Lipids at high latitudes: investigation of sources, environmental controls, and new potential applications of brGDGT-based paleoclimate proxies

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Faculty of Earth Sciences
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Dissertation submitted in partial fulfillment of a *Philosophiae Doctor* degree in Geological Sciences

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Abstract

As high latitude regions continue a decades-long trend of warming at roughly twice the rate of the global average, an understanding of their climatic histories becomes increasingly important for predicting their future. Organic molecular proxies preserved in lake sediment archives offer one avenue for reconstructing key elements of past climates, including their temperature, precipitation, and vegetation regimes. In particular, a class of bacterial membrane-spanning lipids called branched glycerol dialkyl glycerol tetraethers (brGDGTs) form the basis for a paleothermometer that can be applied to reconstruct temperatures as far back as the Cretaceous in sedimentary archives across the globe.

Despite these successes, challenges remain that complicate the development and application of brGDGT-based proxies. First, while they correlate best with temperature and pH, other environmental parameters can influence brGDGT distributions, including seasonality, conductivity, and oxygen availability. Second, it is unknown whether these empirical correlations are the result of a direct physiological response of brGDGT-producing organisms to their environment or an indirect effect resulting from variations in bacterial community composition. Finally, an incomplete understanding of where brGDGTs are produced on the landscape and how they contribute to the sedimentary record hinders our ability to interpret proxies in mixed-source archives.

Herein, I present research addressing each of these three challenges with an emphasis on the Eastern Canadian Arctic and Iceland. First, I develop a technique for grouping brGDGTs based on structural characteristics and show that it can be used to deconvolve the effects of temperature and pH/conductivity. I further find a warm-season bias in brGDGT-derived temperatures and develop calibration equations for temperature and conductivity. Next, I compile >2500 samples from a dozen sample types across the globe and find near-universal trends in the relationships between brGDGTs and temperature, pH, and one another. These commonalities support a physiological basis for observed environmental trends. Finally, by measuring brGDGTs in their intact, polar form, I find that lipid sources in lake catchments can be distinguished and suggest novel applications down core. By advancing our understanding of brGDGTs, my results further our ability to reconstruct key climatic variables from sedimentary archives, especially at high latitudes.
Útdráttur

Lípíð á norðlægum breiddargráðum: rannsókn á uppruna og umhverfisaðstæðum og mögulegrí betingu brGDGT sem nýs veðurvísa í fornloftslagsrannsóknum.

Norðurslóðir hafa á undanförnum áratugum hlýnað um það bil tvöfalt meira en meðaltalið er að jafnaði á heimsvísu, sem gerir rannsóknir og skilning á veðurfarssögu norðurslóða sifellt mikilvægari til að spá fyrir um framtið þeirra. Lifrænar sameindir sem varðveittar eru í seti stöðuvatna bjóða upp á leði til að endurbyggja eða rekja lykilþættir fornloftslag, þar á meðal hitastig, úrkomu og gróðurfar. Flokkur bakteriumnyndandi lípíða, svokölluð „branched glyseról dialkýl glyseról tetraethers“ (brGDGTs) mynda grunn að hitamæli sem hægt er að nota til að endurbyggja hitastig allt aftur til Kritar tímas, til setlögum um allan heim.

Þratt fyrir góðan árangur í rannsóknum á hitanæmi brGDGT skortir enn á almennan skilning á þróun og því notkun á brGDGT sem lifvisi og hitamæli. Í fyrsta lagi, þó að þessi bakteriumnyndandi lípíð tengist best hitastigi og pH, geta aðrar umhverfisbreytur haft áhrif á dreifingu þeirra, þar á meðal árstíðarsveifla, leiðni og súrefnisframboð. Í öðru lagi er ekki vitað hvort fylgni brGDGT og þessara umhverfisþátta eru afleiðing af beinni lifelisfræðilegri svörun brGDGT-framleiðandi lifvera við umhverfi sitt eða öbein áhrif sem stafa af breytileika í samsetningu bakteriusamfélagsins. Einnig skortir fullnægjandi skilning á því hvor í umhverfinu brGDGTs er framleitt og hver þáttur þess er í upphleðslu setlaga, til að unnt sé að túlka það sem öruggan lifvisi/hitamæli í setlögum sem hlaðist hafa upp við breytileg skilyrði og setmyndunarferli.

Í þessari ritgerð er gerð grein fyrir rannsóknum á hverri þessara þriggja áskorana með áherslu á brGDGT í stöðuvötnum og stöðuvatnaseti á Íslandi og norðausturhluta Kanada. Niðurstöður sýna að unnt er að þróa tækni til að flokka brGDGTs út frá byggingareiginleikum og þannig sýna fram á að hægt er að nota þá flokkun til að skilja á milli áhrifa hitastigs og pH/leiðni. Í öðru lagi er sýnt fram á fylgni brGDGT við hitastig hlýrri árstíða (warm-seasons) og kvöðunarjöfnur þróaðar fyrir hitastig og leiðni. Í þriðja lagi eru >2500 sýni af tylíf sýnategunda um allan heim tekin saman og sýnt fram á nær allhliða tengsl brGDGTs og hitastigs, pH og hvors um sig. Þessir sameiginlegu eiginleikar styðja lifelisfræðilegar ástæður fyrir umhverfisþróun bakterianna. Að lokum, með því að mæla brGDGTs í ósnortnu formi, er sýnt fram á að hægt er að greina lípíð uppruna í vatnasvíðum, sem styrkor notkun þeirra sem lifvisi/hitamæla aftur í tíma. Heildarmiðurþróður sýna því fram á, að með auknum skilningar á uppruna og þróun brGDGTs í setlögum, er unnt að draga fram beinar upplýsingar um helstu loftslagsbreytur aftur í tíma, sérstaklega þegar litið er til norðurslóða.
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CHAPTER I: Introduction

1.1 High Latitudes in a Warming World

Recent decades have shown accelerating manifestations of global-scale climate change. Surface temperatures are increasing at a rate that is unprecedented in at least the last 2,000 years (2 ka), with global temperatures in the last decade rising above those of any multi-century period since the last interglacial (LIG; ~125 ka; Fig. 1-1a; IPCC, 2021). Since 1850-1900, the Earth has warmed globally by ~1°C and is on track to warm an additional ~0.5-3.5°C by century’s end (IPCC, 2021). The precise ways in which these rising temperatures manifest in the Earth system has important consequences for human and other life on the planet (IPCC, 2021).
Warming has been particularly pronounced at high northern latitudes, where temperature increases outpace the global average by a factor of two or more (Fig. 1-1b; Ballinger et al., 2020; IPCC, 2021). This magnified warming, known as Arctic amplification, is the result of a complex set of physical mechanisms and feedbacks, including reduced albedo through ice loss and vegetation greening and changes in humidity and cloud formation (Serreze and Barry, 2011; Pithan and Mauritsen, 2014). Snow cover, vegetation, marine and terrestrial ice cover, and precipitation regimes in the Arctic have already displayed significant changes as a result (Box et al., 2019 and references therein). Along with their immediate impacts on humans and wildlife, such changes have the potential to accelerate positive warming feedbacks and affect the climate at lower latitudes (Overland et al., 2016; Post et al., 2019; Cohen et al., 2019).

As the globe begins to diverge from historical analogs, our ability to constrain its possible futures becomes increasingly reliant upon the paleoclimate approach, in which climates of the past are reconstructed to inform our understanding of the climates of the future (Miller et al., 2010a; Tierney et al., 2020). In the Arctic, such efforts have revealed multiple periods of past warmth over the Cenozoic (Miller et al., 2010b). Of these periods, the Holocene Thermal Maximum (HTM, ~8-6 ka) and last interglacial (LIG; ~125 ka) may especially serve as important analogs for the coming century due to their temperature ranges (Fig. 1-1) and relative recency. With an emphasis on these time periods and high latitude regions, I therefore aim herein to refine and develop tools for paleoclimate reconstructions to better inform our understanding of our warming world.

### 1.2 The PACEMAP and ILLUME projects

The work in this thesis represents one component of two collaborative projects aiming to constrain the possible futures of the Arctic by reconstructing their past. The first, Predicting Arctic Change through Ecosystem MoleculAr Proxies (PACEMAP), focuses on the Eastern Canadian Arctic, and especially on Baffin Island. The second, IceLand Landscape
reconstruction with Molecular proxies (ILLUME), is centered in Iceland. PACEMAP and ILLUME target warmer-than-present interglacial periods as analogs for future warmth and rely primarily on biomarker proxies preserved in lake sediment archives. Here, I outline the scope of both projects and my role within them.

### 1.2.1 Study Sites

Two regions that are important for our understanding of high latitude climate change are the Eastern Canadian Arctic (ECA) and Iceland. With low mean annual air temperatures (MAT; Fig. 1-2), substantial late-season snow and ice cover, and tundra ecosystems dipping to some of their southernmost latitudes (Walker et al., 2005), the ECA is a region of cold climate extremes. It is also one of the areas on the planet that is most sensitive to both past (e.g., Brierley et al., 2020; Otto-Bliesner et al., 2021) and modern/future (Fig. 1-1; IPCC, 2021) warming, largely due to the effects of Arctic amplification.

![Mean Annual Temperature (1981-2010)](image)

**Figure 1-2.** Mean annual air temperature (1981-2010) of the Eastern Canadian Arctic. Study sites are shown in red. (Data: NCEP/NCAR North American Regional Reanalysis, NOAA/ESRL Physical Sciences Division).

Though Iceland sits at a comparable latitude, its maritime climate is characterized by warmer temperatures and higher precipitation, in large part due to its position at the northern end of the Atlantic Meridional Overturning Circulation (Wunsch, 1980; Buckley and Marshall, 2016). This major heat advection system buffers winter temperatures and keeps MATs in Iceland above 0°C except at higher altitudes (Fig. 1-3). However, northerly currents bring cold water and occasional sea ice to Iceland’s north coast, counteracting this southerly warmth and placing Iceland in a climatically sensitive region that can provide valuable paleoclimate records.
Recent interglacial periods in these two regions may serve as analogs for future climate and vegetation regimes in the Arctic. The Quaternary temperature histories of both are driven to first order by summer insolation changes in the northern hemisphere associated with periodic fluctuations in the Earth’s orbit (c.f., Miller et al., 2010b). Widespread glaciations in the northern hemisphere were punctuated by interglacial periods of relative warmth roughly every 100 kyr. On Baffin Island, some lake sediment archives from the last and penultimate interglacials (LIG and PIG, ~125ka and ~240ka, respectively) have survived the interceding glacial activity (e.g., Briner et al., 2007). Proxy reconstructions from these sediments have suggested LIG summer temperatures of ~+2-7°C compared to 20th century averages and the northward expansion of shrubs (Crump et al., 2021 and references therein).

While cooler than the LIG, the Holocene Thermal Maximum (HTM; ~8-6 ka) of the present interglacial also saw elevated summer temperatures of ~+1-3°C (Miller et al., 2010b) and expanded vegetation ranges (e.g., Crump et al., 2021, 2019; Geirsdóttir et al., 2020). This early warmth was followed by monotonic cooling and neoglacialization (c.f., Geirsdóttir et al., 2019; McKay et al., 2018; Miller et al., 2005), culminating in the Little Ice Age (LIA; c.f., Mann et al., 2009; Miller et al., 2010b, 2012). In Iceland, landscape instability accompanied this decrease in temperature, with widespread soil erosion preceding human settlement and intensifying during the LIA (Geirsdóttir et al., 2020). The extent to which this shift represents the crossing of a threshold in the climate system versus the effect of human activity remains an important question to address (Geirsdóttir et al., 2020).
1.2.2 Research Approach and Proxies

The warmer-than-present temperatures of the geologically recent HTM, LIG, and PIG make them potential analogs for future Arctic conditions. However, temperature reconstructions alone cannot fully characterize the Arctic system. Increases in temperature are likely to drive observed increases in both rainfall and vegetation greening. These variables can in turn feed back to cause further warming through, for example, decreasing surface albedo, changing snowpack characteristics, and increasing relative humidity (Sturm et al., 2005; Miller et al., 2010a; Serreze and Barry, 2011). Reconstructions of the coupled evolution of temperature, vegetation, and precipitation are therefore key to constraining the possible futures of high latitude regions.

Molecular proxies preserved in lake sediment archives offer one avenue for such reconstructions. Ancient environmental DNA preserved in lake sediments can be used to reconstruct local vegetation communities in the Arctic as far back as the LIG (e.g., Crump et al., 2021, 2019). The isotopic compositions of leaf waxes can provide insight into relative humidity and precipitation amounts and seasonality (e.g., Thomas et al., 2016, 2018). Evidence of human activity can be found in the form of polycyclic aromatic hydrocarbons produced from the burning of organic matter (e.g., D’Anjou et al., 2012) and fecal sterols produced by animals such as sheep (e.g., Bull et al., 2002). Finally, the tetraether lipids of bacterial cell membranes form the basis for a quantitative paleothermometer for reconstructing summer warmth (e.g., Crump et al., 2019; Harning et al., 2020).

