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Microbial lipid signatures in Arctic deltaic sediments - insights into methane cycling and climate variability

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Abstract.

Glycerol Dialkyl Glycerol Tetraethers (GDGTs) are ubiquitous biomolecules whose structural diversity or isotopic composition is increasingly used to reconstruct environmental changes such as air temperatures or pCO$_2$. Isoprenoid GDGTs, in particular GDGT-0, are biosynthesized by a large range of Archaea. To assess the potential of GDGT-0 as a tracer of past methane cycle variations, three sediment cores from the Mackenzie River Delta have been studied for iGDGT and diploptene distribution and stable carbon signature. The absence of crenarchaeol, high GDGT-0 vs crenarchaeol ratio, and $^{13}$C-enriched carbon signature of GDGT-0 indicate production by acetoclastic methanogens as well as heterotrophic Archaea. The oxidation of methane seems to be dominated by bacteria as indicated by the high abundance of $^{13}$C-depleted diploptene. Branched GDGTs, thought to be produced by heterotrophic bacteria, are dominated by hexa- and penta-methylated 5- and 6-methyl compounds. The presence of 5,6-methyl isomer IIIa’’ points towards in situ production of brGDGTs, with only a minor input from soil branched GDGT brought by the Mackenzie River. Carbon isotopic compositions of brGDGTs are in agreement with heterotrophic producers, likely living during summer. The reconstructed temperatures using a global lake calibration reflect recorded summer air temperature ($\pm 2.14 ^\circ$C) during the last 60 years, and further highlight the absence of warming in summer in this region during the last 200 years. Oxygen availability and connection time to the Mackenzie River also seem to control the distribution of branched GDGT with an increase in 6-methyl and 5,6-methyl isomers with increased period of anoxia.

Keywords: GDGT, methane cycle, Mackenzie River, temperature reconstruction, carbon isotopes
1 Introduction

The Mackenzie delta region in the Canadian Arctic is highly sensitive to climate change, and has already recorded a warming of 6 °C in mean annual temperature since 1970 (based on Environment and Climate Change Canada). This warming exclusively manifests itself in winter temperature, whereas the summer temperature has been stable (based on Environment and Climate Change Canada). The Mackenzie River delta is characterized by thousands of small shallow lakes (mean depths from about 0.5 m to 4.5 m) (Emmerton et al., 2007) that are frozen from late September to early June (Droppo et al., 1998). These lakes are connected with the main river channels to different degrees and have been classified into three categories by Lesack and Marsh (2007): “No closure” lakes are continuously linked to the river, “low closure” lakes have a connection that occurs at least once a year during the freshet, and “high closure lakes”, characterized by a higher levees, are only connected to the river during extreme freshets, with recurrence intervals that may be only once per decade. Most inland water bodies of the Mackenzie River delta are a net sink of carbon dioxide (CO₂) and a source of methane (CH₄) (Tank et al., 2008). However, inland water bodies are highly sensitive to temperature changes, as warming will strongly impact water column stratification, aquatic ecosystems as well as the gas exchange dynamics of the lakes. To understand the impact of these arctic wetlands on global greenhouse gas budgets, and project the long-term evolution of this environment, constraining the effects of warming on aquatic ecosystems of the region is essential.

Archaeal and bacterial membrane-spanning lipids, glycerol dialkyl glycerol tetraethers (GDGTs) are ubiquitous biomarkers that are found in extreme environments such as perennially frozen soils, e.g., Siberian permafrost (Kusch et al., 2019), and in surface lake sediments of the Mackenzie delta (Peterse et al., 2014). Isoprenoid GDGTs (iGDGTs) are biosynthesized by a wide range of Archaea, with some iGDGTs such as crenarchaeol and
crenarchaeol regioisomer having a narrow phylogenetic source, i.e., ammonia-oxidizing Thaumarchaeota (Schouten et al., 2013). In contrast, GDGT-0 is found in many cultivated strains of Archaea (Oger and Cario, 2013; Schouten et al., 2013; Villanueva et al., 2014; Elling et al., 2017), but it is particularly abundant in methanogens and methanotrophs (Koga et al., 1993; Pancost et al., 2001; Bauersachs et al., 2015; Sollai et al., 2019). In marine and freshwater realms, GDGT-0 has been used as an indicator of the presence of methanotrophic and methanogenic Archaea. Several ratios have been built to infer the emission of CH₄ such as the methane index (Zhang et al., 2011), or the relative abundance of GDGT-0 versus crenarchaeol (Blaga et al., 2009). Carbon isotopic measurements of iGDGTs have also shed light on the influence of Archaea on methane cycling in lacustrine and marine environments as the stable carbon isotopic composition of lipids reflect the carbon source of the producer, and methane is usually $^{13}$C-depleted (Pancost et al., 2000; Sinninghe Damsté et al., 2009a; Weber et al., 2015; Colcord et al., 2017). Methyl-branched GDGTs (brGDGTs) are ubiquitous bacterial membrane lipids that are abundant in soils (e.g., De Jonge et al., 2014a; Weijers et al., 2007) and lakes (e.g., Blaga et al., 2009; Colcord et al., 2015; Dang et al., 2016; Foster et al., 2016; Russell et al., 2018). In soils, they are inferred to be produced by members of the phylum Acidobacteria, based on cultures (Sinninghe Damsté et al., 2014, 2018) and on their environmental distribution (Weijers et al., 2010). In lakes, they are likely produced by multiple groups of bacteria (Weber et al., 2018). The degree of methylation of the brGDGTs reflects an adaptation to temperature, with higher fractional abundance of hexa- and pentamethylated GDGTs (GDGT-II and GDGT-III) in colder soils (Weijers et al., 2007; Jonge et al., 2014; Colcord et al., 2015; Zink et al., 2016), and permafrost soils dominated by GDGT-IIIa (Kusch et al., 2019). BrGDGTs can be recovered from geological archives such as paleosoils or lacustrine sediments, but their dual provenance in lake sediments can be problematic as the relative abundances of brGDGTs in soils has been shown to differ from those in lake sediments under the same temperature (e.g.,
Martin et al., 2020; Tierney and Russell, 2009; Zink et al., 2016). The source of brGDGTs can thus be inferred by investigating the distribution of the brGDGTs. In marine coastal environments, three ratios have been developed: the weighted average number of cyclopentane moieties and the degree of methylation of the brGDGTs (Sinninghe Damsté, 2016), the ratio of GDGT-IIIa over GDGT-IIa (Xiao et al., 2016), and the BIT index (Hopmans et al., 2004). In freshwater environments, only the ratio of GDGT-IIIa/GDGT-IIa has thus far been used to assess the origin of brGDGTs, utilizing values for modern soils and lake sediments (Martin et al., 2019). In addition, compound-specific $^{13}$C values (Weijers et al., 2007; Naeher et al., 2014a; Colcord et al., 2017; Weber et al., 2018) have been proposed to as a means to distinguish lake- or soil-derived sources through comparison with $^{13}$C values of bulk organic carbon (OC) and other biomarkers. Once the provenance is determined, corresponding lacustrine or soil calibration can be applied, with numerous studies constructing a local, region-specific calibration using lake surface sediments or suspended particulate matter (SPM, e.g., Foster et al., 2016; Pearson et al., 2011; Sun et al., 2011; Tierney et al., 2010; Zink et al., 2016) or other proxies such as pollen (Dugerdil et al., 2020) or haptophyte biomarkers (Harning et al., 2020). Alternatively, calibration based on large spatial scale variations (Loomis et al., 2012; Russell et al., 2018) can be used. Soil-specific calibrations have also been constructed (De Jonge et al., 2014b; Peterse et al., 2012; Watson et al., 2018), and have been found to yield reliable results in temperate environments.

