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Carbon isotopic composition of intact branched GDGT core lipids in Greenland lake sediments and soils

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ABSTRACT

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are the basis of the MBT and CBT indices used as proxies for past changes in temperature and soil pH. Although these compounds were initially thought to originate solely from anaerobic soil bacteria, there is now convincing evidence that they are also produced within aquatic environments. However, the specific source organism(s) remain unknown. We utilize here spooling wire microcombustion-isotope ratio mass spectrometry (SWiM-IRMS) to measure the $\delta^{13}C$ composition of intact brGDGTs from a series of lake sediments and soils, including samples exhibiting in situ production of brGDGTs. The results show close parallels between the $\delta^{13}C_{brGDGT}$ values and OM in the downcore profiles for sediment sequences from two lakes. In addition, the $\delta^{13}C_{brGDGT}$ values were consistently more negative than those of OM for the lakes and soil samples from the surrounding watershed. The values are consistent with a mixture of heterotrophic sources and preferential utilization of ^{13}C-depleted carbon sources. The $\delta^{13}C$ values of individual brGDGT components confirm in situ production of brGDGTs within lacustrine dysoxic environments and suggest that their association with an elevated CH_4 level during the summer may reflect methanotrophy.

1. Introduction

Non-isoprenoid, branched glycerol dialkyl glycerol tetraethers (brGDGTs) were originally recognized as a series of nine structures (IA-C, IIA-C and IIIA-C) that vary in the number of both their cyclopentyl moieties and methyl branches (Sinninghe Damsté et al., 2000; Weijers et al., 2006). Studies of brGDGTs in a wide range of soils led to the presumption that they are produced by anaerobic soil bacteria and to recognition that their composition varies systematically in response to changes in environmental temperature and soil pH (Weijers et al., 2007). Thus, their sedimentary distributions, expressed as the cyclisation ratio of branched tetraethers (CBT) and the methylation index of branched tetraethers (MBT), have become widely used proxies serving as measures of ancient soil pH and paleotemperature (e.g. D'Anjou et al., 2013; de Wet et al., 2016).

The widespread occurrence of brGDGTs in lacustrine, fluvial and marine environments, in addition to soils and peat (reviewed by Schouten et al., 2013), has prompted efforts to extend the soil-based paleotemperature calibrations to brGDGT distributions in lacustrine sedimentary sequences. The resulting temperature reconstructions have, however, often proven unrealistic, frequently resulting in a "cold bias" (e.g. Zink et al., 2010; Blaga et al., 2010; Pearson et al., 2011). It appears that anomalies in the utility of brGDGTs as a temperature proxy can be explained by mounting evidence that they are produced not only in soils but also in aquatic environments (e.g. Tierney and Russell, 2009; Tierney et al., 2012; Bechtel et al., 2010; Schoon et al., 2013; Buckles et al., 2014; Colcord et al., 2015; Weber et al., 2015), which undoubtedly complicates, and in some cases invalidates, the use of proposed brGDGT paleotemperature calibrations. These considerations demonstrate the need for a better understanding of the biological origin of brGDGTs and elucidation of controls on their production to advance the development of brGDGTs as robust molecular proxies.

The brGDGTs, like many other biomarkers (e.g. Ourisson et al., 1979; Brassell et al., 1983), were identified in geological samples (e.g. Sinninghe Damsté et al., 2000; Weijers et al., 2006) prior to their identification in biota, although the presence of structure Ia has since been confirmed in two strains of *Acidobacteria* (Sinninghe Damsté et al., 2011). Yet, the overall phylogenic range and environmental habitats of source organism(s) that produce brGDGTs remain unknown, which further hinders informed application of brGDGT-based proxies. In addition, the structural variety of brGDGTs has recently expanded following the discovery of isomers with methyl groups at varying positions: C-6 (brGDGT IIa′, IIb′ and IIc′; De Jonge et al., 2014), C-6 and C-6′ (brGDGT IIIa′, IIIb′,

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and IIIc′; De Jonge et al., 2014), and at C-5 and C-6′ (brGDGT IIIa″; Weber et al., 2015); none of these compounds are specifically included within the original MBT and CBT indices. Moreover, the occurrence of some of these novel brGDGTs in lacustrine sediments, but not in soils from lake catchments, potentially confirms additional and distinct aquatic sources for brGDGTs (Weber et al., 2015).

The development of compound-specific carbon isotopic measurements (e.g. Hayes et al., 1990; Freeman et al., 1990; Eglinton and Eglinton, 2008) and the subsequent application of the approach has proven invaluable as a tool for recognition and distinction of specific origins and biochemical pathways (e.g. C_3 vs. C_4; Calvin cycle vs. Krebs-TCA cycle; methanotrophy) of individual sedimentary biomarkers (e.g. Hayes, 1993; Summons et al., 1998; Huang et al., 1999; Hinrichs et al., 2000; Bian et al., 2001). Current evidence for the carbon isotopic composition of brGDGTs derives solely from measurement of their alkyl core lipids, which can be isolated after ether cleavage to remove the glycerol end groups (Pancost and Damsté, 2003; Weijers et al., 2010; Weber et al., 2015). Data for the $\delta^{13}C$ composition of alkyl chains in brGDGTs reveal differences between soils of dominantly C_4 rather than C_3 origin (−18‰ vs. −28‰, respectively; Weijers et al., 2010) and distinctive signatures for brGDGTs originating from lacustrine sediments vs. soils (avg. −45‰ vs. −27.5‰; Weber et al., 2015). Thus, compound-specific isotopic analyses of brGDGTs can advance understanding of their likely biological origins, biosynthetic pathways, and carbon source(s). However, the alkyl core lipids do not represent the entirety of the carbon isotopic signatures, which are amenable to measurement by adaptation of the spooling wire microcombustion-isotope ratio mass spectrometry (SWiM-IRMS) method used for isoprenoid GDGTs (Pearson et al., 2016).