The PACEMAP and ILLUME projects aim to reconstruct coupled changes in temperature, vegetation, and precipitation using these proxies in lake sediment archives. Both downcore reconstructions and modern proxy development are key to the success of these projects (Fig. 1-4). Detailed vegetation surveys of modern lake catchments will be used to test the preservation of plant DNA in lake sediments. Trends in modern lake water isotopes will inform the influences of seasonality and evaporation on leaf wax isotopes. Finally, temperature calibrations for tetraether lipids will be refined through the use of in situ recording of air, soil, and lake water temperatures, repeat measurements of lake water chemistry, and exhaustive sampling of study sites. This final goal is the purpose of this thesis.
Figure 1-4: Schematic of key proxies and sampling approaches for the PACEMAP (top) and ILLUME (bottom) projects. (Figures courtesy of Sarah Crump and Áslaug Geirsdóttir).
1.3 The brGDGT Paleothermometer – Applications and Challenges

Branched dialkyl glycerol dialkyl tetraethers (brGDGTs; Fig. 1-5) are a class of membrane-spanning lipids whose distributions are empirically related to environmental parameters such as temperature and pH (Weijers et al., 2007b). The lipids’ prevalence in natural systems (e.g., soil, peat, lacustrine, and marine; see Chapter 4) coupled with a preservation potential on the order of tens of millions of years (Super et al., 2018) has allowed for their application in numerous sedimentary archives across the globe (e.g., Loomis et al., 2017; Lu et al., 2019; Naafs et al., 2018). In high latitude settings, pioneering works reconstructed temperatures of the Paleocene-Eocene thermal maximum from marine sediment archives in the Arctic Ocean (Weijers et al., 2007c). More recently, the brGDGT paleothermometer has been extended to lacustrine settings, with paleotemperature records being generated in, for example, the ECA (Crump et al., 2019), Iceland (Harning et al., 2020), Greenland (Kusch et al., 2019a), and the Russian Arctic (Lindberg et al., 2021).

![brGDGT-I, II, and III structures](image)

**Figure 1-5: Structures of the 15 commonly measured “core” brGDGTs. Top: brGDGT-Ia (black structure only), IIa (with red methyl), and IIIa (with red and blue methyls). Solid and dashed lines indicate the positions of methylations in 5- and 6-methyl isomers, respectively. Middle and lower structures are analogous for mono- and bicyclized compounds.**

Despite these successes, however, at least three major gaps exist in our understanding of brGDGTs that complicate their use in paleoclimate research. First, the precise relationships between brGDGT distributions and temperature, pH, and other environmental parameters are still being understood. Early studies found that the number of methylations on brGDGT hydrocarbon backbones was related to temperature, while the number of cyclopentane rings correlated to pH (Fig. 1-5; Weijers et al., 2007a). These relationships have formed the basis for brGDGT-based paleotemperature and paleo-pH proxies to this day. However, other environmental parameters have been shown to interfere with brGDGT-derived temperatures. In lakes, for example, seasonality (e.g., Shanahan et al., 2013), conductivity/salinity (e.g., Raberg et al., 2021; Wang et al., 2021), dissolved oxygen concentration (e.g., Weber et al., 2018), nutrient availability (e.g., Loomis et al., 2014a), and lake water mixing events (e.g.,
Loomis et al., 2014) have been shown to influence brGDGT distributions. These influences can be difficult to recognize, especially in a paleo record, and few methods exist for applying corrections. For example, some lipid ratios can indicate terrestrial versus marine sources of brGDGTs (e.g., Xiao et al., 2020) and allow for mixed calibrations to be applied (Dearing Crampton-Flood et al., 2018), but such efforts are in their nascent stages.

Next, it is unclear whether the variations in brGDGT distributions with parameters like temperature and pH is a physiological response of source organisms to their environment or an indirect effect resulting from the restructuring of the bacterial community composition. Early works favored the physiological explanation, drawing analogies to similar adjustments observed for other lipid classes (Weijers et al., 2007b). These results were recently supported by molecular dynamics simulations of a brGDGT-based cell membrane (Naafs et al., 2021). However, environmental genomic studies have provided evidence that bacterial community restructuring goes hand-in-hand with changes in brGDGT distributions (e.g., De Jonge et al., 2019; Weber et al., 2018), suggesting that different sets of microbes may produce different sets of brGDGTs. Ultimately, our ability to address this question is hindered by the incomplete identification of brGDGT source organisms, which prevents controlled experimentation in a laboratory setting.

Finally, while the ubiquity of brGDGTs on the landscape is an important advantage, it also poses a challenge as lipids from various sources can become mixed in sedimentary archives. In lake sediments, for example, brGDGTs can be derived from catchment soils (e.g., Peterse et al., 2014), inflowing rivers (e.g., Guo et al., 2020), the lake water column (e.g., Van Bree et al., 2020), and even the sediment itself (e.g., Tierney et al., 2012). As brGDGTs in each of these locations can be expected to record a different set of environmental conditions, deconvoluting their relative contributions becomes an important element of interpreting a brGDGT-based proxy record.

1.4 Scope and goals of this dissertation

The goal of this dissertation is to develop and refine brGDGT-based paleoclimate proxies for use in high latitude lake sediment archives, specifically those collected as part of the PACEMAP and ILLUME projects. To this end, I present four chapters of study. The first, Chapter 2, aims to characterize the PACEMAP and ILLUME study sites through the use of drone surveys, water chemistry measurements, and \textit{in situ} temperature logging. The next three chapters (Ch. 3-5) each address one of the three major challenges in brGDGT research outlined above. In Chapter 3, I propose a novel technique for deconvoluting the influences of temperature and pH/conductivity on brGDGT distributions. I further examine the effects of seasonality on brGDGTs and generate temperature calibrations for use at high latitudes. In Chapter 4, I compile lipid and environmental data from over a dozen globally distributed sample media. I show that near-universal trends exist across these disparate environments and postulate that an underlying physiological response gives rise to these commonalities. In Chapter 5, I examine a rarely studied form of brGDGTs (intact polar brGDGTs) at Lake Qaupat, Baffin Island and other sites in the ECA. I show that these intact lipids may be specific to different sources in the lake catchment (e.g., soil versus lake water) and propose their use for deconvolving brGDGT signals downcore. Finally, in Chapter 6, I summarize the findings of this thesis, identify important remaining challenges, and discuss future directions.
CHAPTER II: Characterization of Study Sites

2.1 Introduction

The PACEMAP and ILLUME projects currently employ 20 Canadian and 28 Iceland study sites, respectively (Table 2-1; Introduction Figs. 1-2 and 1-3). To provide a basic characterization of these sites across the PACEMAP and ILLUME projects, we undertook field campaigns to map the topography, bathymetry, and landscape cover of our lake sites using drone imagery, record in situ air, soil, and lake water temperatures across multiple years, and take repeat measurements of lake water chemistry parameters. Here, I outline our methods, provide basic results, and reference repositories where full datasets are available.
Table 2-1: Study sites of the PACEMAP (Canada) and ILLUME (Iceland) projects.

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<th>Lake</th>
<th>Abbreviation</th>
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<th>Longitude ('W)</th>
<th>Elevation (m asl)</th>
<th>Max Depth (m)</th>
<th>Lake Area (ha)</th>
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* Soil-only site
2.2 Drone Surveys

We used a Mavic Pro drone (DJI) to map our primary study sites for the PACEMAP project. We used the Maps Made Easy app (Drones Made Easy) to autopilot the drone along a gridded flight path that provided maximum coverage of the immediate lake catchment. Geotagged images taken periodically along this flight path were stitched together using the Maps Made Easy online interface (www.mapsmadeeasy.com). Outputs include high-resolution (~6 cm) true color imagery, digital elevation models (DEMs; absolute elevations referenced at drone takeoff points), and 3-dimensional renderings. True color imagery was further used in combination with manual water depth measurements to generate approximate bathymetric maps (Martha Raynolds, unpublished results). Sample results of the drone mapping campaign for Lake Qaupat, Baffin Island are provided in Figure 2-1. Larger true color and bathymetric maps are shown in the Appendix (Section 8.1.1) for all lakes surveyed (QPT, 3LN, BIR, BRO, CF8, AFR). Additionally, a bathymetric map for Lake ARQ is provided based off satellite imagery (Section 8.1.1). Full resolution versions of all files, along with 3D imagery, are provided in a data repository (Section 8.1.2).
Figure 2-1: Sample results of drone mapping campaign. Survey was conducted at Lake Qaupat, Baffin Island on July 22, 2018. A) True color imagery; B) topography; C) approximate bathymetry; D) digital elevation model. Distortions are present in B) and D) due to light scattered from the surface of the lake.

2.3 Water Chemistry Measurements

Lake water chemistry was measured using two methods. First, we used a multiparameter probe (HydroLab HL4, OTT HydroMet) to measure in situ temperature, pH, dissolved oxygen (DO), and electrical conductivity of the water column at 0.5-1 m resolution. These measurements were repeated during each field campaign, resulting in multiple years of summer water chemistry data for many lakes. For some lakes, profiles of the water column were additionally taken through the ice during February (Iceland) or May (Baffin Island) field campaigns. DO measured during the ice-covered season was generally depleted with respect to ice-free conditions. Lakes were on average more acidic in Canada (pH = 5.4 ± 0.1) than Iceland (pH = 6.7 ± 0.1). Sample water chemistry profiles for Lake CF8 are plotted in Figure 2-2 and all data are provided (Section 8.1.2).

![Water chemistry profiles for Lake CF8](image)

Figure 2-2: Sample water chemistry profiles. Water column dissolved oxygen (DO), pH, raw conductivity, and temperature at Lake Clyde Forelands 8 (CF8) during May 2019 (solid line; ice covered), August 2019 (short dashed; ice-free), and August 2017 (long dashed; ice-free).

In addition to these in situ measurements, lake surface (and sometimes bottom or middle) water samples (~0.5 L) were collected for analysis of total phosphorous (TP; acid persulfate digestion), chloride, nitrate, and sulfate (Cl⁻, NO₃⁻, and SO₄²⁻; ion chromatography), and
total/dissolved organic carbon and total nitrogen (TOC/DOC and TN; combustion using a Shimadzu TOC-L/TNM-L analyzer). Analyses were performed at the Arikaree Laboratory (University of Colorado at Boulder). Canadian and Icelandic lake sites displayed similar average values for most species, with slightly higher concentrations of total phosphorous and sulfate in Iceland on average (Fig. 2-3). Birch Lake in Canada contained sulfate concentrations two orders of magnitude higher than most other lakes (109 mg/L). TOC/DOC, TN, and TP values for Canadian and Icelandic lakes were typical for their climate zone, with values generally falling below the global average (Chen et al., 2015).

![Figure 2-3: Summary of water chemistry measurements. Average lake water chloride, nitrate (NO₃⁻), sulfate (SO₄²⁻), total nitrogen, total/dissolved organic carbon (TOC/DOC), and total phosphorous of Canadian and Icelandic lake sites. Birch Lake in Canada was excluded from the sulfate boxplot due to its anomalously high concentrations (109 mg/L).](image)

### 2.4 Temperature Logging

Temperature loggers (Thermochron DS1922L or DS1925L ibuttons, Maxim Integrated Products) were used to record air, soil, and lake water temperatures at 1- to 6-hour intervals and 0.0625°C resolution. Air temperature loggers were positioned beneath heat shields and mounted at 2 m above the ground surface. Soil temperature loggers were double wrapped in plastic bags (Whirlpak), placed inside plastic containers (PolyCon), sealed with electrical tape, and buried at 10 cm depth. Water temperature loggers were similarly double wrapped, placed inside 50 mL containers (Nalgene or Falcon), sealed with electrical tape, and suspended at known depths in the lake.
Air, soil, and water column temperatures are plotted for representative Canadian (Lake CF8; Fig. 2-4) and Icelandic (Lake SVID; Fig. 2-5) sites. Air temperatures at Lake CF8 displayed the high seasonality characteristic of Canadian sites. (Air temperatures were not collected on Iceland due to the abundance of existing data from meteorological stations.) Soils displayed a high degree of both spatial and temporal variability, with soils around the same lake sometimes exhibiting substantially different temperature profiles (e.g., Fig. 2-4). These variations are likely the result of differences in snow and vegetation cover, which insulate or shade soils, decoupling them from air temperatures to various degrees (c.f., Myers-Smith et al., 2011). Water temperatures revealed seasonal water column stratification regimes, overturning events, and the onset and duration of ice cover (e.g., Fig. 2-4).

![Temperature Diagram](image)

**Figure 2-4:** Air, soil, and water column temperatures at a representative Canadian lake site (CF8). Air temperature loggers were positioned at a height of 2 m. Soil loggers were buried at 10 cm depth. Water column loggers were attached to sediment traps (ST) at depths of roughly 7 m (Lower), 4.4 m (Middle) and 2.75 m (Upper) in 8 m of water.
Long-term temperature records from nearby meteorological stations were used to calculate 30-year air temperature normals for all sites. Where air temperature logger data was available (Lakes BIR, BRO, CF8, QPT, and SAL), we estimated local normals by pinning logger data to meteorological records (Fig. 2-6). To achieve this, we first interpolated logger data to hourly resolution. We then calculated the difference between the interpolated and meteorological records (Department of Environment, Government of Canada). Finally, we subtracted the mean monthly differences from the 30-year meteorological station normals (1971-2000) to estimate the site-specific monthly temperature normals. For the remaining Canadian and all Icelandic sites, we generated 30-year climate normals for the roughly same time period (1970-2000) using the WorldClim database (Fick and Hijmans, 2017).