Peterse et al. (2014) determined that most of the brGDGTs in Mackenzie delta lake surface sediments originated from in situ production within the lakes. This contrasted sharply with the Mackenzie River where most of the brGDGTs originated from erosion and transport of soils within the catchment. Here, we investigate three sediment cores retrieved from lakes in the middle (MD-1 and MD-2) and upper delta (UD-4) that have previously been studied from a sedimentological and biogeochemical perspective ($n$-alkanes and fatty acid biomarkers, bulk...
OC $^{14}$C and $^{13}$C (Lattaud et al. submitted). The core-tops of these lakes have previously been examined by Vonk et al. (2015) as part of a study to assess sediment dispersal and deposition patterns in the Mackenzie delta. Based on these prior investigations it was determined that low closure lakes have the clear potential to record past climate changes. Here, we use brGDGTs determine the provenance and assess climate signals recorded (last 200 yr) in one low closure lake (UD-4) in comparison with a no closure (MD-2) and a high closure (MD-1) lake. Furthermore, iGDGTs and their stable carbon isotopes are studied to investigate past methane cycling in these lakes.

2 Material and methods

2.1 Study sites

The Mackenzie delta is located in the Canadian arctic, at latitudes above 67°N (Fig. 1), with a subpolar drainage basin that lies primarily (~75%) within the continuous and discontinuous permafrost zones. Within the central Mackenzie Delta (i.e., the area between Aklavik and Inuvik), summers are cool and short, with ice-free conditions lasting from early June to late September. The mean summer (June – August) and winter (November – February) air temperature for Inuvik are 11.4 °C and -25.5 °C, respectively (based on Environment and Climate Change Canada) with a mean annual (MAT) average of -9.2 °C. The MAT in the south of the basin is much warmer, averaging 0.7 °C at Fort McMurray. The Mackenzie River flows northward from areas of relative warmth toward frozen northern regions. It crosses several large lakes such as Great Slave Lake and Great Bear Lake that act as efficient sediment traps (Carson et al., 1998; Carrie et al., 2009), and consequently most of the sediment reaching the Mackenzie delta originates north of these lakes. The freshet, i.e. spring flood, which removes snow cover from terrestrial surfaces, warming the ground and accelerating active-layer
development (Mackay, 1963), accounts for 60% and 90% of annual water and sediment discharge, respectively (Drenzek et al., 2007), and is responsible for seasonal inundation of lakes within the delta.

Figure 1 (a) Localization of the Mackenzie delta lakes with (b) zoom of Inuvik area insert and (c) diagram illustrating the degree of closure of the lakes.

Three representative lakes were chosen for the present study: MD-1, a high closure lake in the middle delta with an area of ~ 5.9 ha and a mean depth of 0.74 m; UD-4, a low closure lake from the upper part of the delta (surface area, ~ 12.5 ha; mean depth, 2.2 m), and MD-2, a no closure lake close to Inuvik in the middle delta (surface area, ~ 700 ha; mean depth, 2.2 m) (Lesack and Marsh, 2010; Vonk et al., 2015). These lakes have been previously studied for their sedimentological properties (Lattaud et al., submitted) and have not always been in the same connection state as now. Specifically, UD-4 was high closure before transitioning to low closure before ca. 1890 C.E. (i.e., below 90 cm), while MD-2 was low closure before ca. 1928 C.E. (i.e., below 110 cm).
2.2 Methods

2.2.1 Lakes sampling

Mackenzie delta lake sediment cores were sampled in March 2009 with a push-corer (system built in-house at the Geology and Geophysics department, Woods Hole Oceanographic Institution, WHOI) as described by Vonk et al. (2015). Cores were shipped in cooled conditions, split lengthwise and sliced every centimetre.

2.2.2 Lipid extraction and analysis

The sediments were freeze-dried and subsequently extracted with the EDGE system as described in Lattaud et al. (submitted). Briefly, the total lipid extract was saponified, the neutral fraction liquid-liquid extracted, and separated on a silica gel column into three fractions. The polar fraction was filtered using a polytetrafluoroethylene 0.45 µm filter prior to analysis. As GDGTs in the environment have predominantly glycosidic bounds, they are not cleaved to core GDGTs by base hydrolysis (done in this study). Hence, the fraction of GDGTs analysed in this study are mostly fossilized core GDGTs, plus a small fraction of the intact polar lipid GDGT pool.

The GDGTs were analysed with high performance liquid chromatography (LC)/atmospheric pressure chemical ionization–mass spectrometry (MS) on an Agilent 1260 Infinity series LC-MS according to Hopmans et al. (2016). Selective ion monitoring of the [M + H]+ was used to detect and quantify the different GDGTs, according to Huguet et al. (2006), except that a similar response factor was assumed for the GDGTs and the internal standard.