Here, we present the first compound-specific carbon isotopic ($\delta^{13}C_{brGDGT}$) measurements of brGDGTs, obviating the need for ether cleavage and thereby preserving the entire carbon isotopic signature, including the glycerol moieties that are probably ^{13}C enriched based on fractionation associated with their likely biosynthetic pathways (cf. Pearson et al., 2016). The samples investigated originate from two lakes and their associated soils from Greenland (Colcord et al., 2015), which afforded the opportunity to (i) explore spatial and temporal variation in the $\delta^{13}C$ composition of brGDGTs from these lacustrine sediments, (ii) examine their relationship to the $\delta^{13}C$ values for brGDGTs in local soils, and (iii) compare both sets of $\delta^{13}C_{brGDGT}$ data with the associated bulk organic matter (OM; $\delta^{13}C_{TOC}$).

2. Material and methods

2.1. Study site

Lower Epidote Vein Valley (EVV) Lake and Upper EVV Lake are in a valley that extends southwest from the terminus of the Russell Glacier to the Søndre Strømfjord near Kangerlussuaq, Greenland (67°00′N, 50°40′W), which is characterized by a Low Arctic continental climate. They are ca. 100 m apart and both are small (< 3 ha) and shallow (< 6 m deep). They showed no evidence of any surface connectivity, inflow, or outflow during sampling (Cadieux et al., 2016; Fig. 1), an observation supported by their distinct aqueous chemistry (Goldman et al., 2015; Cadieux et al., 2016). The sediment records also exhibit marked differences in organic geochemical signatures (Colcord et al., 2015; Fig. 2), including the brGDGT distributions for both their surface sediments and downcore sediment profiles, which result in disparities in paleotemperature estimates (Colcord et al., 2015), in stark contrast with the expectation that their temperature history would be equivalent given their close proximity.

2.2. Sediment and soil samples

Short sediment cores from Upper EVV (38 cm) and Lower EVV (24 cm) lakes were collected from the center of each in April 2012 via push corer and sub-sampled at 2 cm intervals (Colcord et al., 2015). Samples were stored frozen prior to extraction and geochemical analysis. In addition, 11 samples (1–11 in Fig. 1) of the top 10 cm of soil immediately below the base of vegetation were collected from the lakes' shared watershed.

2.3. Bulk carbon isotopic analysis

Sediment subsamples were reacted with excess 1 N HCl to remove inorganic carbon, rinsed to neutral pH and dried, following routine practices employing standard laboratory methods. Each sample was analyzed using elemental analysis – isotope ratio mass spectrometry (EA-IRMS) at Indiana University's Stable Isotope Research Facility, normalizing values for carbon isotope composition to the Vienna Peedee Belemnite (VPDB) standard. The standard deviation for all $\delta^{13}C_{TOC}$ values from lake and soil samples was 0.1‰.

2.4. Initial brGDGT distribution analysis

All samples (1.5–6.0 g) were freeze-dried and extracted ultrasonically (cf, Hopmans et al., 2000). Each total lipid extract (TLE) was separated using Al_2O_3 column chromatography into three fractions: F1, hexane/CH_2Cl_2 (9:1 v/v); F2, hexane/CH_2Cl_2 (1:1 v/v) and F3, CH_2Cl_2/MeOH (1:1 v/v). The polar fraction (F3) was dried under N_2 and filtered through a Teflon syringe filter (0.45 μm) using hexane/isopropanol (99:1 v/v). The brGDGTs were then identified and quantified via high performance liquid chromatography-mass spectrometry (HPLC-MS) at the University of Massachusetts Biogeochemistry Laboratory (modified from D'Anjou et al., 2013).