Figure 2-5: Soil and water column temperatures at a representative Icelandic lake site (SVID). Soil loggers were buried at 10 cm depth. Water column loggers were attached to sediment traps (ST) at depths of roughly 18.8 m (Lower) and 2 m (Upper) in 20.8 m of water.
The resulting air temperature normals and 1- to 2-year in situ soil and water column records highlight some of the defining characteristics of the Canadian and Icelandic study sites. Overall, temperature loggers indicated extreme seasonality in Canada, with winters (December/January/February (DJF)) characterized by their long duration (~9 months of ice cover; e.g., Fig. 2-4) and low air temperatures (-26 ± 3 °C; Fig. 2-7) and brief but substantially warmer summers (June/July/August (JJA); 5 ± 2 °C; Fig. 2-7). Air normals in Iceland displayed only slightly warmer summer temperatures (JJA = 8 ± 1°C) but far milder winters (DJF = -2 ± 2°C; Fig. 2-7). These air temperature trends were for the most part mirrored in soil and water column temperatures in both regions (Fig. 2-7). Notably, however, winter soil temperatures in Canada were highly variable (standard deviation = 6°C; Fig. 2-7), while winter lake water temperatures in all locations were buffered at ~0-4°C by ice formation. All data from temperature loggers is available in Section 8-2-2.
Figure 2-7: Summary of temperature logger results. Average temperatures of air (1971-2000 or 1970-2000 average), soil (10 cm depth, single year between 2017 and 2020), and water column (averaged over depths, single year between 2017 and 2020). Soil and water column temperatures were recorded by temperature loggers in situ. Air temperatures were a combination of in situ and modeled measurements.

2.5 Discussion

The data we generated through the use of drone surveys, water chemistry measurements, and in situ temperature logging provided us with a basic, but informative characterization of the PACEMAP and ILLUME study sites. Drone surveys allowed for high resolution mapping of the landscape topography, vegetation cover, and lake bathymetry. Water chemistry measurements revealed (seasonal) lake stratification regimes and nutrient limitations. Temperature logging provided in situ data across highly variable landscapes, markedly reducing the uncertainties associated with extrapolation of temperatures from climate models or meteorological stations. These data improved our understanding of our study sites, aided in interpretation of our results, and guided subsequent field campaigns. They also provide useful baseline measurements for future studies, such as repeat sampling or long-term monitoring campaigns.
CHAPTER III: Revised fractional abundances and warm-season temperatures substantially improve brGDGT calibrations in lake sediments

Abstract. Distributions of branched glycerol dialkyl glycerol tetraethers (brGDGTs) are frequently employed for reconstructing terrestrial paleotemperatures from lake sediment archives. Although brGDGTs are globally ubiquitous, the microbial producers of these membrane lipids remain unknown, precluding a full understanding of the ways in which environmental parameters control their production and distribution. Here, we advance this understanding in three ways. First, we present 43 new high-latitude lake sites characterized by low mean annual air temperatures (MATs) and high seasonality, filling an important gap in the global dataset. Second, we introduce a new approach for analyzing brGDGT data in which compound fractional abundances (FAs) are calculated within structural groups based on methylation number, methylation position, and cyclization number. Finally, we perform linear and nonlinear regressions of the resulting FAs against a suite of environmental parameters in a compiled global lake sediment dataset (n = 182). We find that our approach deconvolves temperature, conductivity, and pH trends in brGDGTs without increasing calibration errors from the standard approach. We also find that it reveals novel patterns in brGDGT distributions and provides a methodology for investigating the biological underpinnings of their structural diversity. Warm-season temperature indices outperformed MAT in our regressions, with Months Above Freezing yielding the highest-performing model (adjusted R^2 = 0.91, RMSE = 1.97°C, n = 182). The natural logarithm of conductivity had the second-strongest relationship to brGDGT distributions (adjusted R^2 = 0.83, RMSE = 0.66, n = 143), notably outperforming pH in our dataset (adjusted R^2 = 0.73, RMSE = 0.57, n = 154) and providing a potential new proxy for paleohydrology applications. We recommend these calibrations for use in lake sediments globally, including at high latitudes, and detail the advantages and disadvantages of each.

3.1 Introduction

Paleotemperature records from lake sediment archives are highly sought after in studies of terrestrial paleoclimate. Bacterial branched glycerol dialkyl glycerol tetraether (brGDGT) lipids have solidified themselves as an important tool in this pursuit (Fig. 8-15; Schouten et al., 2013). First isolated from peat (Sinninghe Damsté et al., 2000), these membrane lipids have since been measured in increasingly diverse settings, from marine, soil, lacustrine, and riverine locations (Hopmans et al., 2004; Weijers et al., 2006; Pearson et al., 2011; De Jonge

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1 A version of this chapter is published:
et al., 2014b, respectively) to hot springs, fossil bones, groundwater, deep ocean trenches, and methane seeps (Li et al., 2014; Dillon et al., 2018; Ding et al., 2018; Xiao et al., 2020; Zhang et al., 2020, respectively). Their ubiquity in nature has given them widespread applicability as environmental proxies; brGDGTs have been used to reconstruct temperature in a variety of archives including lake sediments (e.g. de Wet et al., 2016), marine sediments (e.g. Dearing Crampton-Flood et al., 2018), peat (e.g. Zheng et al., 2017), loess (e.g. Lu et al., 2019), and fossil bone (e.g. Zhao et al., 2020). They have additionally been used to reconstruct lake water pH (e.g. Cao et al., 2017). As the microbial producers of brGDGTs remain elusive (Sinninghe Damsté et al., 2018), these paleoclimate reconstructions currently rely on empirical calibrations at both the regional and global level.

At the heart of brGDGT calibrations is the observation that the degree of alkyl-chain methylation and cyclization are correlated to environmental temperature and pH, respectively. These relationships were first quantified by the Methylation index and Cyclization ratio of Branched Tetraethers (MBT and CBT) in a global soil dataset (Weijers et al., 2007b). The authors proposed physiological explanations for both connections, positing that an increase in methylation number will enhance membrane fluidity, a desirable trait in cold environments, while a greater number of cyclic moieties could improve proton permeability, an advantageous adaptation at high pH. These physiological responses have precedent in other bacterial lipid classes (Reizer et al., 1985; Beales, 2004; Yuk and Marshall, 2004) and appear to function for brGDGTs as well. However, genomic analyses of environmental samples (Weber et al., 2018; De Jonge et al., 2019; Van Bree et al., 2020) have suggested that differences in brGDGT distributions may also stem from shifts in bacterial community composition. Variations in the position of alkyl-chain methylations (Fig. 8-15; De Jonge et al., 2014a) further complicate the picture, with most studies showing 5-methyl brGDGT isomers to correlate better with temperature than their 6-methyl counterparts (e.g. Russell et al., 2018), but others arriving at the opposite result (e.g. Dang et al., 2018). These isomeric variations have additionally been shown to correlate with pH in lake sediments (Dang et al., 2016). These discoveries highlight the multifaceted nature of the empirical relationship between brGDGTs and environmental gradients and the need for further study.

Without a clear mechanistic understanding of the dependencies of brGDGTs on environmental parameters and no brGDGT-producing model organisms currently available for laboratory experimentation, researchers have relied on statistical methods to construct empirical brGDGT calibrations. The majority of recent calibrations have employed a variety of statistical techniques to construct linear or polynomial regressions using brGDGT fractional abundances (FAs; De Jonge et al., 2014a; Martínez-Sosa et al., 2020b; Pérez-Angel et al., 2020). The fractional abundance $f_{x_i}$ of a compound $x_i$ in a set of $n$ compounds is defined as,

$$f_{x_i} = x_i / (x_1 + x_2 + \cdots + x_n) \quad (3 - 1)$$

where any $x$ is the absolute abundance of the given compound in the set. For brGDGTs, these FAs are traditionally calculated using all 15 commonly measured compounds (Fig. 8-15),

$$f_{x_i} = x_i / (Ia + Ib + Ic + IIa + IIb + IIc + IIIa + IIIb + IIIc + IIa' + IIb' + IIc' + IIIa' + IIIb') \quad (3 - 2)$$
where $x_i$ is any given compound in the denominator. By grouping together all 15 common brGDGTs, this approach makes no prior assumptions about the relationships between the compounds themselves and maximizes the degrees of freedom available when exploring a dataset. As relationships between brGDGTs and environment parameters are not yet fully understood, this indiscriminate approach is appropriate. However, by lumping compounds of various types and abundances into the denominator, the approach can also dampen meaningful trends and obscure important relationships, especially for less abundant molecules. This adverse effect has been recognized for other lipid biomarkers and has led to, for example, the exclusion of crenarchaeol from the TEX$_{36}$ index (Schouten et al., 2002) and tetra-unsaturated alkenones from the U$^{13}$C$_37$ index (Prahl and Wakeham, 1987). Numerous ratio-based indices have been developed for brGDGTs that similarly exclude low-abundance (e.g. MBT; Peterse et al., 2012) or problematic (e.g. MBT’5Me; De Jonge et al., 2014a) compounds. However, a selective approach to fractional abundance calculations has been hitherto unexplored.

On the other side of the calibration equations are the environmental variables that are regressed against brGDGT indices and FAs. Mean annual air temperature (MAT) has been the traditional target of brGDGT calibrations in lake sediments (e.g. Tierney et al., 2010; Loomis et al., 2012). It was recognized early on, however, that brGDGT-derived temperatures in cold regions may more accurately reflect warm-season temperatures (Sun et al., 2011; Pearson et al., 2011), an hypothesis that was strongly supported in high-latitude lake sediments (Shanahan et al., 2013; Peterse et al., 2014; Foster et al., 2016). Since the methodological advances that allowed for the separation of 5- and 6-methyl isomers (De Jonge et al., 2014a) and the development of new calibrations, both modern (Hanna et al., 2016; Dang et al., 2018; Cao et al., 2020) and paleo (Super et al., 2018; Thomas et al., 2018; Crump et al., 2019; Harning et al., 2020) studies have continued to support a warm-season bias. Additionally, a recent Bayesian calibration found the mean temperature of Months Above Freezing (MAF) to be the only mode to significantly correlate with brGDGT distributions in a global lake sediment dataset (Martinez-Sosa et al., 2020b). However, as the source organisms of brGDGTs remain incompletely known, other measures of warm-season temperature such as peak warmth or cumulative heat may prove to be more biologically relevant. Furthermore, the nature of the warm-season bias has yet to be tested thoroughly in the regions in which it is most pronounced – namely, those with low MAT and high seasonality. As these are the regions that are currently experiencing the most rapid climate change (Landrum and Holland, 2020), their temperature histories are of high interest (Miller et al., 2010a) and the quantification of the brGDGT warm-season bias is an important target of study.

Outside of temperature, lake water pH is the most common focus of calibration studies (e.g. Russell et al., 2018). However, numerous other variables including conductivity (Tierney et al., 2010; Shanahan et al., 2013), dissolved oxygen (DO; Colcord et al., 2017; Weber et al., 2018; van Bree et al., 2020; Yao et al., 2020), nutrient availability (Loomis et al., 2014a), and lake mixing regime (Loomis et al., 2014b; Van Bree et al., 2020) have been shown to be potentially important controls on brGDGT distributions. Modern calibrations do not currently exist for these environmental variables, largely due to the complexity of the relationships and data limitations.

Uncertainties in the physical sources of brGDGTs on the landscape add an additional challenge for calibration efforts. BrGDGTs produced in soils and rivers can contribute to the lacustrine lipid pool to varying degrees, and the exact proportion of soil- and lake-derived
brGDGTs at a particular site requires detailed study to quantify (e.g. Naeher et al., 2014; Guo et al., 2020; van Bree et al., 2020). Further complicating the matter, multiple sources of brGDGTs have been observed within lakes, including at varying depths (e.g. Yao et al., 2020) and within the sediment itself (e.g. Buckles et al., 2014). The intensive studies required to disentangle these various sources are absent for the vast majority of lakes in most calibration studies, undoubtedly contributing to their reported uncertainties. Furthermore, without a clear understanding of where brGDGTs are being produced, it can be difficult to determine which environmental variables are most appropriate to reconstruct. For example, lacustrine brGDGTs were successfully calibrated to lake water temperature at a site in Greenland (Zhao et al., 2021), but such an effort would likely be less successful for a lake with substantial soil input.

In this study, we aim to improve lake sediment calibrations for brGDGTs in three ways. First, we extend the global calibration dataset to include high-latitude sites by adding surface sediment from 43 lakes in the Eastern Canadian Arctic, Northern Quebec, and Iceland. Second, we selectively group brGDGTs based on their three structural variables – methylation number, methylation position, and cyclization number – and use FAs calculated within these structural sets to investigate isolated relationships between environmental parameters and each structural variable in turn. Finally, we analyze the relationship between the compiled global dataset and MAT, four warm-season temperature indices, pH, conductivity, DO, and lake geometry and generate empirical calibrations for use in lake sediments globally.

