turnover and a heterotrophic life style of their source organism(s). *Biogeosciences*. 2010;7(9):2959-2973. doi:10.5194/bg-7-2959-2010
53. Lengger SK, Sutton PA, Rowland SJ, et al. Archaeal and bacterial glycerol dialkyl glycerol tetraether (GDGT) lipids in environmental samples by high temperature-gas chromatography with flame ionisation and time-of-flight mass spectrometry detection. *Org Geochem*. 2018;121:10-21. doi:10.1016/j.orggeochem.2018.03.012
54. Sutton PA, Rowland SJ. High temperature gas chromatography–time-of-flight-mass spectrometry (HTGC–ToF-MS) for high-boiling compounds. *J Chromatogr A*. 2012;1243:69-80. doi:10.1016/j.chroma.2012.04.044
55. Worthington P, Blum P, Perez-Pomares F, Elthon T. Large-Scale Cultivation of Acidophilic Hyperthermophiles for Recovery of Secreted Proteins. *Appl Env Microbiol*. 2003;69(1):252-257. doi:10.1128/AEM.69.1.252-257.2003
56. Weber Y, Damsté JSS, Hopmans EC, Lehmann MF, Niemann H. Incomplete recovery of intact polar glycerol dialkyl glycerol tetraethers from lacustrine suspended biomass. *Limnol Oceanogr Methods*. 2017;15(9):782-793. doi:10.1002/lom3.10198

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
57. Schouten S, Huguet C, Hopmans EC, Kienhuis MVM, Sinninghe Damsté JS. Analytical methodology for TEX₈₆ paleothermometry by High-Performance Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry. *Anal Chem*. 2007;79(7):2940-2944. doi:10.1021/ac062339v
 58. Smittenberg RH, Hopmans EC, Schouten S, Sinninghe Damsté JS. Rapid isolation of biomarkers for compound specific radiocarbon dating using high-performance liquid chromatography and flow injection analysis—atmospheric pressure chemical ionisation mass spectrometry. *J Chromatogr A*. 2002;978(1):129-140. doi:10.1016/S0021-9673(02)01427-9
 59. Patwardhan AP, Thompson DH. Efficient Synthesis of 40- and 48-Membered Tetraether Macrocyclic Bisphosphocholines. *Org Lett*. 1999;1(2):241-244. doi:10.1021/ol990567o
 60. Sessions AL, Jahnke LL, Schimmelmann A, Hayes JM. Hydrogen isotope fractionation in lipids of the methane-oxidizing bacterium *Methylococcus capsulatus*. *Geochim Cosmochim Acta*. 2002;66(22):3955-3969. doi:10.1016/S0016-7037(02)00981-X
 61. Sessions AL. Isotope-ratio detection for gas chromatography. *J Sep Sci*. 2006;29(12):1946-1961. doi:10.1002/jssc.200600002
 62. Chikaraishi Y, Naraoka H. $\delta^{13}\text{C}$ and δD relationships among three n-alkyl compound classes (n-alkanoic acid, n-alkane and n-alkanol) of terrestrial higher plants. *Org Geochem*. 2007;38(2):198-215. doi:10.1016/j.orggeochem.2006.10.003
 63. Blumenberg M, Seifert R, Reitner J, Pape T, Michaelis W. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. *Proc Natl Acad Sci*. 2004;101(30):11111-11116. doi:10.1073/pnas.0401188101

- Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>
64. Zhang Z, Sachs JP. Hydrogen isotope fractionation in freshwater algae: I. Variations among lipids and species. *Org Geochem*. 2007;38(4):582-608.
doi:10.1016/j.orggeochem.2006.12.004
65. Osburn MR, Dawson KS, Fogel ML, Sessions AL. Fractionation of Hydrogen Isotopes by Sulfate- and Nitrate-Reducing Bacteria. *Front Microbiol*. 2016;7.
doi:10.3389/fmicb.2016.01166
66. Zhang X, Gillespie AL, Sessions AL. Large D/H variations in bacterial lipids reflect central metabolic pathways. *Proc Natl Acad Sci*. 2009;106(31):12580-12586.
doi:10.1073/pnas.0903030106
67. Campbell BJ, Sessions AL, Fox DN, et al. Minimal Influence of [NiFe] Hydrogenase on Hydrogen Isotope Fractionation in H₂-Oxidizing *Cupriavidus necator*. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01886
68. Dawson KS, Osburn MR, Sessions AL, Orphan VJ. Metabolic associations with archaea drive shifts in hydrogen isotope fractionation in sulfate-reducing bacterial lipids in cocultures and methane seeps. *Geobiology*. 2015;13(5):462-477.
doi:10.1111/gbi.12140
69. Leavitt WD, Murphy SJ-L, Lynd LR, Bradley AS. Hydrogen isotope composition of *Thermoanaerobacterium saccharolyticum* lipids: Comparing wild type with a nfn-transhydrogenase mutant. *Org Geochem*. 2017;113:239-241.
doi:10.1016/j.orggeochem.2017.06.020
70. Leavitt WD, Flynn TM, Suess MK, Bradley AS. Transhydrogenase and Growth Substrate Influence Lipid Hydrogen Isotope Ratios in *Desulfovibrio alaskensis* G20. *Front Microbiol*. 2016;07. doi:10.3389/fmicb.2016.00918

Lengger, S.K., Weber, Y., Taylor, K.W.R., Kopf, S.H., Berstan, R., Bull, I.D., Mayser, J.-P., Leavitt, W.D., Blewett, J., Pearson, A., Pancost, R.D., n.d. Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry.

Rapid Communications in Mass Spectrometry 2020, **e8983**. <https://doi.org/10.1002/rcm.8983>

71. Sinninghe Damsté JS, Rijpstra WIC, Foesel BU, et al. An overview of the occurrence of ether- and ester-linked iso-diabolic acid membrane lipids in microbial cultures of the Acidobacteria: Implications for brGDGT paleoproxies for temperature and pH. *Org Geochem.* 2018;124:63-76. doi:10.1016/j.orggeochem.2018.07.006