2.5. Isolation of brGDGTs

The isolation of brGDGTs closely follows that of Pearson et al., 2016, who isolated isoprenoid GDGTs for compound specific ^{13}C analysis. After initial brGDGT analysis via HPLC-MS, F3 was dried under N_2 and dissolved in 3% isopropanol in hexane (95 μl). The low concentration of brGDGTs in the soil samples necessitated combination of the polar fractions (11 original samples reduced to 5 combined samples), which was guided by both their proximity to each lake and similarities in the carbon isotopic composition of the OM. Each F3 fraction was separated via normal phase HPLC using a ZORBAX NH_2 column (Agilent #880952-708, 4.6 × 250 mm, 5 μm) to isolate the brGDGTs. Samples were eluted isocratically with 1.35% isopropanol in hexane with the following instrument settings: 90 μl injection, 1.0 ml/min, 30 °C. Fractions were collected at 1 min intervals from 30 to 42 min. EtOAc (1 ml) was added to each of the resulting samples, and 50 μl were analyzed via flow injection analysis (FIA) to confirm the ions present and the amount of brGDGTs in each fraction. Fractions with the same brGDGT ions were combined and dried under N_2. The resulting samples were dried under N_2 and dissolved in 95 μl EtOAc/MeCN (65:35 v/v). Samples were further purified via reversed phase HPLC using a ZORBAX Eclipse XDB-C8 column (Agilent #993967-906, 4.6 × 150 mm, 5 μm). The elution gradient was 80:20 MeCN/water to 100% EtOAc with the following instrument settings: 90 μl injection, 1 ml/min, 30 °C. Three fractions were collected from 22.4 to 25.6 min, calibrated to 1 min pre- and post-brGDGT elution (F1, F3), with a 1.2 min middle fraction

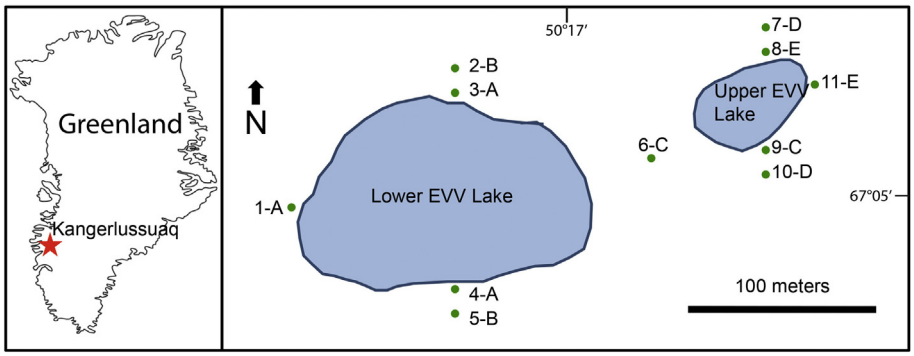

Fig. 1. Location of study area east of Kangerlussuaq, Greenland (67°00N, 50°40W) as previously described (Colcord et al., 2015). Sediment cores were recovered from Lower EVV Lake and Upper EVV Lake. Soil samples were collected from 11 locations designated by the green circles within the lakes' shared watershed. Their numbers refer to the individual locations, whereas letters indicate which soil samples were combined for $\delta^{13}C$ analysis of their brGDGTs as described in the text.

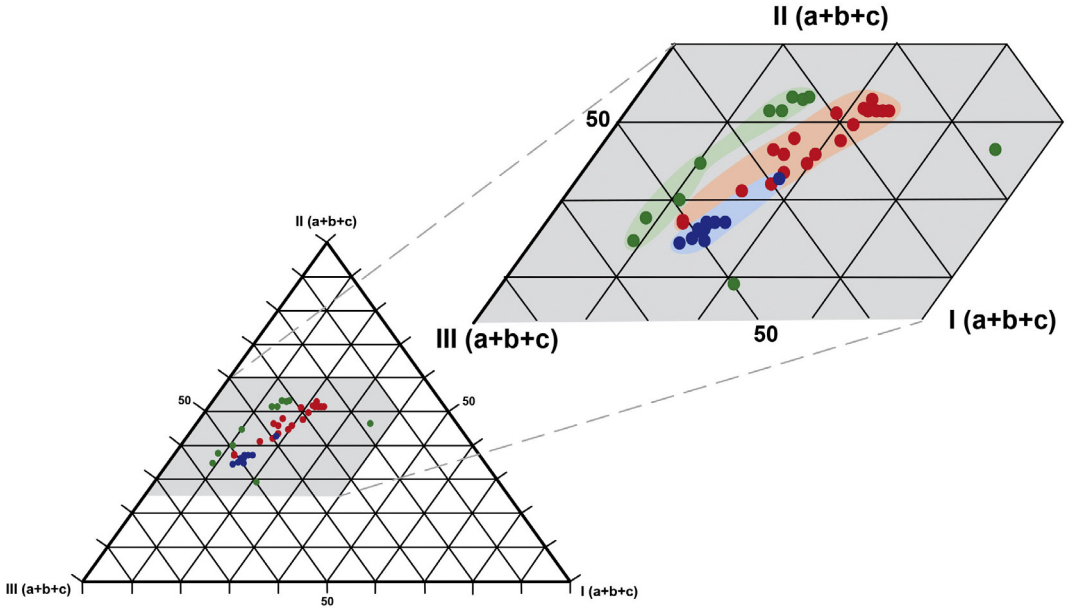

Fig. 2. Proportion of brGDGT types I (right axis), II (left axis), and III (bottom axis) from Upper EVV Lake sediments (red), Lower EVV Lake sediments (blue) and soils (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(F2) to collect the purified brGDGTs. Isolation and carbon isotopic analysis of brGDGTs was completed at Harvard University's Laboratory for Molecular Biogeochemistry and Organic Geochemistry.