### 3.2 Methods

#### 3.2.1 Study sites and sample collection

Surface sediments (0-0.5, 0-1, or 0-2 cm; Ekman box corer or core-top sediment) were collected from 43 lakes (26 from Iceland; 16 from Baffin Island, Arctic Canada; 1 from Northern Quebec; Fig. 3-1 insets) between 2003 and 2019. For 28 of these lakes, water temperature, pH, conductivity, and DO were measured at the time of sampling during the summers of 2017-2020 using a multiparameter probe (HydroLab HL4, OTT HydroMet). These parameters were additionally measured beneath the lake ice in Feb-May of 2018-2020 for 11 lakes. All but one of these lakes experienced depleted bottom water oxygen levels under the ice relative to ice-free conditions. We therefore assume that our ice-free water chemistry profiles do not capture the minimum DO (DO_{min}) levels experienced by our Canadian and Icelandic lakes and exclude them from our analysis of DO_{min}, with the exception of the site from Northern Quebec, which contained a summer oxycline. All other water chemistry parameters were averaged across all depths and seasons before being used in calibrations.
Previously published data from 36 lakes in Central Europe (Weber et al., 2018), 65 lakes in East Africa (Russell et al., 2018), and 38 lakes in China (Dang et al., 2018; Qian et al., 2019; Ning et al., 2019; Cao et al., 2020), were added to the dataset for a total of 182 data points (Fig. 3.1). Average brGDGT FAs were used for lakes with multiple surface sediment samples. Lake surface areas (SAs) were taken from published datasets or estimated using DigitalGlobe imagery. Lake volumes were estimated by approximating each lake basin as a hemiellipsoid (volume \( = \frac{4}{3} \times SA \times \text{maximum depth} \)). Water chemistry parameters were taken from the literature where available or else excluded from our analyses. The number of datapoints for each environmental parameter is available in Table S1. Some parameters were correlated in the dataset, most notably conductivity and pH \((r = 0.75; \text{Table} \ S2)\).

### 3.2.2 Sample extraction and analysis

Roughly 1 g of freeze-dried sediment was extracted using either an accelerated solvent extractor (ASE 200 DIONEX; 10 samples) or a modified Bligh and Dyer (BD; 33 samples) method. We provide a comparison of both extraction methods below. For the ASE method, samples were extracted twice using 9:1 (v:v) dichloromethane:methanol (DCM:MeOH) at 100°C and 2,000 psi. Total lipid extracts (TLEs) were redissolved in 99:1 (v:v) hexane:isopropanol (Hex:IPA) and filtered (0.45 μm, PTFE) before analysis. The remaining 33 samples were extracted using a modified BD procedure (Wörmer et al., 2013). Briefly, sediment was vortexed and sonicated in Mix A (DCM:MeOH:50mM Phosphate buffer (aq., pH 7.4) [1:2:0.8, v:v:v]). The mixture was then centrifuged at 3,000 rpm and 10°C for 10 minutes and the supernatant was collected in a glass separatory funnel. The process was
performed twice with Mix A, twice with Mix B (DCM:MeOH:5% Trichloroacetic acid buffer (aq., pH 2) [1:2:0.8, v:v:v]), and once with Mix C (DCM:MeOH [1:5, v:v]). Equal volumes of HPLC-grade water and DCM were added to induce separation. The organic fraction was collected and dried under a nitrogen stream. The aqueous phase was washed once with DCM and the organic fraction was added to the extract. The TLE was then redissolved in 99:1 (v:v) Hex:IPA and filtered (0.45-μm, PTFE) before analysis.

We analyzed brGDGTs using a Thermo Scientific UltiMate 3000 high-performance liquid chromatography instrument coupled to a Q Exactive Focus Orbitrap-Quadrupole high-resolution mass spectrometer (HPLC-MS) via an atmospheric pressure chemical ionization (APCI). We achieved chromatographic separation using a slightly modified version (Harning et al., 2019; Crump et al., 2019; Pérez-Angel et al., 2020) of the HPLC method described by Hopmans et al. (2016). Due to observed deterioration of chromatography over time, we lowered the initial concentration of eluent B from 18% to 14% to maintain optimal separation of the 5- and 6-methyl isomers. A C46 GDGT internal standard (Huguet et al., 2006) was added to the TLE immediately after extraction and was used to quantify brGDGT yields. Peaks with a signal-to-noise ratio of at least 2:1 were considered for analysis.

### 3.2.3 Comparison of ASE and BD Extraction Methods

To ensure that brGDGT distributions were agnostic to our extraction method, we extracted three surface sediments, two suspended particulate matter (SPM) samples (2.5 L lake water filtered onto 0.3 μm glass fiber filters), and three soils from Baffin Island using the ASE and BD methods in parallel (Fig. S1). The mean difference in MBT$^{5\text{Me}}$ (Eq. A3) between the two extraction methods was 0.006 ± 0.004, or 2 ± 2%. This translates to a MBT$^{5\text{Me}}$-derived temperature difference of 0.2 ± 0.2°C using recent calibrations for soils (Naafs et al., 2017) and lake sediments (Russell et al., 2018), which is well below their respective RMSEs of 5.3°C and 2.14°C (Fig. S2). To test for compound-specific differences, we calculated percent differences in FAs between the two methods. For compounds with FAs > 0.05 in the ASE method, the mean absolute percent difference compared to BD was 4 ± 3%. For the lower abundance compounds (FA ≤ 0.05), this difference was higher (19 ± 17%). No biases in the FA differences were found in either case (difference < standard deviation).

We further extracted the BD sample residue with the ASE method to determine if any brGDGTs remained after BD extraction. On average, we recovered only an additional 0.8 ± 0.6% brGDGTs. These residual brGDGTs had a similar MBT$^{5\text{Me}}$ to that of original BD extract (mean difference = 0.02 ± 0.01, equivalent to 0.5 ± 0.4°C) and were not present in high enough abundances to significantly affect the overall BD distributions (Fig. S2). We therefore conclude that there are no significant differences between samples extracted with the two methods and treat them identically in the analyses that follow.

### 3.2.4 Air Temperatures

Monthly air temperature averages were gathered using the following methods. For nine sites on Baffin Island, one year of in situ two-meter air temperature data from five temperature loggers (one- to four-hour resolution, Thermochron iButtons, Maxim Integrated Products) was converted to a 30-year monthly climate normal (1971 to 2000) using a transfer function to relate local data to nearby meteorological stations (Department of Environment, Government of Canada). For the remaining Canadian sites as well as all sites in Iceland, we
used the WorldClim database (Fick and Hijmans, 2017) to generate 30-year climate normals for the same time period (1970 to 2000). Monthly temperatures for Central European sites were derived using monthly altitudinal lapse rates constructed from climate normals (1970 to 2013) of 148 meteorological stations (Federal Office of Meteorology and Climatology: MeteoSwiss). Monthly temperature data was not available for the East African lakes. However, the seasonality (standard deviation of monthly temperatures) of these lakes is low (0.5 ± 0.2 °C in the WorldClim database, or < 2% of range of the dataset). We therefore approximate all monthly temperatures to be equivalent to MAT for these lakes. Monthly temperature data from all other studies were either published or provided by the authors.

We used the above monthly air temperatures to calculate MAT and four warm-season temperature indices. Three of these indices represent an average temperature for the warmer portion of the year: mean temperature of Months Above Freezing (MAF), Mean Summer Temperature (MST; mean of June, July, and August in the Northern Hemisphere and December, January, and February in the Southern Hemisphere), and mean Warmest Month Temperature (WMT). These indices capture average temperatures for the warm season, but are unaffected by its duration. We therefore additionally calculate the Summer Warm Index (SWI), defined as the cumulative sum of all monthly temperatures above 0°C. This index represents an important control on vegetation patterns at high latitudes and is a useful alternative to the Growing Degree Days Above 0°C (GDD0) index when daily temperature data is not available (e.g. Raynolds et al., 2008). For our five in situ temperature loggers in the Eastern Canadian Arctic for which sub-daily temperature data is available, GDD0 and SWI are highly correlated (R2 = 0.998). We also quantified shoulder season temperatures by calculating averages of the first and last, or first and last two, positive degree months. However, the monthly resolution of the dataset proved too coarse to apply this method robustly.

### 3.2.5 Statistical Methods

To construct calibrations between brGDGT FAs and environmental variables, we used the following method. Each FA was first regressed alone against the environmental variable being investigated. Compounds with a correlation p-value ≥ 0.01 were considered non-significant and removed from further analysis (Pérez-Angel et al., 2020). Fits were then constructed from the remaining compounds using two independent approaches. The first approach was stepwise forward selection/backwards elimination (SFS/SBE) using the MASS package (Venables and Ripley, 2002) in R (Team R Development Core, 2021). This approach finds the best fit by sequentially adding (SFS) or removing (SBE) terms in a generalized linear model and evaluating the resulting fit using the Bayesian Information Criterion (BIC; Schwarz, 1978). The approach is common for constructing brGDGT calibrations (e.g. Dang et al., 2018; Russell et al., 2018), but it is not exhaustive. We therefore additionally used the leaps package (Lumley, 2020) in R to evaluate all possible linear combinations of fitting variables (the “combinatorial” approach, Pérez-Angel et al., 2020). We again used the BIC to select the best fit. For both methods, we imposed the additional criterion that each of the resulting fitting variables must itself be statistically significant (p < 0.01). To help avoid overfitting, we additionally used the adjusted R² to evaluate calibration performances. Some of our variables (conductivity, depth, surface area to depth, and volume) spanned multiple orders of magnitude. We performed regressions against the natural logarithm of these variables, which greatly improved their normality.
We applied this calibration procedure to the FAs of each brGDGT structural set and subset defined below (Sect. 3.3). In the subset-specific calibrations, it was sometimes possible for a single compound to dominate (FA = 1; see section S1.4). These samples were generally clear outliers resulting from the low natural abundances of all other members of the subset and they were therefore removed from the subset-specific calibration models. We additionally tested for linear regressions against a number of previously defined brGDGT indices. A summary of these indices and their definitions is provided in the Appendix (Section 8.2.1).

All correlations reported in the text and figures were significant (p < 0.01) except those marked with an asterisk or with a \( p\)-value provided. All \( R^2 \) values reported in the text and figures are adjusted \( R^2 \).

### 3.3 Partitioning brGDGTs into structural sets for FA calculations

The structure of brGDGTs can vary in three (currently observed) ways: methylation number, methylation position, and cyclization number. These variations result in 15 commonly measured brGDGTs (Fig. 8-15). The standard approach in brGDGT analysis is to calculate fractional abundances using all 15 of these compounds (Eq. 3-2). By mathematically mixing brGDGTs with varying methylation number, methylation position, and cyclization number, however, this approach risks convolutioning the influences of disparate environmental variables. Here, we present a method of grouping brGDGTs that highlights one type of structural variation (e.g. methylation number) while holding one or both of the others constant. By calculating FAs within each group, we aim to deconvolve the influences of temperature, pH, and other environmental variables on the structural variations of brGDGTs.
Figure 3-2: Schematic of the basic (a-c) and combined (d-g) brGDGT structural sets. Fractional abundances are calculated within each boxed group independently (Eq. (3-3 to 3-9) and Table 8-1). Schematic structures highlight the defining alkyl-chain moieties, with cyclopentane rings filled in for emphasis and C6 methylations denoted in red. Complete structures are available in Fig. 8-14.

To explore how changes in methylation number alone relate to environmental parameters, we constructed the Methylation (Meth) set (Fig. 3-2a). This set was generated by grouping brGDGTs with the same number of cyclopentane rings and the same methylation positions. Fractional abundances calculated within the Meth set solely reflect changes in methylation number, while ring number and isomer designation are held constant. Within the Meth set, we defined the Meth-5Me and Meth-6Me subsets as those that contained only 5- and 6-methyl lipids, respectively. As the tetramethylated brGDGTs (Ia, Ib, and Ic) are neither 5-methyl nor 6-methyl isomers, we generated versions of these two subsets that excluded (Meth-5Me, Meth-6Me) and included (Meth-5Me+, Meth-6Me+) these compounds (Fig. S3a-d). We found that the correlation between 5-methyl isomers and temperature was significantly improved when the tetramethylated compounds were included in their FA calculations, while the opposite was true for the 6-methyl compounds (see discussion in Sect. 3.4.2.2). We therefore grouped the tetramethylated compounds with the 5-methyl
compounds (and not with the 6-methyl compounds) when generating the Meth set. FAs for the Meth set were calculated using Eq. (3-3),

\[
f_{xy}^{\text{Meth}} = \frac{xy}{\sum_{n=1}^{III} ny}; \quad f_{xy}^{\prime} = \frac{xy^\prime}{\sum_{n=1}^{III} ny^\prime}
\]

where \( f_{xy} \) and \( xy \) are the fractional and absolute abundances of the brGDGT with Roman numeral \( x \) (I, II, or III) and alphabet letter \( y \) (a, b, or c) and tetramethylated compounds are grouped with 5-methyl isomers.

We next defined the Cyclization (Cyc) set to examine the relationship of brGDGT ring number with environmental variables (Fig. 3-2b). The Cyc set was formed by grouping brGDGTs with the same number and position of methylations. With these variables held constant, variations in the Cyc FAs reflect only variations in the number of cyclopentane moieties. We defined the Cyc-5Me and Cyc-6Me subsets as those containing only 5- and 6-methyl isomers, respectively (Fig. S3e-f). FAs for the Cyc set were calculated using Eq. (3-4),

\[
f_{xy}^{(c)}_{\text{cyc}} = \frac{xy^{(c)}}{\sum_{m=a}^{c} xm^{(c)}}
\]

where \( f_{xy} \) and \( xy \) are the fractional and absolute abundances of the 5- or 6-methyl brGDGT with Roman numeral \( x \) (I, II, or III) and alphabet letter \( y \) (a, b, or c).