Diploptene was quantified on a HP 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID), and a VF-1 MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The temperature program started with a 1 min hold time at 50 °C, followed by a
10 °C min⁻¹ ramp to 320 °C and a 5 min hold time at 320 °C. Quantification was based on an internal standard (C₃₆ n-alkane) of known concentration.

### 2.2.3. GDGT carbon isotopic analysis

Before stable carbon isotope analysis, GDGTs from MD-2 and UD-4 were isolated from the polar fraction using semi-preparative normal phase HPLC following the method of Gies et al. (2020). Fractions were collected from 16 to 18 min for GDGT-0 and 36 to 48 min for the brGDGTs.

GDGTs from MD-1 were purified using semi-preparative normal phase HPLC following the method of Pearson et al. (2016).

Purity of the prepped fractions was assessed using HPLC flow injection analysis (FIA) relative to a dilution series of the C₄₆-GDGT standard (Huguet et al., 2006). ¹³C measurements of the GDGT were performed at Harvard University as described in Pearson et al. (2016). In summary, the GDGT fractions are purified by reverse phase (RP) HPLC to remove non-GDGT material and the fractions containing the isolated GDGT (F2) and the minute before (F1) were collected.

Values of δ¹³C_{GDGT} are measured by Spooling Wire Microcombustion-IRMS (SWiM-IRMS; Pearson et al., 2016; Sessions et al., 2005). The precision measurements was ± 0.2‰. The ratio of F2/F1 indicates the level of background contamination in a sample with low ratio (< 2) indicating possible contamination.

### 2.2.4. Diploptene carbon isotopic analysis

The carbon isotope composition of diploptene was measured in duplicate by Gas Chromatography-isotope ratio mass spectrometry (GC-IRMS) on a Thermo Trace GC (1310) coupled with a Thermo Delta-V plus system at the Climate Geology group at ETH Zurich. The
GC was equipped with a RTX-200 MS capillary column (60 m x 0.25 mm i.d., 0.25 μm film thickness) and temperature program was as follows: ramp from 40 °C to 120 °C at 40 °C min⁻¹, followed by a 6 °C min⁻¹ ramp to 300 °C and 12 min hold time at 320 °C. Duplicates were measured when possible.

3. Results

3.1. GDGTs and diploptene distribution

3.1.1. iGDGT

The isoprenoid GDGTs are dominated by GDGT-0 (Fig. 2), especially in MD-1 (high closure) where it accounts for 90 ± 2% of all iGDGTs. GDGT-0 comprises 49 ± 4% of iGDGTs in MD-2 (no closure) and 80 ± 12% of iGDGTs in UD-4 (low closure). The total amount of iGDGT is higher in MD-1 (18.5 ± 17.7 μg g<sub>TOC</sub>⁻¹) followed by UD-4 (10.8 ± 7.2 μg g<sub>TOC</sub>⁻¹) and MD-2 (6.8 ± 3.6 μg g<sub>TOC</sub>⁻¹), largely due to the increased abundance in GDGT-0. Only trace amounts of crenarchaeol and crenarchaeol isomer are detected in all lakes (0.01 – 0.6 μg g<sub>TOC</sub>⁻¹ in MD-1, 0.01 – 0.1 μg g<sub>TOC</sub>⁻¹ for UD-4 and 0.1 – 4 μg g<sub>TOC</sub>⁻¹ for MD-2).

3.1.2. BrGDGTs

All sampled lakes have a similar distribution of brGDGT lipids (Fig. 2), with brGDGT-IIIa and GDGT-IIIa’ as the most abundant components, followed by GDGT-IIa, GDGT-Ia and GDGT-IIa’. In MD-2 lake sediments (no closure), hexamethylated, pentamethylated and tetramethylated components represent 46 ± 2%, 38 ± 2% and 17 ± 1%, respectively, of all the brGDGT (15 brGDGT are quantified, see appendix 1). Similar distributions are encountered in the other lake sediments, with 47 ± 5%, 38 ± 3% and 16 ± 2% for UD-4 (low closure), and 43 ± 2%, 40 ± 1% and 18 ± 1% for MD-1 (high closure) (hexa, penta and tetramethylated compounds, respectively). Structural isomers of the hexamethylated compounds exhibit different patterns among the lakes, with GDGT-IIIc X-methyl (structure not identified,
compound eluting between the GDGT-IIIc and GDGT-IIIc’, further called the IIIcX) present in MD-2, while IIIa’ and IIIcX are found in UD-4, and GDGT-IIIb X-methyl (structure not identified, compound eluting between the GDGT-IIIb and GDGT-IIIb’, IIIbX) is present in MD-1. The concentration of brGDGTs is similar between MD-1 and MD-2 but is more variable (22.5 ± 21.5 µg gTOC⁻¹ and 19.2 ± 10.4 µg gTOC⁻¹), respectively) and higher in UD-4 (33.7 ± 29.0 µg gTOC⁻¹).

**Figure 2** GDGT composition in Mackenzie delta lakes (a) fractional abundance and (b) ternary diagram of the hexa-, penta- and tetra-methylated branched GDGT in Mackenzie lakes in comparison with a global lakes and soils dataset (Pearson et al., 2011 for lakes, Foster et al. 2016 for Antarctic lakes and De Jonge et al., 2014b for soils).

**3.1.3. Diploptene**
Diploptene (17β(H), 21β(H)-hop-22 (29)-ene) is detected at all depths in UD-4 (low closure) and MD-1 (high closure), and at some depths in MD-2 (no closure). Its concentration is higher in MD-1 and UD-4 than in MD-2 (110 ± 50 µg g$_{TOC}^{-1}$, 130 ± 80 µg g$_{TOC}^{-1}$ and 50 ± 20 µg g$_{TOC}^{-1}$ respectively). The concentration of diploptene in MD-1 increases from the oldest part of the record towards the top, reaching a maximum around 14 cm (200 µg g$_{TOC}^{-1}$). In UD-4 the concentration is higher in the oldest part of the core, around 90 cm (reaching 340 µg g$_{TOC}^{-1}$) before decreasing until 28 cm (lowest concentration 40 µg g$_{TOC}^{-1}$), and then increasing again to the top of the core. Diploptene concentration in MD-2 is higher at the bottom of the core (140 µg g$_{TOC}^{-1}$) then decreases and remains stable until the core top (average of 40 ± 20 µg g$_{TOC}^{-1}$, n = 64).