2.6. Compound-specific carbon isotopic analyses of brGDGTs

Each isolated brGDGT fraction was dried under N_2 and dissolved in EtOAc (25 or 50 µl, depending on brGDGT concentration) and the carbon isotopic composition determined via SWiM-IRMS (Pearson et al., 2016). Briefly, the wire was first moved through a heated chamber (950 °C) to remove any contaminants. Next, five aliquots (1 µl) of each sample were loaded at intervals of 30 s onto the moving wire, enabling 5 replicate measurements. The wire moved each aliquot sequentially into a glass drying chamber where the solvent was evaporated, leaving the dry sample on the wire. Continued movement of the wire took the sample into a second heated chamber (900 °C) where it was combusted, with the resultant CO_2 gas directed into the IRMS instrument. Carbon isotopic values of the brGDGTs were normalized using two standards of known isotopic composition, C_{46} GTGT and crenarchaeol (Pearson et al., 2016), which were also analyzed as sequences of five aliquot loadings in concert with samples and in dilution series to correct for sample size effects. After analysis was complete, any extra sample material was reanalyzed using FIA to confirm the identity and purity of the compound(s).

3. Results

3.1. Carbon isotopic composition of OM

3.1.1. Lake sediments

OM from the 19 Upper EVV Lake sediment samples, which span the past ca. 2580 yr (Colcord et al., 2015), has a carbon isotopic composition ranging from −28.1‰ to −31.2‰ (avg. −29.3‰; Fig. 3; Table 1). The lowest value (−31.2‰) occurred in the surface sediments (0–2 cm; Fig. 4). OM from the 12 Lower EVV Lake sediment samples, which span the past ca. 2380 yr (Colcord et al., 2015), displayed values ranging from −22.8‰ to −25.6‰ (avg. −24.6‰; Fig. 3). For this lake, the surface sediment (0–2 cm) had the highest value (−22.8‰; Fig. 4). Thus, the isotopic composition values of the OM in the sediments were entirely distinct (Fig. 3) despite the close proximity of the two lakes (Fig. 1).

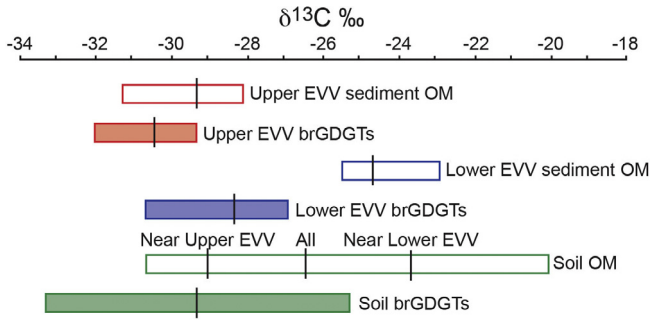

Fig. 3. Range of $\delta^{13}C$ values for the sedimentary OM from Upper EVV and Lower EVV Lakes, for all samples of soil OM and those near to the two lakes, and for the brGDGT constituents within each group of samples. Vertical bars represent the average composition for each sample category.

3.1.2. Soils

OM from the 11 soil samples surrounding the two Lakes has a carbon isotopic composition that ranges from −20.0‰ to −30.5‰ (avg. −26.4‰; Fig. 3; Table 1). Samples from the eastern region of the valley (Fig. 1; soils #7–11), closer to Upper EVV Lake, had an average carbon isotopic composition of −28.9‰ whereas those from the western region of the valley (Fig. 1; soils #1–5), closer to Lower EVV Lake, had an average of −23.7‰. These differences parallel the isotopic composition of the surface sediments from Upper and Lower EVV Lakes, respectively (Fig. 4).

3.2. Carbon isotopic composition of brGDGTs

3.2.1. Upper EVV Lake

In total, the carbon isotopic composition of brGDGTs from 5 samples from Upper EVV Lake were determined, yielding values

Table 1
Values from Lower EVV Lake, Upper EVV Lake, and the surrounding soils for the carbon isotopic composition of bulk OM (TOC) and brGDGTs, as well as the MBT and CBT indices. Details of the soil sample numbering and lettering are explained in Section 3.2.3 and Fig. 1.