Third, we defined the Isomer (Isom) set to isolate changes in the relative abundances of brGDGT isomers (Fig. 3-2c). The Isom set was constructed by grouping brGDGTs with the same number of methylations and cyclizations. Its FAs are solely a measure of the relative abundances of 5- and 6-methyl isomers, without the convoluting influence of ring or methylation number variations. The isomeric diversity of brGDGTs is large, however, and there are structural variations that are not controlled for within this set. For example, hexamethylated brGDGTs have two isomers: one with methylations on different alkyl chains (e.g. at C5 and C5’) and another with methylations on the same chain (e.g. C5 and C24; De Jonge et al., 2013). As these compounds coelute, they cannot be treated independently at this time. Additionally, it is unclear whether brGDGT-IIIa” (Weber et al., 2015), which contains both a C5 and a C6’ methylation, should be grouped with 5- or 6-methyl brGDGTs, or whether the three recently-identified 7-methyl brGDGTs (Ding et al., 2016) should be included in the Isom series. As these compounds are rarely reported, we excluded them from our analysis here, but suggest the possibility of expanding the Isom set to include them in the future should more data become available. The Isom FAs were calculated using Eq. (3-5),

\[
f_{xy}^{(c)}_{\text{isom}} = \frac{xy^{(c)}}{\sum_{\text{isomers}} xy}
\]

where \( f_{xy} \) and \( xy \) are the fractional and absolute abundances of the 5- or 6-methyl brGDGT with Roman numeral \( x \) (I, II, or III) and alphabet letter \( y \) (a, b, or c) and “isomers” refers to 5- and 6-methyl brGDGTs. The Isom set contained groups of two compounds each, making their FAs redundant. We therefore used only the 6-methyl FAs in our analysis.
The Meth, Cyc, and Isom sets each allow only one structural component of brGDGTs to vary. It is possible, however, that two structural alterations occur in tandem in response to the same environmental variable. We therefore defined three additional sets that hold one variable constant while allowing the other two to vary. The first is the Meth-Isom (MI) combination set (Fig. 3-2d). In this set, brGDGTs with the same ring number are grouped together, while both methylation number and position are allowed to vary. The Cyc-Isom (CI; Fig. 3-2e) is analogously constructed by holding methylation number constant, while the Meth-Cyc (MC; Fig. 3-2f) set holds methylation position constant (again treating tetramethylated brGDGTs as 5-methyl compounds, Fig. S3g-j). Finally, we defined the Full set (Fig. 3-2g), which takes the standard approach of allowing all three structural characteristics to vary freely by grouping all 15 commonly measured brGDGTs together.

The FAs of the combined sets are calculated using Eqs. (3-6 to 3-9),

$$f_{xy}^{(t)}_{MI} = \frac{xy^{(t)}}{\sum_{isomers} \sum_{n=1}^{III} ny}$$  \hspace{1cm} (3-6)

$$f_{xy}^{(t)}_{CI} = \frac{xy^{(t)}}{\sum_{isomers} \sum_{m=a}^{c} xm}$$  \hspace{1cm} (3-7)

$$f_{xy}^{(t)}_{MC} = \frac{xy}{\sum_{n=1}^{III} \sum_{m=a}^{c} nm}; \quad f_{xy}^{(t)}_{MCI} = \frac{xy}{\sum_{n=1}^{III} \sum_{m=a}^{c} nm}$$  \hspace{1cm} (3-8)

$$f_{xy}^{(t)}_{Full} = \frac{xy^{(t)}}{\sum_{isomers} \sum_{n=1}^{III} \sum_{m=a}^{c} nm}$$  \hspace{1cm} (3-9)

where $f_{xy}$ and $xy$ are the fractional and absolute abundances of the 5- or 6-methyl brGDGT with Roman numeral $x$ (I, II, or III) and alphabet letter $y$ (a, b, or c) and tetramethylated compounds are treated as 5-methyl isomers. An expanded guide to FA calculations is provided in Table 8-1.

As a proof of concept, we show that the fractional abundance of just one compound, brGDGT-Ia, can be calculated within different structural sets to provide either a strong temperature or pH correlation, without a strong cross-correlation. When the standard FA is calculated using all 15 compounds ($f_{Ia_{Full}}$), a moderate correlation is found with MAF ($R^2 = 0.61$) and none with pH ($R^2 = 0.04$, $p = 0.014$; Fig. 3-3). When cyclization is held constant by using the MI set, correlation with MAF increases ($R^2 = 0.75$) while that with pH remains uncorrelated ($R^2 = 0.13$). The temperature correlation increases further when isomer designation is controlled for as well ($f_{Ia_{Meth-5Me+}}$, $R^2 = 0.88$), with an $R^2$ nearly matching that of MBT$^{5Me}$ ($R^2 = 0.89$) and the pH correlation remaining low ($R^2 = 0.27$). In contrast, the correlation with temperature disappears for the analogous 6-methyl subset ($f_{Ia_{Meth-6Me+}}$, $R^2 = 0.08$). Finally, allowing only ring number to vary ($f_{Ia_{Cyc}}$) effectively erases the correlation with temperature ($R^2 = 0.18$) and instead provides a correlation with pH that, while modest, is already higher than any reported for a lake sediment calibration to date ($R^2 = 0.59$).
Figure 3-3: Adjusted R² values for a linear regression of environmental parameters against the fractional abundance (FA) of brGDGT-Ia calculated within different structural sets. Colors denote the strengths of the relationships, from the minimum to the maximum observed coefficients of determination, with white being the median of the dataset. “Compounds” denote all brGDGTs used in the FA calculation. All values are significant (p < 0.01) unless marked with an asterisk.

### 3.4 Results and Discussion

#### 3.4.1 Distributions of brGDGTs in Icelandic and Canadian lake sediments

The new Icelandic and Canadian lake sediments bolster the global dataset on the cold end, extending the lowest MAT from -0.2°C to -18°C and containing 28 of the 30 coldest samples by MAT. The low temperatures are reflected in the brGDGT distributions of these sediments (Fig. 3-4), which contain on average a higher fIIIa<sub>Full</sub> and lower fIa<sub>Full</sub> than the other samples in this dataset. Additionally, the Canadian and Icelandic datasets provide important end-member samples, including those with the highest fIIIa<sub>Full</sub>, fIIb<sub>Full</sub>, and fIIc<sub>Full</sub> and the lowest or second-lowest fIa<sub>Full</sub>, fIb<sub>Full</sub>, and fIc<sub>Full</sub>. The new samples also contain some of the lowest fractional abundances of 6-methyl isomers, providing low end-member values for fIa<sub>Full</sub>, fIIb<sub>Full</sub>, fIIc<sub>Full</sub> and below-average values for the hexamethylated 6-methyl brGDGTs.
Figure 3-4: Average fractional abundances (calculated within the standard Full set) of new samples from Canada and Iceland as compared to the average of the other samples in this study (Others).

3.4.2 Temperature relationships with brGDGTs

In this section, we show that two adjustments can be made to significantly improve the correlations between brGDGT FAs and temperature in lake sediments: 1) replace MAT with a warm-season temperature index and 2) use FAs calculated within the Meth structural set. The effect of these two adjustments on one representative compound, brGDGT-Ia, is shown from left to right in Fig. 3-5. The relationship between temperature and fla is improved from a weak but significant correlation with a large error ($R^2 = 0.49$; RMSE = 7.00°C) to a stronger correlation with a smaller error ($R^2 = 0.88$; RMSE = 2.42°C). The two adjustments are detailed in the following sections.

![Figure 3-5: From left to right, the effects of substituting the Months Above Freezing (MAF) warm-season index for Mean Annual Temperature (MAT) and flaMeth for flaFull on the relationship between the fractional abundance of brGDGT-Ia and temperature.](image)

Warm-season temperatures outperform MAT

To assess the possibility of a warm-season bias in brGDGT temperature reconstructions, we tested the relationships between brGDGTs and five air temperature indices: MAT, MAF, MST, WMT, and SWI (Sect. 3.2.4). The correlation between these variables and the FA of one representative compound, flaMeth, is shown in Fig. 3-6. In all cases, substituting MAT with a warm-season temperature variable draws samples with strong seasonality into the main body of data. MAF performs best for brGDGT-Ia ($R^2 = 0.88$), with high-seasonality lakes falling progressively out of alignment for SWI ($R^2 = 0.84$), MST ($R^2 = 0.64$), and WMT ($R^2 = 0.60$). This result was upheld when performing temperature calibrations; MAF outperformed all other measures of temperature examined in this study (Sect. 3.4.4.1).
A possible explanation for the success of the MAF temperature index is that the activity of brGDGT-producing microbes may be heavily depressed under lake ice and/or in frozen soils (Pearson et al., 2011; Shanahan et al., 2013; Peterse et al., 2014; Cao et al., 2020). However, studies employing sub-seasonal sampling of sediment traps and suspended particulate matter in two mid-latitude lakes have shown that brGDGTs are produced within the water column throughout the year, despite the presence of ice cover (Woltering et al., 2012; Loomis et al., 2014b). These and other studies employing similar sampling techniques (Hu et al., 2016; Miller et al., 2018; Weber et al., 2018; Van Bree et al., 2020) have additionally found production of brGDGTs to be dependent on the degree and timing of lake mixing versus stratification. Though heightened biological activity may still be the underlying driver of the observed warm-season bias, these depth- and time-resolved studies paint a complex picture of brGDGT production in lakes that precludes a simple explanation. Unfortunately, as knowledge of the timing, extent, and temperature of ice cover and mixing events is lacking for the vast majority of lakes in this study, these effects cannot be tested here. We therefore stress the empirical nature of our MAF calibrations and the need for further study.

Temperature and the Methylation set
The Methylation set provided the strongest relationships between brGDGT FAs and MAF (Fig. 3-7). This set both strengthened existing correlations (e.g. fIIaMeth, Fig. 3-7a) compared to the standard approach (Full set, R² values in parentheses in Fig. 3-7) and generated new ones (e.g. fIIbMeth, Fig. 3-7e). Furthermore, many relationships between FAs and temperature were revealed to be qualitatively similar regardless of ring number. For example, the FAs of all tetramethylated compounds (fIIaMeth, fIIbMeth, fIIcMeth) had a strong positive linear relationship with temperature (Fig. 3-7a-c), while those of the 5-methyl pentamethylated compounds all had a noisier negative relationship (Fig. 3-7d-f). The hexamethylated FA trends were less clear in part due to their lower abundances, but all were negatively correlated with temperature and nonlinearities were apparent (Fig. 3-7g-l). These analogous trends show that the number of methylations responds similarly to temperature regardless of the number of cyclopentane rings. Within a paleoclimate lens, this observation opens up the possibility of independent temperature calibrations for un-, mono-, and bicyclized brGDGTs (Sect. 3.4.4.1). From a biological standpoint, it could imply that methylation and cyclization play their biological roles independently, as the former appears to vary more or less freely of the latter.
Figure 3-7: Relationships between the average air temperature of months above freezing (MAF) and brGDGT FAs calculated within the Meth set. $R^2$ values are provided for each subplot, with $R^2$ values for the standard Full FAs given in parentheses for comparison. $P$-values were < 0.01 except where marked with an asterisk. Note that plots of IIa’, IIb’, and IIc’ are redundant because they exactly mirror those of IIIa’, IIIb’, and IIIc’, respectively, and are therefore not shown.

The brGDGT temperature response appears to be agnostic to methylation position as well (Fig. 3-7g-i versus j-l), but only when the tetramethylated brGDGTs (Ia, Ib, and Ic) are excluded from 6-methyl FA calculations. The Meth-5Me+ subset (Fig. S3a) showed strong relationships between 5-methyl brGDGTs and MAF ($R^2 \leq 0.88$; Fig. S4). On the other hand, the analogous Meth-6Me+ subset (Fig. S3c) was broadly uncorrelated with MAF ($R^2 \leq 0.29$; Fig. S5). At present, there is no known mechanism whereby an additional methylation at the C5 position would have an influence on membrane physiology in a way that a methylation at C6 would not (though the magnitude of the effect may be stronger closer to the less fluid membrane surface; see discussion in Section S3). At first glance, then, the markedly different responses of the Meth-5Me+ and Meth-6Me+ subsets to changes in temperature do not appear to support a physiological basis for the empirical relationship between temperature and brGDGT methylation number. However, when tetramethylated brGDGTs were excluded from the FA calculations for these compounds (Meth-5Me and Meth-6Me subsets; Figs. S3b and d), statistically significant and qualitatively similar temperature relationships did become visible for both isomer types (Figs. S6-7). An analogous result was found for the MC set (Figs. S3g-j and S8-11). It is not clear at this time why the inclusion of tetramethylated brGDGTs improved temperature correlations for 5-methyl compounds but weakened them for 6-methyl compounds. The discrepancy may imply one or a combination of the following: 1) the isomers are produced by different organisms; 2) the isomers serve distinct biological functions, either in addition to or apart from a temperature response; or 3) the currently measured tetramethylated brGDGTs (Ia, Ib, and Ic) are not the precursors of 6-methyl brGDGTs. Regardless, this result allows us to combine the higher-performing Meth-5Me+ and Meth-6Me subsets to generate the Meth set (Fig. 3-2a), which maximizes the temperature responses of all 15 commonly measured brGDGT (Fig. 3-7).