3.2. Carbon isotopes

3.2.1. GDGT-0 and diploptene

The δ$^{13}$C value of GDGT-0 is $^{13}$C-enriched compared with that of the brGDGT lipids, with MD-1 (high closure) exhibiting higher δ$^{13}$C values than UD-4 (low closure, -22.6 ± 0.2‰, n = 3 versus -26.2 ± 0.2‰, n = 6, Fig. 3). In MD-1, the δ$^{13}$C value of GDGT-0 decreases with increasing depth while it stayed constant for UD-4.

Most of MD-2 (no closure) samples are below detection limit for diploptene δ$^{13}$C measurements. When measurable, the δ$^{13}$C value of diploptene is significantly lower than that of the GDGTs (Fig. 3), in average -47.0 ± 3.3‰ in MD-2 (n = 2), -51.6 ± 2.5‰ in UD-4 (n = 13) and -56.5 ± 1.8‰ in MD-1 (n = 24). In MD-1 (high closure), diploptene is more $^{13}$C-enriched at the top of the cores than deeper downcore, exhibiting a trend similar to the GDGTs.

In UD-4 (low closure), δ$^{13}$C values of diploptene increase at the bottom of the core.

3.2.2. BrGDGTs
All branched GDGTs have been pooled for $^{13}$C measurements, with 3 cm resolution. MD-2 (no closure) and UD-4 (low closure) have similar $\delta^{13}$C values of $-29.0 \pm 0.3\%$ (Fig. 3c) ($n = 9$) while MD-1 (high closure) is more $^{13}$C-depleted ($-31.1 \pm 0.2\%$; $n = 3$). In MD-1, the $\delta^{13}$C value of the brGDGTs decreases with increasing depth while it stayed constant with depth in the other lakes (Fig. 3c).

**Figure 3** Carbon stable isotope compositions of (a) GDGT-0, (b) diploptene (three samples could not be run in duplicate and therefore do not have error bars), (c) brGDGT, and (d) bulk TOC (Lattaud et al., submitted) in the Mackenzie delta lakes. Asterisks (*) indicate low F2/F1, and therefore $\delta^{13}$C_{GDGT} values influenced more strongly by background carbon.
4 Discussion

4.1. Origin of GDGTs in Mackenzie Lakes

4.1.1. iGDGT reflect in situ methane production

The distribution of the iGDGTs is dominated by GDGT-0 (Fig. 2), comprising more than 90% of all iGDGTs. Previous studies have shown that the number of cyclopentane rings in GDGTs from surface sediments substantially increases with increasing lake temperature (Schouten et al., 2002), hence the predominance of GDGT-0 is not a surprise in arctic lakes (MAT = -8.4 ± 1.7 °C, 1960 – 2020, Inuvik weather station). However, the absence of crenarchaeol is surprising, and the observed distribution differs from those of permafrost samples of Kusch et al. (2019), and more closely resembles those found in the deep anoxic regions of Lake Chala, Africa (Sinninghe Damsté et al., 2009b) and Lake Rotsee, Switzerland (Naeher et al., 2014a).

GDGT-0 can be produced by a wide range of Archaea; Thaumarchaeota (Oger and Cario, 2013; Schouten et al., 2013; Villanueva et al., 2014; Elling et al., 2017), thermophilic and mesophilic Crenarchaeota, as well as in methanogens and Euryarchaeota that mediate the anaerobic oxidation of methane (Koga et al., 1993; Pancost et al., 2001; Bauersachs et al., 2015). The near absence of crenarchaeol in addition to the low concentration of GDGT-1 to 3 points toward a precursor other than the lacustrine Thaumarchaeota, and methanogenic or methanotrophic Archaea are a likely predominant source of GDGT-0 in Mackenzie delta lakes.

Blaga et al. (2009) proposed the ratio of GDGT-0 over crenarchaeol is an indicator of methanogenesis in lakes. A value above 2 was suggested as indicative of the presence of anaerobic methanogenesis, although few studies clearly demonstrated a strong correlation (Naeher et al., 2014a). In the Mackenzie delta, MD-1 (high closure) has an average GDGT-0/crenarchaeol ratio of 152 ± 51 while the ratios for UD-4 (low closure) and MD-2 (no closure) are of 17 ± 8 and < 1.6 ± 0.3, respectively (Fig. 5). These ratios for the high closure and low closure lakes are much higher than those reported by Blaga et al. (2009) in European lakes, but
similar to Pan et al. (2016) in marine hydrothermal deposits and to some lakes such as Lake Honghu (China) with low oxygen content (Dang et al., 2016) and eutrophic Lake Rotsee (Switzerland) (Naehler et al., 2014b). The low values in MD-2 could indicate limited methanogenesis. Biomass from high emergent macrophyte productivity in Mackenzie delta lakes during summer is rapidly oxidized under the ice during winter, creating anoxia. The protracted interval of water column anoxia as well as the abundance of CO$_2$(aq) – due to high organic matter input and freezing of the lake – create an ideal habitat for anaerobic methanogens in the water column or sediment-water interface. In early summer, when the lake water column is oxic, these organisms could persist deeper in the sediment. Their 16S rRNA genes have been detected in the water column of some lakes of the delta (Bergstresser, 2018) and in the water column and sediments of other Arctic thermokarst lakes (Heslop et al., 2015; Matheus Carnevali et al., 2015; Crevecoeur et al., 2016). However, Bergstresser (2018) only detect few reads (one OTU linked to methanogenic Archaea), which they attributed to the use of a non-specific 16S rRNA primer.