Sample	$\delta^{13}C_{TOC}$	$\delta^{13}C_{brGDGTs}$					MBT	CBT
		(all)	(1050 Da)	(1036 Da)	(1022 Da)	std.dev.		
Lower Lake								
0–2 cm	−22.8	−26.8				0.4	0.163	0.734
2–4 cm	−23.5						0.156	0.723
4–6 cm	−24.6						0.146	0.694
6–8 cm	−25.2	−30.5				0.3	0.148	0.717
8–10 cm	−25.4						0.134	0.692
10–12 cm	−24.6						0.143	0.603
12–14 cm	−23.8	−27.7				0.3	0.145	0.640
14–16 cm	−24.9						0.184	0.621
16–18 cm	−25.2		−28.2	−28.3		0.3	0.148	0.713
18–20 cm	−25.6						0.142	0.742
20–22 cm	−25.0	−28.0				0.0	0.162	0.686
22–24 cm	−25.2						0.142	0.766
Upper Lake								
0–2 cm	−31.2	−32.0				0.1	0.124	1.190
2–4 cm	−29.3						0.123	1.235
4–6 cm	−29.1						0.158	1.153
6–8 cm	−28.6						0.169	1.305
8–10 cm	−28.7		−29.9	−30.7		0.1	0.174	1.339
10–12 cm	−28.5						0.191	1.342
12–14 cm	−28.1		−29.3	−30.2		0.2	0.155	1.199
14–16 cm	−28.2						0.213	1.070
16–18 cm							0.182	1.121
18–20 cm	−28.4						0.217	1.152
20–22 cm	−29.0						0.214	1.156
22–24 cm	−29.3		−29.3	−30.1		0.2	0.177	1.155
24–26 cm	−30.2						0.211	1.083
26–28 cm	−30.0		−30.8	−31.4		0.2	0.230	1.056
28–30 cm	−29.9						0.236	1.063
30–32 cm	−29.6						0.213	1.305
32–34 cm	−29.9						0.225	1.180
34–36 cm	−30.4						0.199	1.261
36–38 cm	−28.3						0.198	1.056
Soil Samples								
#1	−21.7						0.09	0.66
#3	−20.0						0.11	0.59
#4	−22.2						0.09	0.68
Soil A				−25.2	−25.5	0.2		
#2	−27.6						0.10	0.50
#5	−27.3						0.13	0.96
Soil B				−28.4	−29.8	0.2		
#6	−27.1						0.15	0.74
#9	−27.5						0.14	0.84
Soil C		−27.9				0.1		
#7	−28.3						0.21	1.14
#10	−28.1						0.36	1.47
Soil D			−29.3			0.3		
#8	−30.5						0.14	1.41
#11	−30.0						0.16	1.47
Soil E				−31.9	−33.5	0.2		

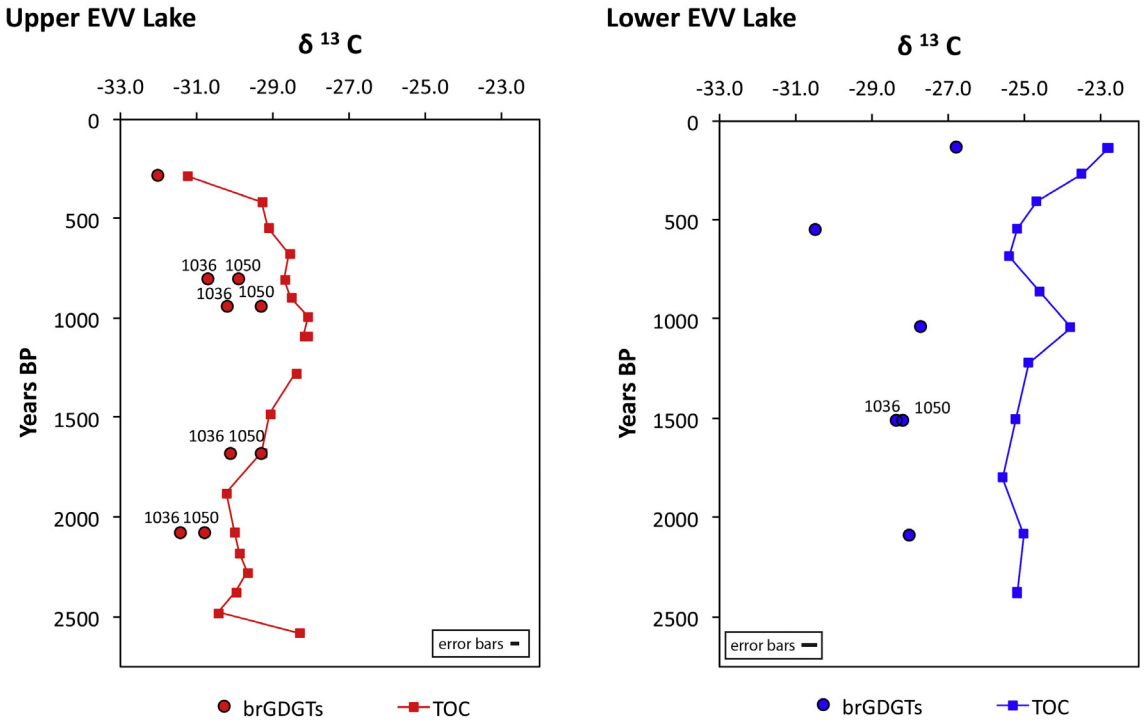

Fig. 4. Downcore profiles for $\delta^{13}C$ of sedimentary organic matter (TOC; connected squares) and $\delta^{13}C$ values for constituent brGDGTs (circles; specific compounds IIa and IIIa labeled as 1036 and 1050, respectively) for Upper EVV Lake (red) and Lower EVV Lake (blue). Error bars for the brGDGT $\delta^{13}C$ values are shown for each lake. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