While the Meth set highlights the relationships between brGDGT FAs and temperature, it simultaneously weakens those with other environmental variables. FAs calculated in the standard Full set contain conductivity and pH dependencies ($R^2 \leq 0.66$ and 0.50, respectively; Figs S25 and S32) that are greatly reduced in the Meth set ($R^2 \leq 0.40$ and 0.28, respectively; Figs S19 and S26). This is evidence that many of the conductivity and pH relationships visible in the Full FAs are in fact due to the mathematical mixing of brGDGTs with different cyclization numbers and isomer designations. Holding these variations constant in the Meth FAs largely removes the effects of these environmental variables (e.g. fIIaMeth in Fig. S19d). DO dependencies are weak in both the Full and Meth sets, but slightly weaker in the latter ($R^2 \leq 0.40$ and 0.35, respectively, Figs S39 and S33). The Methylation set thus improves compound-specific correlations with temperature while decreasing their dependencies on other environmental variables.
3.4.3 Conductivity and pH relationships with brGDGTs

While pH is the traditional secondary target of brGDGT calibrations after temperature, numerous works have suggested that conductivity plays an important role in controlling brGDGT distributions (Tierney et al., 2010; Shanahan et al., 2013). Conductivity and pH often plot nearly colinearly in principal component analyses (Shanahan et al., 2013; Russell et al., 2018; Dang et al., 2018) and were moderately correlated \((r = 0.75)\) in this dataset (Table S2). This correlation is not surprising, as pH will affect conductivity both directly (through the concentration of \(H_3O^+/OH^-\)) and indirectly (by altering the solubility of various ions). Furthermore, both pH and conductivity can be affected by the same factors, such as catchment lithology and precipitation chemistry (cf. Wetzel, 2001). BrGDGT FAs in this study showed similar relationships with pH and conductivity, but also displayed potentially important differences. We therefore treat them separately in our analyses but discuss them together in this section.

The Isomer set and conductivity

Conductivity provided the strongest compound-specific correlations with brGDGT FAs after temperature (Figs. 3-8 and S23). Of the basic brGDGT sets (Meth, Cyc, and Isom), the Isom set had the highest statistical performance (Fig. 3-8). The FAs of all 6-methyl brGDGTs showed a positive linear correlation with conductivity in this set, with coefficients of determination as high as \(R^2 = 0.70\) (Fig. 3-8d). Furthermore, this positive correlation was broadly independent of both methylation number and cyclization number, indicating that methylation position varies with conductivity irrespective of other structural properties. The 6-methyl brGDGTs were also all positively correlated with pH, but more weakly so \((R^2 \leq 0.54,\) Fig. S28). A relationship between brGDGT isomers and pH in lake sediments has been previously observed and quantified by the isomerization of branched tetraethers (IBT, Eq. A14; Ding et al., 2015) and the Isomer Ratio of 6-methyl isomers (IR\(_{6Me}\), Eq. A8; Dang et al., 2016). However, these indices were more closely tied to conductivity (IBT \(R^2 = 0.65,\) IR\(_{6Me}\) \(R^2 = 0.66\)) than pH (IBT \(R^2 = 0.55,\) IR\(_{6Me}\) \(R^2 = 0.49\)) in our dataset. These results indicate that isomer abundances are primarily dependent on conductivity, but have some relation to pH as well. Temperature correlations were also present in the Isom subset \((R^2 \leq 0.57\) with MAF, Fig. S14). Further study is needed to elucidate whether temperature acts as a secondary control on isomer abundances or whether these correlations are simply due to the inherent connection between MAF and conductivity in our dataset \((r = 0.70,\) Table S1). DO provided little to no correlation in the Isom set \((R^2 \leq 0.38,\) Fig. S35).
Figure 3-8: Relationships between the natural logarithm of conductivity and brGDGT FAs calculated within the Isom set. $R^2$ values are provided for each subplot, with $R^2$ values for the standard Full FAs given in parentheses for comparison. P-values were < 0.01 except where marked with an asterisk. Note that plots of 5-methyl compounds are redundant because they exactly mirror those of their 6-methyl counterparts; only the 6-methyl FAs are shown.

The Cyclization set and pH

The Cyclization set highlighted the relationship between brGDGTs and pH. As pH increased, all cyclized brGDGTs were found in greater relative abundance (Fig. 3-9). This result reinforces previous observations that higher ring number is associated with higher pH, as has been quantified by the CBT (Weijers et al., 2007b), #rings$_{tetra}$ (Sinninghe Damsté, 2016), Degree of Cyclization (DC; Baxter et al., 2019), and related indices (see Appendix (Section 8.2.1)). However, this pH relationship has thus far been demonstrated primarily in soils; the single-compound FAs of the Cyc subset already provide the strongest pH correlations ($R^2 = 0.61$, Fig. 3-9b) yet reported in lake sediments. The Cyc set also reveals that compounds with structural similarities exhibit analogous responses to pH. For example, all monocyclized brGDGTs show a nonlinear increase with pH (Fig. 3-9b, e, h, k, n), while unicycled brGDGTs all exhibit a nonlinear decrease (Fig. 3-9a, d, g, j, m). These trends are apparent regardless of methylation number or methylation position, suggesting that ring number is broadly independent of both. This independence may imply that alkyl-chain
cyclization serves its biological function(s) regardless of the number and position of methylations present. It also allows for the construction of independent pH calibrations for tetra-, penta-, and hexamethylated brGDGTs (Sect. 3.4.4.2).

![Figure 3-9: Relationships between pH and brGDGT FAs calculated within the Cyc set. $R^2$ values are provided for each subplot, with $R^2$ values for the standard Full FAs given in parentheses for comparison. P-values were < 0.01 except where marked with an asterisk.](image)
Though the Cyc set FAs were most strongly correlated with pH ($R^2 \leq 0.61$), they also exhibited robust relationships with conductivity ($R^2 \leq 0.57$; Fig. S20). All cyclized compounds showed positive correlations with conductivity, and this increase was largely independent of methylation number or position. These results indicate that ring number is primarily dependent on pH, but is correlated with conductivity in a similar manner. The brGDGT indices showed an analogous result; all cyclization indices (CBT and related indices, #ringStetra and related indices, and DC; see Appendix (Section 8.2.1)) correlated most strongly with pH, but also exhibited weaker relationships with conductivity. The Cyc set exhibited little to no correlation with either temperature ($R^2 \leq 0.25$) or DO ($R^2 \leq 0.08$), suggesting that neither of these environmental variables plays an important role in controlling brGDGT cyclization.

The Combined Cyclization-Isomer set strengthens both conductivity and pH trends

Though the strongest conductivity trends were displayed by the Isom set ($R^2 \leq 0.70$), correlations were also present in the Cyc FAs ($R^2 \leq 0.57$, Fig. S20). Similarly, the Cyc set contained the highest pH dependencies ($R^2 \leq 0.61$), but notable relationships were visible in the Isom set as well ($R^2 \leq 0.54$, Fig. S28). To take advantage of all of these conductivity and pH relationships, we therefore used the combined Cyc-Isom set, which holds only methylation number constant while allowing both cyclization number and methylation position to vary (Fig. 3-2). Both conductivity and pH trends were strengthened in this combination set ($R^2 \leq 0.73$ and $0.62$, Fig. S23 and S30, respectively), especially for the uncyclized compounds. However, temperature correlations were also increased in the CI set ($R^2 \leq 0.60$, Fig. S16), a potentially convoluting influence that may not be desired.

### 3.4.4 Calibrations

For each set, combined set, and subset defined in Sect. 3.3, we performed linear and quadratic regressions against temperature, conductivity, pH, dissolved oxygen, and lake geometry variables using SFS/SBE and combinatoric fitting methods. We found temperature and conductivity to provide the strongest empirical calibrations with brGDGTs, followed by pH and dissolved oxygen, and discuss our recommended calibrations below.

**Temperature Calibrations**

For each structural set and subset defined in Section 3.3, we performed regressions of subset-specific brGDGT FAs against five temperature variables to generate multiple global-scale calibrations. Of the temperature indices that we tested, MAF provided the fit with the highest statistical significance ($R^2 = 0.91$), followed closely by MST ($R^2 = 0.90$), SWI ($R^2 = 0.89$), and WMT ($R^2 = 0.88$). MAT provided calibrations with high statistical performance as well ($R^2 \leq 0.87$). However, these fits showed clear seasonality biases in their residuals, resulting in substantial over-estimations of MAT for cold sites (Fig. S40). Furthermore, they often relied heavily on low-abundance compounds as fitting variables (IIc, IIb’, IIIc, IIIc’, IIIb, and IIIb’). We therefore do not recommend a brGDGT MAT calibration and focus our discussion on the warm-season temperature indices, especially MAF. We note, however, that MAF is equal to MAT in warmer climates or lower latitudes and that MAF calibrations may be interpreted as reconstructing MAT in these cases.
Methylation number was the single most important structural variable for temperature calibrations. The Meth set, which allowed only methylation number to vary, provided a MAF calibration ($R^2 = 0.90$, Fig. 3-10b) that was on par with other recent global and regional lake sediment calibrations ($R^2 = 0.85$ to 0.94; Dang et al., 2018; Russell et al., 2018; Martinez-Sosa et al., 2020b). The MI, MC, and Full sets, which additionally allowed for changes in cyclization number and/or methylation position, added little to the calibration performance ($R^2 = 0.90$ to 0.91, Fig. 3-10a). Furthermore, sets which held methylation number constant – Cyc, Isom, and CI – performed markedly worse ($R^2 = 0.51$, 0.63, and 0.67, respectively, Fig. 3-10a). Analogous results were found using RMSE to compare fit performance (Fig. S41a). These results indicate that effectively all of the temperature dependence of the 15 commonly measured brGDGTs is captured by methylation number alone.

Figure 3-10: Summary of temperature calibrations. A) Performance (adjusted $R^2$) of all linear and quadratic fits for the mean air temperature of months above freezing (MAF) and brGDGT fractional abundances (FAs) calculated within the basic (Meth, Cyc, Isom; left of dashed line) and combined (Meth-Isom (MI), Cyc-Isom (CI), Meth-Cyc (MC), and Full sets; right of dashed line) structural sets. Results of both the SFS/SBE and combinatoric fitting methods are plotted. The fit we suggest for general use (Meth set, quadratic, SFS/SBE; Eq. 3-10) is bolded and marked with an asterisk in a) and plotted in b). “Est. MAF” is the MAF temperature estimated using this suggested fit.

Within the Meth set, independent calibrations were generated for the Meth, Methb, and Methc subsets as well. These subsets consist of only un-, mono-, and bicycized compounds, respectively (Fig. 3-2a). As the FAs of these subsets were calculated independently, we were able to test for temperature calibrations of each subset alone. The Meth subset provided the strongest of the subset MAF fits ($R^2 = 0.88$). This calibration has the notable advantage of employing only the 3 most abundant brGDGTs typically found in nature, Ia, Ila, and IIIa, which may allow for temperatures to be reproduced with high fidelity from even organic-lean samples. Furthermore, since it uses only non-cyclized, 5-methyl brGDGTs, it may be...
less subject to influence by the environmental factors that impact cyclization numbers and isomer ratios. MAF calibrations were also obtained using only the monocyclized (Methb, \( R^2 = 0.79 \)) and bicyclized (Methc, \( R^2 = 0.74 \)) brGDGTs. These fits represent, to our knowledge, the first calibrations that make use of only cyclized brGDGTs. This is a noteworthy result as it shows conclusively what can be seen by eye in Fig. 3-7; the relationship between temperature and methylations is a broad feature of brGDGTs that is present regardless of the number of rings on the carbon backbone. These un-, mono-, and bicyclized calibrations may find use in the case that one or more brGDGTs are suspected to be influenced by variables other than temperature.

Of the brGDGT temperature indices that we tested (see Appendix (Section 8.2.1)), MBT’\textsuperscript{5Me} performed best. This index correlated better with MAF (\( R^2 = 0.89 \)) than any other warm-season variable (SWI \( R^2 = 0.84 \); MST \( R^2 = 0.70 \); MAT \( R^2 = 0.70 \); WMT \( R^2 = 0.66 \)). The slope and intercept of the MAF/MBT’\textsuperscript{5Me} calibration (MAF = -0.5 (± 0.4) + 30.4 (± 0.8) * MBT’\textsuperscript{5Me}) were similar to the MAT/MBT’\textsuperscript{5Me} calibration presented by Russell et al. (2018) (MAT = -1.21 + 32.42 * MBT’\textsuperscript{5Me}). This may suggest that MBT’\textsuperscript{5Me}-derived temperatures using the Russell et al. (2018) calibration in cold regions are best considered to reconstruct MAF rather than MAT. Though MBT’\textsuperscript{6Me} was previously found to correlate well with temperature in highly alkaline lakes on a regional scale (\( R^2 = 0.75 \); Dang et al., 2018), it was not correlated with any temperature variable in our global dataset (\( R^2 \leq 0.12 \)). Finally, we note that the Community Index (De Jonge et al., 2019), which was associated with bacterial community changes in geothermally heated Icelandic soils, is identical to our \( \text{fla}_{\text{Meth}} \). This may suggest that the strong connection between \( \text{fla}_{\text{Meth}} \) and MAF could also be driven at least in part by changes in microbial community composition. However, genomic data is not currently available for the majority of the sites in this study to test this hypothesis.

MAF provided the strongest temperature calibrations at the global level in this study. Unlike for MAT, these calibrations yielded unbiased residuals at the cold end (Fig. 3-10b), encouraging their application in cold regions with high seasonality in particular. However, we note that the range of MAF values in our combined Canadian and Icelandic dataset is small (6.6°C, or 3.2°C without the warm and cold endmembers) compared to that of MAT (22.9°C), especially in relation to the MAF calibration error (RMSE = 2.14°C, Fig. 3-10b). While the global-scale MAF calibrations presented here are valid for application at high latitudes, they may therefore prove unable to resolve smaller temperature variations in paleoclimate archives from those regions. Regional high-latitude calibrations aimed at improving this temperature resolution are the subject of ongoing work.