Several other cyclic iGDGT ratios have been defined in order to trace the presence of methanotrophic archaea (Blaga et al., 2009; Zhang et al., 2011), i.e., GDGT-2 over crenarchaeol and GDGT-1, -2, -3 over crenarchaeol (methane index), respectively. For the first ratio, values $> 0.2$ points toward methanotrophy (Blaga et al., 2009), while for the methane index, values $> 0.5$ indicate significant production of iGDGT by methanotrophic Euryarchaeota (Zhang et al., 2011). For the Mackenzie delta, GDGT-2/Crenarchaeol ratios exceed the threshold value for methanotrophy for MD-1 (high closure) and UD-4 (low closure) (0.7 ± 0.3 and 0.3 ± 0.1, respectively), while MD-2 (no closure) fall below this threshold (Fig. 5). The methane index is relatively high in the high closure lake sediments of the Mackenzie delta (MD-1, 0.7 ± 0.1) in comparison with those from low and no closure lakes (MD-2 0.3 ± 0.1 and UD-4 0.4 ± 0.1, respectively). Both ratios thus indicate the potential presence of
methanotrophic Archaea in MD-1 (high closure). The anaerobic oxidation of methane is characterised by a low energy yield (Nauhaus et al., 2002) and, so far, three communities of Euryarchaeota (ANME-1, -2, -3) were identified in consortium with sulphate-reducing bacteria (SRB) that provide electron acceptor (SO$_4^{2-}$). But other oxidants (e.g., Fe(III), Mn(IV)) are also plausible (e.g., Beal et al., 2009). In high closure lakes, iron and sulfate concentrations are elevated (Geeves, 2019), and anoxia and high methane concentrations could favor the presence of methanotrophic Archaea.

![Figure 4](image)

**Figure 4** GDGT ratios (a) GDGT-0/crenarchaeol, (b) GDGT-2/crenarchaeol in Mackenzie delta lakes.

We measured the carbon isotopic composition of GDGT-0 in order to assess the role of Archaea in the methane cycle in the Mackenzie River delta lakes. In low closure (UD-4) and high closure (MD-1) lakes, where GDGT-0 was present in sufficient abundance for isotopic
measurement, $\delta^{13}$C$_{\text{GDGT-0}}$ values are $^{13}$C-enriched compared to brGDGTs, with higher values for MD-1 (-22.5 ± 0.2‰) than for UD-4 (-26.6 ± 0.3‰). These compositions are similar to those of the $\delta^{13}$C$_{\text{TOC}}$ (-22.7 ± 0.3‰ and -26.0 ± 0.4‰, respectively), which could point towards the production by heterotrophic Archaea in the lakes although the fractionation is very small. GDGT-0 could also be produced by acetoclastic or hydrogenotrophic methanogenic Archaea, utilizing acetate or dissolved CO$_2$ as their carbon source for biosynthesis. In the latter, lipid $\delta^{13}$C values would reflect that of lake water CO$_2$(aq) after accounting for fractionation during carbon fixation and biosynthesis. Although $\delta^{13}$C$_{\text{CO2(aq)}}$ values are not directly available for the lakes examined in this study, they can be calculated from $\delta^{13}$C values of dissolved inorganic carbon (DIC) (assuming about a 10‰ fractionation between DIC and CO$_2$(aq) at 10 °C, Mook et al., 1974). During spring to summer there is a decrease in $\delta^{13}$C$_{\text{DIC}}$ values due to the decrease in $pCO_2$ (-7.6‰ in June to -13‰ in August for high closure lakes, Tank, 2009), which would yield corresponding $\delta^{13}$C$_{\text{CO2(aq)}}$ values of ~ -17 to -23‰. However, most methanogens are expected to be productive in winter (see above) when high $pCO_2$ (due to under-ice accumulation following the release by permafrost) is expected and the $\delta^{13}$C$_{\text{CO2(aq)}}$ likely $^{13}$C-enriched compared to summer. Unfortunately, the $\delta^{13}$C$_{\text{CO2(aq)}}$ or $\delta^{13}$C$_{\text{DIC}}$ values of the winter lake water are not known. Methanogenesis commonly occurs in anoxic parts of lake sediments and overlying waters, where the dominant mechanism in freshwater environments is via the acetoclastic pathway (Whiticar et al., 1986). The latter results in a -2 to -6‰ fractionation in the produced lipids, while hydrogenotrophic methanogenesis, which has also been reported in cold environments, results in higher fractionation ($\Delta_{\text{lipid}} = -11$ to -20‰, Londry et al., 2008; Penning et al., 2006; Valentine et al., 2004; Whiticar et al., 1986). Furthermore, methylotrophic methanogenesis would result in an even higher fractionation ($\Delta_{\text{lipid}} = -33$ to -46‰), but is rarely reported in freshwater environments due to the lower availability of these substrates. $^{13}$C-CH$_4$ measurements in pore-waters of surface sediment of high closure
lakes suggest dominant hydrogenotrophic methanogenesis with a minor contribution from the acetoclastic pathway (-80 to -50‰, Geeves, 2019), while 16S rRNA sequencing suggests a larger gene copies of Archaea involved in acetoclastic methanogenesis (Bergstresser, 2018). Furthermore, Bergstresser (2018) reported the presence of Methanosaeta (methanogenic Archaea) in the top sediment (0 – 2 cm) of the Mackenzie delta lakes in winter and early spring and the possibility for aerobic methanogenesis to occur in the water-column of high closure lakes.

Our δ^{13}C_{GDGT-0} values suggest a possible contribution of GDGT-0 by non-methanogenic Archaea but are also compatible with a significant contribution by acetoclastic methanogens. The difference in δ^{13}C_{GDGT-0} values between the lakes likely reflects differences in δ^{13}C_{CO_2(aq)} due to contrasting pCO_2 (higher for MD-1 and high closure lakes in general, Lesack et al., 1998; Squires et al., 2009).

No methanotrophic Archaea were reported by Bergstresser (2018). This might be due to the primer used in the study, but this finding is in agreement with the carbon isotopic composition of GDGT-0 (Fig. 3), which is not significantly ^{13}C-depleted compared to the δ^{13}C_{CH_4}.