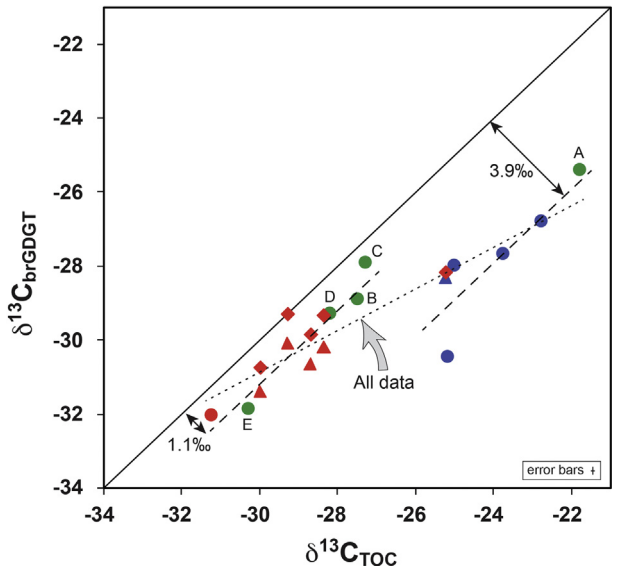

Fig. 5. $\delta^{13}C$ of organic matter (TOC) vs. $\delta^{13}C$ of branched GDGTs (brGDGT) for Upper EVV Lake (red), Lower EVV Lake (blue), and surrounding soils (green, designated by letters corresponding to the five combined soil samples). For some lake samples, sufficient material was present to measure the brGDGT IIa (1036 Da; triangles) and brGDGT IIIa (1050; diamonds) separately; all other measurements are shown as circles. The solid diagonal line represents equal values of $\delta^{13}C$ for TOC and brGDGT, and the position of the data reveals that brGDGTs are consistently more depleted in ^{13}C than TOC. The average ^{13}C depletion for Upper EVV and Lower EVV Lakes and their surrounding soils are −1.1‰ and −3.9‰, respectively, represented by the dashed lines. The correlation for all data is shown by the dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ranging from −29.3‰ to −32.0‰, (avg. −30.4‰, standard deviation ≤ 0.2‰; Fig. 3; Table 1). The surface sediment had the lowest value for brGDGTs, at −32.0‰. The brGDGTs were depleted relative to OM for all samples, with an average offset of 1.1‰ (Figs. 4 and 5). For some samples the concentration of brGDGTs afforded sufficient material to enable determination of discrete isotopic composition for IIa and IIIa (Sinninghe Damsté et al., 2000) characterized *m/z* 1036 and 1050, respectively. The results showed that IIa was consistently more depleted in ^{13}C than IIIa, with $\delta^{13}C$ values differing by an average of 0.8‰ (Fig. 5).

3.2.2. Lower EVV Lake

In total, 5 samples of brGDGTs from Lower EVV Lake ranged in $\delta^{13}C$ values from −26.8‰ to −30.5‰ (avg. −28.2‰, standard deviation ≤ 0.4‰; Table 1). For Lower EVV Lake the brGDGTs from the surface sediment exhibited the highest value (−26.8‰), in contrast to Upper EVV Lake where the surface sediment brGDGTs yielded the lowest $\delta^{13}C$ value (−32.0‰). The brGDGTs were depleted relative to bulk OM for all samples, with an average offset of 3.9‰ (Figs. 4 and 5); only one sample from Lower EVV Lake contained sufficient brGDGTs to obtain separate measurements for the compounds (IIa and IIIa) characterized by *m/z* 1036 and 1050. However, the difference for this sample was only 0.1‰, within the range of analytical error.

3.2.3. Soils

Samples were combined on the basis of the comparability of their locations within the watershed, including proximity to a specific lake, and the similarities in bulk $\delta^{13}C$ compositions and MBT and CBT ratios. Thus, the following samples were combined: Soil A from samples #1, 3 and 4 – proximal to Lower EVV Lake; Soil B from samples #2 and 5 – near Lower EVV Lake; Soil C from samples #6 and 9; Soil D from samples #7 and 10 – near Upper EVV Lake; Soil E from samples #8 and 11 – proximal to Upper EVV Lake (Fig. 1), which enabled carbon isotopic compositions of brGDGTs in a total of 5 combined soil samples (A–E) to be measured. The $\delta^{13}C$ values of brGDGTs from these combined soil samples ranged from −25.2‰ to −33.5‰ (avg. −29.3‰; Fig. 3; Table 1). These values

from the soil samples are all depleted relative to the bulk OM, comparable to the characteristics for brGDGTs from lake sediments (Fig. 5). Separate isotopic measurements for compounds Ia and IIa, with ions at *m/z* 1022 and 1036, respectively, were also possible for those samples that contained sufficient material. For the soil samples, Ia (*m/z* 1022) was always more depleted than II (*m/z* 1036 Da) with an average offset of 1.3‰, although further analyses are needed to confirm the veracity of this difference, given variation in the comparative purity of the brGDGT fractions from the soils.

4. Discussion

4.1. $\delta^{13}C$ composition of brGDGTs supports heterotrophic bacterial origins

BrGDGT Ia has been identified in two strains of *Acidobacteria* (Sinninghe Damsté et al., 2011), consistent with other studies suggesting that brGDGTs are produced by heterotrophic bacteria (e.g. Pancost and Damsté, 2003; Weijers et al., 2010; Weber et al., 2015; Huguet et al., 2017). However, the phylogenetic diversity and occupied niches of source organism(s) that biosynthesize brGDGTs remains unknown and is undoubtedly complex, especially in sedimentary environments receiving a varied input of OM (e.g. Sinninghe Damsté, 2016).