Conductivity and pH Calibrations

Conductivity outperformed pH in our calibrations (\( R^2 = 0.83 \) versus 0.74) and was the second most important predictor of brGDGT distributions in our dataset after temperature. Both cyclization number and methylation position were important to the success of these calibrations. The Cyc set, which allowed only cyclization number to change, provided a conductivity fit with \( R^2 = 0.73 \) (Fig. 3-11a). The Isom set, which isolated trends in isomer abundances, generated a slightly stronger calibration with \( R^2 = 0.76 \). When both of these structural properties were allowed to vary together in the CI set, the calibration was markedly improved (\( R^2 = 0.83 \), Fig. 3-11b). In contrast, methylation number was a poorer predictor of conductivity alone (Meth set \( R^2 = 0.65 \)) and did not improve upon the CI correlation in the combined sets (MI \( R^2 = 0.80 \); MC \( R^2 = 0.83 \); Full \( R^2 = 0.81 \)). Similar results were found when using RMSE to compare fit performance (Fig. S41b). The CI\textsubscript{III} and CI\textsubscript{II} subsets also
provided conductivity calibrations with relatively high statistical performance ($R^2 = 0.75, 0.73$). The CI$_i$ subset performed worse ($R^2 = 0.65$), likely due to the fact that no isomer variations are present in these FAs. These subset-specific fits are the first of their kind, and their success indicates that the relationship between brGDGTs and conductivity is present regardless of methylation number.
Figure 3-11: Summary of pH and conductivity calibrations. Performance (adjusted $R^2$) of all linear and quadratic fits for lake water conductivity (a) and pH (c) and brGDGT fractional abundances (FAs) calculated within the basic (Meth, Cyc, Isom; left of dashed lines) and combined (Meth-Isom (MI), Cyc-Isom (CI), Meth-Cyc (MC), and Full sets; right of dashed lines) structural sets. Results of both the SFS/SBE and combinatoric fitting methods are plotted. The fits we suggest for general use (CI set, quadratic, combinatoric, for both variables; Eqs. 3-12 and 3-13) is bolded and marked with an asterisk in a) and c) and plotted in b) and d). “Est. ln(Conductivity)” and “Est. pH” are the natural logarithm of lake water conductivity and pH estimated using these suggested fits.

In addition to conductivity, the CI set also best captured the relationship between brGDGTs and pH ($R^2 = 0.73$; Fig. 3-11c and d). The addition of methylation number variations in the Full set did not substantially improve the calibration ($R^2 = 0.74$), indicating that the majority of the relationship between brGDGTs and pH is captured by cyclization number and methylation position. Of these two structural variables, cyclization number was more important for pH; the Cyc set calibration ($R^2 = 0.67$) outperformed those from the Isom and Meth sets ($R^2 = 0.59$ for both). Analogous results were found using RMSE (Fig. S41c). The CI, CI$\text{I}_{\text{I}}$, and CI$\text{I}_{\text{II}}$ subsets provided weaker, but significant calibrations with comparable performances to one another ($R^2 = 0.67, 0.68,$ and $0.62$, respectively), indicating again that pH relationships are more or less independent of methylation number.

**Dissolved Oxygen and Lake Geometry Calibrations**

There is increasing evidence that oxygen availability strongly affects lacustrine brGDGT distributions (Colcord et al., 2017; Weber et al., 2018; Van Bree et al., 2020; Yao et al., 2020). We therefore tested for calibrations within our dataset with mean and minimum dissolved oxygen concentration (DO$_{\text{mean}}$ and DO$_{\text{min}}$). As lake morphology can be an important predictor of lake oxygen levels (Hutchinson, 1938; Nürnberg, 1995), we also tested the natural logarithms of maximum water depth (Depth), the ratio of lake surface area to maximum depth (SA/D), and approximate lake volume. None of the DO or lake geometry variables generated strong correlations with brGDGT distributions (Table S3; section S5.1).

In light of increasing evidence that oxygen availability strongly affects lacustrine brGDGT distributions, it is perhaps surprising that we do not find a stronger correlation between brGDGTs and DO. However, the effects of DO on brGDGT distributions appear to be highly site-specific. For example, some detailed studies have found elevated levels of brGDGT-IIIa in low oxygen conditions (Weber et al., 2018; Yao et al., 2020), but another found all of the most common brGDGTs except IIIa in abundance in the oxygen-depleted hypolimnion (Van Bree et al., 2020). A third detailed study found no correlation between brGDGTs and oxygen at all (Loomis et al., 2014b), and no calibration study to date has found regional or global trends. The wide range of possible drivers of DO – mixing regimes, eutrophication state, and ice cover, to name a few – may play a role in the incoherent relationship between brGDGTs and DO in these studies and our own. Additionally, DO measurements are often taken at the time of sampling and are most likely not representative of the annual range. For the Canadian and Icelandic lakes in this study, for example, DO was depleted under lake ice relative to ice-free conditions in 10 out of 11 cases. Few of the lakes in our study have continuous DO monitoring data available, and most are from Central Europe. Therefore, while our study does find some significant correlations between brGDGTs and DO, we do not recommend a calibration for general use and instead highlight the need for further study.
Recommended Calibrations

The Meth, MC, MI, and Full subsets all provided MAF temperature calibrations with comparable $R^2$ (0.90 to 0.91) and RMSE (1.97 to 2.14°C) values (Fig. 3-10a, Table S3). However, the Meth set provided the FAs with the strongest compound-specific relationships with MAF ($R^2 \leq 0.88$) in the modern dataset and exhibited little influence ($R^2 \leq 0.49$) from any other variable examined in this study. In contrast, the MC, MI, and Full sets all exhibited stronger relationships with pH and conductivity (Fig. 3-11a and c). We therefore recommend the highest-performing Meth calibration (Fig. 3-10b; Eq. 3-10; $n = 182$, $R^2 = 0.90$, RMSE = 2.14°C) for general use in lake sediments:

$$MAF \ (°C) = 92.9(±15.98) + 63.84(±15.58) \times fIb_{Meth}^2 - 130.51(±30.73) \times fIb_{Meth}$$

- 28.77(±5.44) $\times fIIa_{Meth}^2 - 72.28(±17.38) \times fIIb_{Meth}$
- 5.88(±1.36) $\times fIIc_{Meth}^2 + 20.89(±7.69) \times fIIIa_{Meth}^2$
- 40.54(±5.89) $\times fIIIa_{Meth} - 80.47(±19.19) \times fIIIb_{Meth}$

(3)

Importantly, Eq. (3-10) contains terms for $fIIc_{Meth}$ and $fIIIb_{Meth}$, which may be equal to zero in organic-lean samples (e.g. Fig. 3-7f and h) and could thus result in unrealistic values for MAF. In these cases, we recommend applying subset-specific calibrations that exclude bicycized (Eq. S1; $n = 182$, $R^2 = 0.89$, RMSE = 2.19°C) or both mono- and bicycized (Eq. S2; $n = 182$, $R^2 = 0.88$, RMSE = 2.33°C) compounds. These calibrations offer flexibility in downcore applications with minimal sacrifice of statistical performance. Subset-specific calibrations are also available for mono- and bicycized compounds alone (Eq. S3-4).

The Full set MAF calibration provided the highest $R^2$ and lowest RMSE in our dataset. This fit may be applicable in settings with good conductivity or pH control and may be useful for comparison with previous calibrations. It is therefore provided in Eq. (3-11) ($n = 182$, $R^2 = 0.91$, RMSE = 1.97°C):

$$MAF \ (°C) = -8.06(±1.56) + 37.52(±2.35) \times fIa_{Full} - 266.83(±98.61) \times fIb_{Full}^2$$

+ 133.42(±19.51) $\times fIb_{Full} + 100.85(±9.27) \times fIIa_{Full}^2$

+ 58.15(±10.09) $\times fIIIa_{Full}^2 + 12.79(±2.89) \times fIIIa_{Full}$

(3)

The calibrations presented in Eqs. (3-10), (3-11), and (S1-4) allow for the quantitative reconstruction of warm-season air temperatures from lake sediment archives, including those at high latitudes. The statistical performance of these fits is comparable to recently published calibrations (Russell et al. (2018): $R^2 = 0.94$, RMSE = 2.14°C, $n = 65$; Martínez-Sosa et al. (2020): $R^2 = 0.85$, RMSE = 2.8°C, $n = 261$; Dang et al. (2018): $R^2 = 0.91$, RMSE = 1.10°C, $n = 39$). We do not recommend an independent MAT calibration due to residual seasonality biases (Fig. S40), though we note again that MAT is often identical to MAF in warm or low-seasonality settings.

Independent calibrations were generated for lake water conductivity and pH. However, we re-emphasize that these two variables are fundamentally related (Section 3.4.3), which may cause them to vary in tandem in a paleo record. While we provide separate calibrations for conductivity and pH here, an examination of downcore trends in both reconstructed variables is recommended, along with an understanding of their relationship at the modern site.
variables downcore. Importantly, this advantage is gained without sacrificing the statistical
therefore provide some protection against the unwanted influences of other environmental
modern dataset. The application of calibrations such as Eqs. (3-12; n = 143, R² = 0.83,
structural set FAs separate the effects of temperature from pH and conductivity in the
structural set calibrations for MAF, conductivity, and pH presented above offer potential
a chromatographic methods which improves upon the error of previously available
studies by providing a globally
6Previous work has demonstrated the value of brGDGT-derived pH records in studies of terrestrial paleoclimate (Tyler et al., 2010; Cao et al., 2017; Fastovich et al., 2020). However, these studies relied on calibrations generated from soils and/or analyses in which the 5- and 6-methyl isomers were not separated. The fit presented in Eq. (3-13) may improve such studies by providing a globally-distributed pH calibration in lake sediments using the latest chromatographic methods which improves upon the error of previously available calibrations (RMSE = 0.80, Russell et al., 2018).

The structural set calibrations for MAF, conductivity, and pH presented above offer potential advantages over traditional calibrations constructed using the standard Full set. The structural set FAs separate the effects of temperature from pH and conductivity in the modern dataset. The application of calibrations such as Eqs. (3-10), (3-12), and (3-13) could therefore provide some protection against the unwanted influences of other environmental variables downcore. Importantly, this advantage is gained without sacrificing the statistical performance of the calibration. Furthermore, the subset-specific calibrations such as Eqs.
(S1) and (S2) use only the most abundant compounds, making it easier to apply brGDGT proxies to organic-lean samples where only some compounds are typically present above the detection limit.

### 3.5 Conclusions

We have shown that brGDGT structural sets and warm-season temperature indices improve compound-specific correlations with environmental parameters while advancing our biological understanding of the lipids themselves. Grouping brGDGTs into structural sets based on methylation number, methylation position, and cyclization number elucidated the relationships between environmental variables and brGDGT structures. These sets revealed that methylation number fully captures the relationship between brGDGT distributions and temperature. They also showed the relative abundance of 5- and 6-methyl isomers to be dependent on conductivity and cyclization number to be primarily tied to pH. The deconvolved relationships provided by these subsets allowed for the generation of calibrations with temperature and pH that relied on fewer compounds with robust modern trends, without sacrificing statistical performance. They additionally revealed conductivity to be the second-most important variable in controlling brGDGT distributions and provided a calibration for this oft-overlooked variable, which may find use as a proxy for precipitation/evaporation balances or hydrologic changes.

The structural sets also provided insight into the biological underpinnings of brGDGT structural diversity. The Meth, Cyc, and Isom sets gave evidence that methylation number, cyclization number, and methylation position vary more or less independently of one another across environmental gradients. They further revealed that the inclusion of tetramethylated compounds (Ia, Ib, Ic) enhances the temperature dependencies of 5-methyl compounds but erases those of their 6-methyl counterparts. Finally, the results linking methylation number to temperature and cyclization number to pH are consistent with a physiological explanation of brGDGT distributions (Weijers et al., 2007b). However, an explanation for the connection between conductivity and methylation position has, to our knowledge, not yet been proposed, and encourages further study. As the microbial producers of brGDGTs have yet to be identified and cultured, the structural set approach thus provides a valuable tool for investigating controls on brGDGT diversity with a biological lens.

Warm-season temperatures outperformed MAT as the most important predictors of brGDGT distributions, particularly in high-latitude environments. We introduced 43 new lake sediment samples from sites with low MAT and high seasonality. In conjunction with a global dataset, these samples showed a clear warm-season bias in brGDGT temperature relationships, with MAF providing the strongest fits in our dataset. The warm-season bias may suggest a direct or indirect connection to heightened primary productivity in the summer. Alternatively, it may be the result of a more complex relationship with dynamic lake processes such as mixing events. While further study is needed to unravel these complications, the strong empirical calibrations presented here support the use of the brGDGT paleotemperature proxy to quantitatively reconstruct warm-season air temperatures from high-latitude lake sediments.

In summary, the use of brGDGT structural sets and warm-season temperature indices deconvolved relationships between brGDGT structure and environmental gradients,
revealed trends with biological implications, and tied brGDGT distributions to warm-season temperatures. Furthermore, they allowed for the construction of temperature and pH calibrations that rely on a smaller number of compounds with clearer modern trends compared to the traditional approach, without sacrificing statistical performance. Finally, we used the new structural set framework to generate the first brGDGT conductivity calibration in a global lake sediment dataset. Our results thus allow brGDGTs to be used to quantitatively reconstruct warm-season air temperatures and lake water conductivity and pH from lake sediment archives and provide a new methodology for the study of brGDGTs in the future.