Anaerobic methanotrophy is rare in freshwater environments due to low sulfate (i.e., electron acceptor) concentrations. In contrast, Bergstresser (2018) detected large amounts of bacteria known to mediate the aerobic oxidation of methane (i.e., MOB), which are especially abundant during winter. MOB produce hopanoids that are depleted in ^{13}C compared to lipids produced by photosynthetic autotrophs (e.g., sterols, n-alkanes; Pancost et al., 2000). In agreement with this observation, we find large amount of diploptene (17β(H), 21β(H)-hop-22(29)-ene) in MD-1 (high closure) and UD-4 sediments (low closure) with δ^{13}C values of -51.6 ± 2.5‰ and -56.6 ± 1.8‰, respectively, comparable with values reported for Alaskan Arctic thermokarst lakes (Davies et al., 2016). Hence, it seems that the oxidation of methane occurs aerobically and is
primarily mediated by bacteria, but further studies that target the methanotrophic archaea (using specific primers or biomarkers) are needed.

4.1.2. Provenance of brGDGT lipids

BrGDGTs in lake sediments can derive from the watershed, after soil erosion, and from in situ production within the lake water column itself. Elucidating the source of these lipids is necessary before paleoclimate information can be robustly retrieved from their distributions.

In a ternary plot for globally distributed soils and lakes (Fig. 2), the GDGT distribution of Mackenzie lake sediments plot towards cold soils and lakes, similar to that observed in Antarctic lakes (Foster et al., 2016). The dominant brGDGTs are the non-cyclopentane containing pentamethylated as well as hexamethylated 5 and 6 methyl brGDGTs (IIa, IIa’, IIIa and IIIa’, respectively; Fig. 2) which have been suggested to be indicative of in situ production in lakes (Blaga et al., 2009; Tierney et al., 2010; Pearson et al., 2011; Sun et al., 2011; Shanahan et al., 2013; Peterse et al., 2014; Russell et al., 2018). Tetramethylated brGDGTs Ia, Ib and Ic are usually characteristic for soil input (Fig. 2, e.g., Weijers et al., 2007; Zink et al., 2016; Russel et al., 2018). However, soils from cold areas such Alaska and Svalbard) and permafrost soils from Siberia (Kush et al., 2019) have a higher IIIa proportion than temperate and tropical soils (Weijers et al., 2007). One soil from the Mackenzie River delta has been measured (Peterse et al., 2014) and has 42% IIIa + IIIa’, but the analysis has been done with an older protocol without differentiating 6-methyl isomers from 5-methyl isomers. Taking the pH of this soil (6.5) and the associated IR value (0.49, Yang et al., 2015) into account, there is likely equal proportions of IIIa and IIIa’ in this soil sample, which differs from the lake samples (Fig. 2). Furthermore, the 5,6-methyl brGDGT IIIa’’ in UD-4 sediments (low-closure lake, Fig. 2) indicates the presence of in situ production, as this compound was exclusively reported from
lake sediments and water column suspended particulate matter and is always below detection limit in soils (De Jonge et al., 2014b; Weber et al., 2015, 2018; Martin et al., 2019).

The BIT index is consistently above 0.9 for the three lakes (0.99 in soil, Peterse et al., 2014), but these high values in the lakes likely reflect the low crenarchaeol concentration (Fig. 2) rather than an indicator of soil input. The IIIa/IIa ratio (Xiao et al., 2016; Martin et al., 2019) is $1.6 \pm 0.4$ for UD-4, $1.5 \pm 0.2$ for MD-2 and $1.4 \pm 0.3$ for MD-1, which also points toward a lacustrine in situ production (the reported threshold is $>0.9$ for in situ aquatic production, in one soil of the Mackenzie region it is 1.2, Peterse et al., 2014). The Mackenzie River transports large quantities of soil-derived organic matter (Vonk et al., 2019), and is therefore a potential source of soil-derived brGDGTs, especially to the no-closure lakes. However, the distribution of brGDGTs in Mackenzie River SPM differs from that in lakes with IIa (IIa + IIa’) and IIIa (IIIa = 15% and IIIa’ = 19%) as main brGDGTs (34% each, Peterse et al., 2014) and IIIa/IIa equal to $1.0 \pm 0.1$ ($n = 6$; Peterse et al., 2014). Furthermore, as Peterse et al. (2014) noted, the concentration of brGDGTs in the lakes (total brGDGTs $19 – 33 \ \mu g \ g^{-1}_{TOC}$) are significantly higher than in the Mackenzie River (total brGDGTs $0.4 – 3.9 \ \mu g \ g^{-1}_{TOC}$) where soil brGDGTs could originate. In conclusion, the brGDGT distribution indicates a dominant in situ lacustrine production in all studied lakes, irrespective of their degree of closure, and with no apparent changes in provenance downcore.

The production of IIIa” has been previously found to be confined to the anoxic part of the water column of Lake Lugano and Hinterburg, Switzerland (Weber et al., 2015, 2018). It is also reported in sediments from Lake St Front, France (Martin et al., 2019). In the Mackenzie lake sediments it is only found in lake UD-4 (low closure). Mackenzie delta lake bottom waters experience anoxia during the winter season when ice effectively seals the lakes and large amounts of organic matter is respired (Squires et al., 2009). However, the other studied lakes also experience anoxia in winter yet do not show any IIIa” production, suggesting that this
GDGT it is not a specific marker for anoxia. IIIbX, a brGDGT with an unknown structure, has not been reported before and is only present in lake MD-1, whereas IIICX, another brGDGT with unknown structure, is found in lakes UD-4 and MD-2 but has not been reported in other lakes (Fig. 2). As the proportion of IIIb isomers and IIIC isomers increase with increasing pH, and MD-1 has a higher pH than the other lakes, the presence of IIIbX could tentatively be attributed to increased pH. However, this inference, together with the curious presence of IIICX in MD-2 and UD-4, requires more study, in particular through analysis of SPM from the lakes.