The temporal variations in the $\delta^{13}C$ values for brGDGTs in sediments from both Upper and Lower EVV lakes closely follow those for OM in the same samples (Fig. 4). These data further substantiate the prevailing view that brGDGTs are produced by heterotrophic bacteria, resulting in brGDGT $\delta^{13}C$ values strongly dependent on the composition of the OM that serves as their carbon source. This is exemplified by the $\delta^{13}C$ composition of the surface sediments from the two lakes, which demonstrate antithetic trends: the minimum and maximum values for both OM and brGDGTs occur in the surface sediments of Upper EVV Lake and Lower EVV Lake, respectively.

The isotopic composition of brGDGTs and OM for individual sediment horizons from both lakes exhibits a consistent offset, averaging 1.1‰ and 3.9‰ for Upper EVV and Lower EVV lakes and their environs, respectively (Fig. 5). The different offsets for the two lakes may perhaps be explained as a result of preferential utilization of specific components within the OM that are more ^{13}C-depleted by the bacteria producing the brGDGTs (e.g. Rinnan and Bååth, 2009). The net effect of this preferential utilization would result in a greater offset for sediments from Lower EVV Lake because their bulk OM is more ^{13}C enriched than that from Upper EVV Lake. Hence, the data from both lakes combined display a greater isotopic difference between brGDGTs and OM as the OM becomes more ^{13}C enriched (Fig. 5, dotted line). The clear relationship between $\delta^{13}C$ value of brGDGT core lipids and OM for both Greenland lake sediments and soils (Figs. 4 and 5) lends further support to the pivotal role of heterotrophy in the production of these compounds across a wide range of isotopic composition.

4.2. $\delta^{13}C$ composition of brGDGT homologues and the role of methanotrophy

4.2.1. Evidence for the association of brGDGTs with methanotrophy

Convincing evidence for in situ production of brGDGTs within the bottom waters and/or sediment-water interface has been documented for Upper EVV Lake (Colcord et al., 2015). There, it was shown that a strong correlation (R^2 0.987) exists between brGDGT distributions and lake bottom water temperatures for several lakes with hypoxic/anoxic bottom waters, including Upper EVV Lake. The one lake in the study to deviate from this trend was Lower EVV Lake, which is the only lake that has fully oxygenated bottom waters. Thus, it was concluded that there was no substantial in situ brGDGT production within Lower EVV Lake.

Carbon isotopic studies of the relationship between brGDGTs in sediments and surrounding soils from Lake Hinterburg, Switzerland, revealed significant ^{13}C depletion (ca. 16‰ difference) in the brGDGT core lipids recovered by cleavage from sediments (−43 to −46‰) vs. those obtained from surrounding soils (−27 to −28‰; Weber et al., 2015). In addition, the occurrence of a proposed lake-specific brGDGT (IIIa″ provided further evidence for in situ brGDGT production within the lake (Weber et al., 2015). Yet the offset between brGDGTs from sediments and surrounding soils for Upper EVV Lake is relatively small (avg. ca. 1.1‰; Fig. 3) compared with those in Lake Hinterburg (ca. 16‰), despite evidence of in situ production in Upper EVV Lake (Colcord et al., 2015). The difference in the isotopic characteristics of brGDGTs from sediments vs. surrounding soils at Lake Hinterburg compared with Upper EVV Lake appears to reflect the markedly greater ^{13}C depletion for the alkyl cores of brGDGTs from Lake Hinterburg sediments (ca. −46‰), which can be attributed to the uptake of ^{13}C-depleted OC, possibly associated with significant amounts of chemoautotrophy or methanotrophy within the lake (Weber et al., 2015).

4.2.2. Evidence for the minor role of methanotrophy in Upper EVV Lake

Prior studies have found that the concentration of CH_4 in Upper EVV Lake is low for an Arctic lake environment (Cadieux et al., 2016; Goldman et al., 2015) and varies seasonally; it is markedly higher (ca. 50 μM) in the winter when the lake is ice-covered than in the summer (< 10 μM) under open water conditions. The $\delta^{13}C$ and $\delta^{2}H$ values of CH_4 in Upper EVV Lake attest to its microbial origin from methanogenesis in the sediments and to its consumption by methanotrophic oxidation within the water column (Cadieux et al., 2016). The potential influence of dissolved inorganic carbon (DIC) on in situ production of brGDGTs and on their $\delta^{13}C$ composition cannot be assessed because of insufficient data for $\delta^{13}C_{DIC}$ in the water column (Cadieux et al., 2016). However, the difference between the $\delta^{13}C$ composition of brGDGTs in lake sediments vs. soils is less than expected if methanotrophy represents a dominant process in Upper EVV Lake. Indeed, the entire range of $\delta^{13}C$ values for soil brGDGTs (−25.2‰ to −33.5‰) exceeds that of brGDGTs in the sediments (Upper EVV −29.3‰ to −32.0‰; Lower EVV −26.8‰ to −30.5‰; Fig. 3). Although microbial and biogeochemical generation of ^{13}C depleted OM and methylotrophic oxidation of CH_4 occur in Upper EVV Lake (Cadieux et al., 2016), the concentration and isotopic composition of the CH_4 suggest that such processes are minor; specifically, they are far less significant in Upper EVV Lake than in Lake Hinterburg (Weber et al., 2015).