The δ13C values of brGDGTs can shed light on the metabolism of brGDGT producers (Naehler et al., 2014; Weber et al., 2015, 2018). The values found in lake UD-4 (low closure) and MD-2 (no closure) are similar and constant over the length of each cores (−29.0 ± 0.2‰ and −28.9 ± 0.2‰, respectively) and also present a systematic 3‰ offset with δ13C_TOC (−26.0 ± 0.4‰ and −26.6 ± 0.4‰, Lattaud et al., submitted) as was observed in lake SPM before by Weber et al. (2018). The ≈ 3‰ difference between the brGDGTs and the TOC is consistent with the heterotrophic metabolism of Acidobacteria (Kielak et al., 2016), with the breakdown and assimilation of particulate organic carbon in the water column and surface sediments. For the upper part of the MD-1 (high closure) core, the δ13C_TOC is significantly higher (−22.8 ± 0.3‰, > 22 cm) while the δ13CbrGDGT is significantly lower (−31.1 ± 0.2‰). In contrast, δ13C_TOC values in the lower part of MD-1 were markedly lower (−27.9± 0.3‰) while the δ13CbrGDGT values were similar to those in the upper part (−32.7 ± 0.3‰). The top part of the sediment core is a mix of labile carbon (macrophyte-derived and MOB-derived), as indicated by younger TOC 14C ages while deeper in the core only the refractory, pre-aged OC remain (mainly terrestrial higher-plant-derived, Lattaud et al., submitted). This suggests that the lacustrine brGDGT-producers consume labile organic carbon such as a mix of MOB-derived and macrophyte-derived. In no-closure and low-closure lakes characterized by macrophyte production (Squires...
and Lesack, 2002) and less MOB-derived OM, terrestrial plant-derived OM may serve as a more important carbon source for brGDGT producers. Hence, the large difference (~ 9‰) between δ$^{13}$C$_{GDGT}$ and δ$^{13}$C$_{TOC}$ in the upper part of the core from MD-1 points toward the heterotrophic consumption by brGDGT producers of labile, $^{13}$C-depleted MOB-derived organic matter instead of more refractory $^{13}$C-enriched compounds. While in MD-2 and UD-4, brGDGT producers are likely feeding (using fermentation) on more refractory terrestrial-plant derived OM due to the depletion of labile compounds.

**Figure 5** Temperature reconstruction using brGDGTs in (a) MD-1 (high closure), (b) MD-2 (no closure) and (c) UD-4 (low closure). Mean summer temperature at Inuvik, GDGT-
reconstructed summer temperature using equation 1 (Russell et al., 2018) and using equation 3 (Russell et al., 2018).

4.2. Environmental reconstruction using brGDGTs

4.2.1. Calculation of GDGT-based temperatures

Several temperature calibrations exist for lakes, many of which are lake-specific (e.g., Foster et al., 2016; Pearson et al., 2011; Sun et al., 2011; Zink et al., 2016), hindering their use in other locations. The Mackenzie delta is characterized by low annual air temperatures (-8.4 ± 1.7 °C average of the last 60 years at Inuvik weather station) but comparably high summer temperatures (11.9 ± 1.4 °C average of the last 60 years at Inuvik weather station), and the lakes of the delta are only free of ice from June (freshet) to late September. A few calibrations have been performed in the high Arctic (Shanahan et al., 2013; Colcord et al., 2015), but none of these are based on the full suite of 15 brGDGTs (see appendix), owing to changes in the analytical protocol. We therefore adopted the same approach as Shanahan et al. (2013), who used a global lake calibration. We use the multivariate regression that Russell et al. (2018) propose ([Eq. 1] RSME 2.14 °C) based on a set of African lake surface sediments. In addition we also calculate the MBT’5ME index as defined by De Jonge et al. (2014b) [Eq. 2] and apply the calibrations calculated by Russell et al. (2018) ([Eq. 3], RSME = 2.43 °C). We interpret the reconstructed temperatures as mean annual summer temperatures since it is likely, although not proven, that the brGDGTs are mainly produced when the region is free of ice (Shanahan et al., 2013).

\[
\text{MAST} = 23.81 - 31.02 \times IIIa - 41.91 \times IIb - 51.59 \times IIb' - 24.70 \times IIa + 68.80 \times \text{Ib}
\]

(1)

\[
\text{MBT'}_{5\text{ME}} = \frac{Ia+Ib+lc}{Ia+Ib+lc+IIa+IIb+IIc+IIIa}
\]

(2)
The absolute reconstructed temperatures using [Eq. 1] are in the range of those recorded in summer at Inuvik during the last 60 years, supporting the validity of this calibration, while those reconstructed by [Eq. 3] are lower (~ 4 °C lower than recorded temperatures, Fig. 5). The reconstructed temperatures using [Eq. 1] and [Eq. 3] present the same pattern for MD-1 and UD-4 but differ in the bottom part of MD-2. The latter could indicate that [Eq. 2] is influenced by parameters other than temperature, such as oxygen concentration and pH changes (see below). Furthermore, we note that MBT'5Me is, on average, increasing with decreasing connectivity to the river (Fig. 6), implying that this ratio is not only controlled by variations in temperature.

In MD-1 there is a slight increase (+1 °C) in MAST over the record [Eq. 1], but UD-4 present more variation than MD-1 which could reflect the slow sedimentation in MD-1 that buffers large changes in temperature. In UD-4 the most recent part of the record seems to capture the yearly variability recorded in the instrumental record [Eq. 1]. This absence of warming reflects the stability of the summer temperatures in the region, and equation [Eq. 1] seems to be better at capturing yearly variability in temperature. Hence, equation [Eq. 1] is recommended in the Mackenzie delta lakes to reconstruct summer air temperature.

4.2.2. BrGDGTs as indicators of lake hydrology and connectivity

The three lakes studied differ with respect to their connectivity to the river, as well as their size and depth. For example, MD-2 (no closure) is the largest and deepest lake, while UD-4 (low closure) and MD-1 (high closure) have a rather small area and are quite shallow (~1 m deep). Due to this variation in connectivity their nutrient content and chemical properties are also expected to be different. The mean annual pH of the lakes is similar but can significantly vary...
over the course of the year, being as high as ~10 in late summer in high closure lakes while remaining at ~7.8 in no closure lakes (similar to the Mackenzie River, Tank et al., 2008, 2009). A principal component analysis (PCA, Fig. S1), performed for each lake using the fractional abundance of all 15 brGDGT compounds, reveals that in MD-2 (no closure lake) and UD-4 (low closure), the principal component 1 (PC1) (explaining 68% and 52% of the variance, respectively) is represented by IIIa’ and IIIa’”, which may reflect anoxic conditions (Weber et al., 2018), while PC2 (explaining 14% and 25% of the variance, respectively) is represented by IIIa which likely reflects a temperature control (as it is included in [Eq. 1] and [Eq. 2]). In MD-1, the main sources of variation differ, with IIa’ and IIIa having a high (and opposite) loading on PC1 (explaining 58% of the variance) and IIIa’ on PC2 (explaining 33% of the variance). Hence, both temperature and oxygen availability seem to exert significant influence on the distribution of brGDGTs in Mackenzie lakes. Weber et al. (2018) observed more brGDGT isomers (6-methyl and 5,6-methyl) as well as a stronger increase in IIIa’ than in IIa’ in the anoxic part of the water column of Lake Lugano. To assess this, the isomer ratios (IR) have been calculated for the GDGT-IIIa and the GDGT-IIa (IR_{IIIa} [Eq. 5] and IR_{IIa} [Eq. 6]):

\[ IR_{IIIa} = \frac{IIIa'}{IIa' + IIIa} \] (5)

\[ IR_{IIa} = \frac{IIa'}{IIa' + IIa} \] (6)