Further studies are necessary to confirm that the consistent offset between the $\delta^{13}C$ values of brGDGTs IIa (*m/z* 1036) and IIIa (*m/z* 1050) is a true measure of the isotopic difference between these components and not an artifact of HPLC purification, either with respect to incomplete removal of background contaminants during separation or to isotopic fractionation during fraction collection. The 0.8‰ difference in the Upper EVV Lake sediments cannot be attributed to biosynthetic pathways associated with methylation since the sole difference between the IIa and IIIa is that they contain 5 and 6 methyl groups, respectively, which would therefore require a wholly unrealistic difference (> 60‰) in the ^{13}C composition of the methyl groups formed by methylation vs. other carbons in the alkyl core lipids and glycerol end groups. A more plausible explanation is that the isotopic difference between IIa and IIIa may reflect changes in the balance of their production from different carbon sources whose $\delta^{13}C$ composition varies seasonally. For example, the presence of ice cover could mean that heterotrophic production of discrete brGDGTs exploits different pools of OM

during the winter from those utilized under open lake conditions during the summer. Moreover, the differences in concentration and isotope composition of CH_4 for Upper EVV Lake in April vs. July (Cadieux et al., 2016) attest to seasonal variation in this potential carbon source.

Previous interpretation of brGDGT distributions in Upper EVV Lake suggests that they may record lake bottom temperature during summer months (Colcord et al., 2015). Such summer production of in situ brGDGTs would be expected to yield a greater proportion of IIa relative to IIIa, as a result of the correspondence between the abundance of IIIa and colder temperature via the MBT/CBT indices (Weijers et al., 2007). Hence, the difference in the $\delta^{13}C$ values of brGDGTs IIa (*m/z* 1036) and IIIa (*m/z* 1050) in the Upper EVV Lake sediments could perhaps be associated with methanotrophy, given the higher concentration of ^{13}C-depleted CH_4 ($\delta^{13}C$ −66.9‰ to −76.4‰) in sediments from Upper EVV Lake during the summer (Cadieux et al., 2016). In addition, the brGDGTs from the combined sample of soils peripheral to Upper EVV Lake have an average $\delta^{13}C$ value of −32.4‰, which could reflect utilization of CH_4 during episodes of lake expansion, given the high CH_4 concentration in the littoral zone of the lake (Cadieux et al., 2016).

4.2.3. Lack of evidence for methanotrophy in Lower EVV Lake

In comparison, the inventory of CH_4 in Lower EVV Lake is low throughout the water column during ice-free conditions (Cadieux, 2015). Additionally, the difference between the $\delta^{13}C$ values of brGDGTs IIa (*m/z* 1036) and IIIa (*m/z* 1050) measured from Lower EVV Lake sediments (0.1‰) is insignificant and falls within instrumental error. Hence, the absence of evidence for in situ production of brGDGTs in Lower EVV Lake – in contrast to Upper EVV Lake (Colcord et al., 2015) – may reflect differences in their CH_4 inventories. This explanation, albeit based on less pronounced differences in $\delta^{13}C$ values, parallels the interpretation that the ^{13}C-depleted (ca. −46‰) alkyl cores of brGDGTs in Lake Hinterburg may be associated with methanotrophy within the lake (Weber et al., 2015). It is also consistent with the timing and importance of seasonal methanotrophic activity in Arctic lakes (He et al., 2012a,b).

5. Conclusions

This contribution reports the first measurements of the $\delta^{13}C$ composition for intact brGDGT core lipids from Greenland lake sediments and soils. It demonstrates that the approach enables determination of the $\delta^{13}C$ composition of entire brGDGT compounds, unlike existing published methods for isotopic analysis of brGDGTs that measured the $\delta^{13}C$ values of alkyl core lipids after cleavage (Weber et al., 2015).

The ability to measure the $\delta^{13}C$ composition of brGDGTs reveals that they mirror the $\delta^{13}C$ values of OM in both lacustrine sediment and soil samples, although they are always depleted in ^{13}C relative to the associated OM. The data provide further confirmation of the role of heterotrophy in the production of brGDGTs and reflect utilization of ^{13}C depleted carbon sources in their bacterial biosynthesis. The $\delta^{13}C$ values of brGDGTs from Upper EVV Lake sediments and proximal soils, augmented by the isotopic differences between the brGDGTs designated IIa (*m/z* 1036 Da) and IIIa (*m/z* 1050 Da), further suggest the possibility that the brGDGTs reflect a minor contribution from methanotrophy, especially during the summer in this lake. Thus, these isotopic analyses provide further evidence suggesting in situ production of brGDGTs within dysoxic aquatic environments, and illustrate how such investigations can help identify the biological origins of these compounds. Indeed, further application of this analytical capability for obtaining the $\delta^{13}C$ composition for intact brGDGTs using SWiM-IRMS should provide a valuable tool in the elucidation of the biogeochemical and environmental controls on their production.

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