Overall, there is an increase in average downcore IR values with decreased lake connection (significant for IR_{IIa} between all lakes and significant for IR_{IIIa} between NC and HC and LC and HC, t-test < 0.05) with the Mackenzie River (Fig. 6), in agreement with longer anoxic conditions in high closure lakes. This control of the river connectivity also influences the MBT’_{5Me} values, which on average show an increase (significant between LC to HC and NC to HC, t-test < 0.05) with decreasing connectivity (Fig. 6).
In prior studies, brGDGTs have been linked to other environmental parameters than temperature, such as variation in pH (e.g. De Jonge et al., 2014b), with the CBT$_{5Me}$ allowing for the reconstruction of pH in soils and lakes ([Eq. 4] and [Eq. 5]):

\[
C_{BT_{5Me}} = \frac{Ib + IIb}{Ib + IIb + Ia + IIa}
\]  

\[
pH = 7.84 - 1.73 \times C_{BT_{5Me}}
\]

The reconstructed pH in all lakes is similar (6.8 ± 0.1 for MD-2 and UD-4, and 7.0 ± 0.1 for MD-1) and does not vary down core (data not shown). This seems unlikely given the large variability in pH between the lakes. Hence, CBT$_{5Me}$ does not appear to reflect pH variation in the Mackenzie delta lakes and brGDGTs are likely controlled by other environmental parameters such as redox properties but also possibly the trophic state of the lakes.

![Figure 6](image)

**Figure 6** brGDGT ratios in link with the river connection with (a) isomer ratios of GDGT-IIIa and GDGT-IIa and (b) MBT'$_{5Me}$.

5 Conclusion

In a step towards assessment of the effects of recent warming in the Canadian arctic on aquatic ecosystems of the region, and potential feedbacks with respect to greenhouse gas emissions,
we have investigated microbial lipid (GDGT) signatures in sediments from three Mackenzie River delta lakes. The iGDGT compositions are dominated by GDGT-0, a potential marker for the presence of methanogens, in agreement with the quasi absence of crenarchaeol, and corresponding $^{13}$C-signatures are compatible with an acetoclastic methanogenic source along with a significant production by heterotrophic archaea. The abundance and $^{13}$C-depleted signature of diploptene indicates methane consumption via bacterial aerobic oxidation rather than anaerobic oxidation by Archaea. BrGDGT composition reveals a predominant “cold” signature that seems to be dominated by in situ lacustrine production of hexamethylated and pentamethylated brGDGTs, in agreement with the presence of the uncommon IIIa”, IIIb$^X$ and IIIc$^X$ GDGT. Temperature reconstructions using brGDGTs suggest a near stable summer air temperature for the last century, in agreement with recent instrumental records (> 1960 C.E.). A strong control of the lake connectivity on the brGDGT distributions is evident, apparently linked with the oxygen content of the lakes.

**Competing interests**

The authors declare that they have no conflict of interest.

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**Data availability**
Data from this study will be available from the PANGEA repository (doi:).

**Supplement**

**Figure S1:** Principal component analysis of brGDGT on (a) MD-2 (no closure), (b) UD-4 (low closure) and (c) MD-1 (high closure).

**Figure S2:** Carbon stable isotope compositions of brGDGT relatively to the carbon stable isotope compositions of the bulk organic matter (Lattaud et al., submitted). Data from Colcord et al. (2017) are represented for comparison.
References


Heslop, J.K., Walter Anthony, K.M., Sepulveda-Jauregui, A., Martinez-Cruz, K., Bondurant,
A., Grosse, G., Jones, M.C., 2015. Thermokarst lake methanogenesis along a complete
talik profile. Biogeosciences 12, 4317–4331.

Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved
chromatography on GDGT-based palaeoproxies. Organic Geochemistry 93, 1–6.

Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten,
S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and

temperature variations in the Arabian Sea over the last 23 kyr using organic proxies
(TEX$_{86}$ and U$_{37}^K$). Paleoceanography 21. doi:10.1029/2005pa001215

ecology of Acidobacteria: Moving beyond genes and genomes. Frontiers in Microbiology
7, 1–16.

of the Distribution of Component Parts of Polar Ether Lipids in Methanogens. Systematic
and applied microbiology 16, 342–351.

Kusch, S., Winterfeld, M., Mollenhauer, G., Höfle, S.T., Schirrmeister, L., Schwamborn, G.,
Rethemeyer, J., 2019. Glycerol dialkyl glycerol tetraethers (GDGTs) in high latitude
Siberian permafrost: Diversity, environmental controls, and implications for proxy
applications. Organic Geochemistry 136, 103888.

times in the Mackenzie River Delta respectively via two global change mechanisms along
the arctic coast. Geophysical Research Letters 34, 1–6.


Geochemistry 66, 174–181.


Arctic to Antarctic: Renewed potential for the application of GDGT-paleothermometry in lakes. Geochimica et Cosmochimica Acta 75, 6225–6238.


Squires, M.M., Lesack, L.F.W., 2002. Water transparency and nutrients as controls on phytoplankton along a flood-frequency gradient among lakes of the Mackenzie Delta,
western Canadian Arctic. Canadian Journal of Fisheries and Aquatic Sciences 59, 1339–1349.


Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. Organic Geochemistry The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBT 0) in soils from an altitudinal transect at Mount Shennongjia. Organic Geochemistry 82, 42–53.