3.6 Acknowledgements

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4 CHAPTER IV: Near-universal trends in brGDGT distributions in nature

Abstract. Bacterial brGDGT lipids are a prevalent tool in studies of terrestrial paleoclimate. Their distributions correlate empirically with environmental temperature and pH and their ubiquity in terrestrial, freshwater, and marine environments gives them wide applicability. Whether correlations with temperature and pH emerge due to a physiological response of source organisms and/or a shift in bacterial community composition remains an open question with important implications for proxy development and application. We applied a newly described technique for grouping brGDGTs to a globally compiled dataset (n = 2535) consisting of all modern sample media known to host brGDGTs. We found strong resemblances in the relationships between brGDGT fractional abundances and both temperature and pH across nearly all sample types examined. We also found near-universal connections between the brGDGTs themselves. Given the markedly different bacterial communities expected to inhabit these settings, these widespread relationships may suggest physiological and/or biochemical bases for observed brGDGT distributions.

4.1 Introduction

Lipid biomarkers preserved in sedimentary archives are an invaluable tool in studies of Earth’s past climates. Of these, bacterial branched glycerol dialkyl glycerol tetraethers (brGDGTs) have quickly become one of the most popular proxies for reconstructing temperature and pH, in part because they are nearly ubiquitous in the natural world. BrGDGTs have been measured in over a dozen different sample media from the High Arctic (Kusch et al., 2019b) to the deepest ocean trenches (Xiao et al., 2020), including in soil (Weijers et al., 2006), peat (Sinninghe Damsté et al., 2000), groundwater (Ding et al., 2018), hot springs (Schouten et al., 2007), aeolian particulate matter (Fietz et al., 2013), marine hydrothermal (Lincoln et al., 2013) and methane cold seep (Zhang et al., 2020) carbonate, fossil bone (Dillon et al., 2018), speleothem (Yang et al., 2011), and sediment and suspended/settling particulate matter (SPM) from lacustrine, riverine, and marine settings (Hopmans et al., 2004; Blaga et al., 2009; Tierney and Russell, 2009; De Jonge et al., 2014b). This ubiquity, along with a preservation window of at least tens of millions of years (Super et al., 2018), makes brGDGTs applicable in a wide variety of sedimentary archives over geologic timescales, even when other proxies may be absent (Weijers et al., 2007a; Dearing Crampton-Flood et al., 2018; Naafs et al., 2018; Crump et al., 2019; Lu et al., 2019; Harning et al., 2020). However, longstanding questions exist in brGDGT research that complicate their use as reliable, quantitative paleoclimate proxies.

The origin of the connection between brGDGT distributions and environmental parameters is one such pertinent question. Drawing analogies to other bacterial membrane lipid classes, early studies posited that the empirical relationships between brGDGTs and environmental parameters were physiological adaptations of source organisms to variations in external

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2 A version of this chapter is in review:
temperature and pH (Weijers et al., 2007b). Subsequent studies have since shown that changes in brGDGT distributions are also accompanied by shifts in the composition of the associated microbial community, indicating that the empirical correlations between the lipids and environmental parameters may be indirect in nature (Weber et al., 2018; De Jonge et al., 2019; Van Bree et al., 2020; Wu et al., 2021; De Jonge et al., 2021). Whether the variation in a brGDGT paleo record is due to the direct physiological response of microbes to a changing environment or the indirect restructuring of a microbial community has important implications for its interpretation and fidelity (De Jonge et al., 2021). Fortunately, brGDGT-producing source organisms remain incompletely identified and cultured (Sinninghe Damsté et al., 2018), limiting our ability to test these hypotheses through controlled laboratory experimentation. However, some insight can be gained by other means. Mesocosm studies of both incubated soils (Chen et al., 2018) and lake waters (Martínez-Sosa et al., 2020a) have suggested a bacterial community influence. Molecular dynamics simulations have recently produced a split result, supporting the physiological explanation for the brGDGT paleothermometer but leaving open the possibility of a community-driven pH response (Naafs et al., 2021). Here, we take an alternate approach in addressing this long-standing question by compiling the large and diverse body of existing lipid and environmental data and revisiting the empirical trends that exist in nature.

Early studies of brGDGTs in soils found that the number of methyl groups and cyclopentane rings on the alkyl backbone were empirically correlated with temperature and pH, respectively (Weijers et al., 2007b). Subsequent studies have generally upheld these basic relationships but have also found important differences in their details across sample types and regions. These discrepancies have led to independent modern calibrations for different sample types (e.g. soils and peats (Dearing Crampton-Flood et al., 2020), lake sediments (Martínez-Sosa et al., 2021) or SPM (Zhao et al., 2021), bones (Zhao et al., 2020), speleothems (Blyth and Schouten, 2013), and marine sediments (Dearing Crampton-Flood et al., 2018)) and regions (e.g. East Africa (Russell et al., 2018) and China (Dang et al., 2018)) and have necessitated much effort to disentangle the allochthonous versus autochthonous sources of brGDGTs to sedimentary archives (Buckles et al., 2014a; Loomis et al., 2014b; De Jonge et al., 2015b; Sinninghe Damsté, 2016; Dearing Crampton-Flood et al., 2018; Guo et al., 2020; Xiao et al., 2020; Dearing Crampton-Flood et al., 2021). Further complicating the matter, brGDGT distributions are affected by a wide array of other environmental parameters, including oxygen levels (Xie et al., 2014; Liu et al., 2014; Colcord et al., 2017; Weber et al., 2018; Van Bree et al., 2020; Yao et al., 2020; Dugerdir et al., 2021; Lattaud et al., 2021; Wu et al., 2021), salinity/electrical conductivity (Raberg et al., 2021; Wang et al., 2021), seasonality (Kim et al., 2012; Loomis et al., 2014b; Van Bree et al., 2020; Dearing Crampton-Flood et al., 2020), nutrient availability (Loomis et al., 2014a; Hu et al., 2016), and soil chemistry (De Jonge et al., 2021), which can mask or override relationships with temperature or pH. Finally, an observed bias in brGDGT-derived temperatures towards warmer seasons has proven difficult to quantify, with various studies finding summer air temperature (Shanahan et al., 2013), the mean air temperature of months above freezing (MAF) (Dearing Crampton-Flood et al., 2020; Raberg et al., 2021; Martínez-Sosa et al., 2021), growing degree days above freezing (Naafs et al., 2017), or other temperature indices to provide the strongest correlations in modern training sets. Such discrepancies might be expected as these air temperature indices are themselves only proxies for the in situ temperatures that are relevant for brGDGT-producing microbes. A growing body of in situ temperature studies (e.g., in lakes (Zhao et al., 2021) and soils (Wang et al.,
2020; Pérez-Angel et al., 2020)) show promise, but the vast majority of published works currently rely on the more readily available air temperatures. Importantly, brGDGT studies have traditionally relied on fractional abundances (FAs) calculated by dividing the compound of interest by the sum of all 15 commonly measured brGDGTs (“Full set FAs”; Fig. 8-16g; Table 8-2). By mathematically mixing these compounds in the denominator, this approach risks convoluting the influences of multiple environmental parameters on lipid distributions and obscuring individual compounds’ trends (Raberg et al., 2021). We recently proposed an alternative approach in which brGDGT FAs are calculated within structurally-based brGDGT subgroups (Fig. 8-16; Table 8-2) and showed that these groupings deconvolved relationships between the lipids and temperature, pH, and conductivity in a global lake sediment dataset (Raberg et al., 2021). This study revealed that variations in methylation number, as isolated by the Methylation (Meth) structural set (Fig. 8-16a), captured the entirety of the brGDGT temperature response. Similarly, the pH response was fully captured by variations in cyclization number and isomer abundance, as quantified by the Cyclization-Isomer (Cyc-Isom) set (Fig. 8-16e). Here, we apply this new methodology to a compiled global dataset (n = 2535) of all available modern sample media known to contain brGDGTs to assess the uniformity of the relationships between compound FAs and temperature/pH across sample types and environments. We find near-universal relationships between brGDGT FAs, temperature, and pH across this diverse global dataset and discuss the implications of our findings for developing global brGDGT-based proxies that are independent of sample type, building confidence in downcore applications, and advancing our understanding of the origin of the connection between brGDGT lipid distributions and the environment.

4.2 Results

Recent work showed that the Meth structural set (Fig. 8-16a; Table 8-2) best captured the relationship between brGDGT FAs and temperature in a dataset of globally distributed lake sediments (Raberg et al., 2021). We find this result to be upheld for soils, peats, lacustrine SPM, bones, and marine surface sediments (Fig. 4-1). These sample types do not always share the same temperature variable (e.g., air temperature for soils versus sea surface temperature for marine sediments), due primarily to inherent differences between sample types and data availability constraints (see Methods for details). Despite these differences, all 15 compound-specific temperature relationships of these disparate sample types shared visibly similar functional forms (Fig. 4-1), with linear or quadratic fitting coefficients often within one standard error (Fig. 8-17). Limited datasets of lacustrine mesocosm, low dissolved oxygen (DO) SPM (separated due to the marked influence of oxygen levels on brGDGT distributions), and groundwater samples broadly plot within global trends. Notable deviations are visible for low DO SPM (e.g. Fig. 4-1e), groundwater (e.g. Fig. 4-1g), and marine samples (e.g. Fig. 4-1j). The Meth set FAs unanimously provided stronger correlations than the standard Full set FAs (Fig. 8-16g; Table 8-2) as well as better agreement between sample types (Fig. 8-18).
Figure 4-1: Relationships between temperature and brGDGT fractional abundances (FAs) in the Meth set. The Meth set is formed by grouping brGDGTs by cyclization number and isomer designation (schematics at top); FAs are calculated within each group (Raberg et al., 2021). Plots of IIa’, IIb’, and IIc’ exactly mirror those of IIIa’, IIIb’, and IIIc’, respectively, and are therefore not shown. *Temperatures are (necessarily) different for different sample types (see Methods) and are as follows: warmest month air temperature (Soil, Peat, Bone); mean air temperature of months above freezing (Lake Surface Sediment); sea surface temperature (Marine Sediment); in situ water temperature (SPM, Meso/Microcosm, Groundwater). Best fit curves (quadratic for IIa and IIIa; linear for all other compounds) are plotted for each compound, with $R^2$ values provided. $R^2$ values for FAs calculated using the standard approach (grouping all 15 compounds together) are given in parentheses for comparison. P values were < 0.01 except where marked with an asterisk. Open circles denote points with FA = 0 or 1. These points were clear outliers originating from samples with low concentrations of brGDGTs and were excluded from statistical analyses (see Methods).

The Cyc-Isom set (Fig. 8-16e; Table 8-2) best captured the relationships between brGDGTs and pH for soils, peats, and lacustrine sediments and SPM, with compound-specific relationships again exhibiting similar functional forms across sample types (Fig. 4-2). Linear slopes were often within one standard error (Fig. 8-19). However, unlike for temperature, a consistent offset is visible between the terrestrial (soil and peat) and lacustrine (sediment and SPM) datasets (Fig. 4-2). Sample types with limited datasets, including lacustrine microcosm and low DO SPM, riverine sediments and SPM, and groundwater generally plot within these global trends, with notable exceptions for low DO SPM and groundwater samples (e.g., Fig. 4-2e). Lower-pH microcosm samples also deviated, potentially due to the death of brGDGT-producing microbes in these experiments (Martinez-Sosa et al., 2020a). Again, the Cyc-Isom FAs provided stronger correlations and better agreement between sample types than the standard approach (Fig. 8-20).
Figure 4-2: Relationships between pH and brGDGT fractional abundances (FAs) calculated within the Cyc-Isom set. The Cyc-Isom set is formed by grouping brGDGTs by methylation number (schematics at right; methylations at C5 (black) or C6 (red)); FAs are calculated within each group (Raberg et al., 2021). Linear fits are plotted for each compound, with $R^2$ values provided. $R^2$ values for FAs calculated using the standard approach (grouping all 15 compounds together) are given in parentheses for comparison. P values were < 0.01 except where marked with an asterisk. N = 1295 for all subplots.
We additionally found multiple near-universal relationships between pairs of brGDGT FAs (Fig. 4-3). By partitioning brGDGTs into structural sets, these pairs of FAs are guaranteed to be mathematically independent (Raberg et al., 2021). The FAs of uncyclized and monocyclized tetramethylated brGDGTs (fIaMI and fIbMI; Fig. 8-16d; Table 8-2) were tightly correlated in the Meth-Isom set ($R^2 = 0.79$, Fig. 4-3a). Similarly, those of 5-methyl pentamethylated and hexamethylated uncyclized brGDGTs (fIIIaCI and fIIIIaCI; Fig. 8-16e; Table 8-2) were closely related in the Cyc-Isom set ($R^2 = 0.77$, Fig. 4-3b). Finally, 6-methyl isomers of pentamethylated and hexamethylated uncyclized brGDGTs (fIIa'Iosm and fIIIIa'Iosm; Fig. 8-16c; Table 8-2) are present in proportional abundances in the Isom set ($R^2 = 0.80$, Fig. 4-3c). These relationships were broadly consistent across sample types, with linear slopes and intercepts often within error (Fig. 8-21). A comparable analysis is not possible using traditional Full set FAs as they are not mathematically independent.

Figure 4-3: Relationships between selected brGDGT fractional abundances (FAs). FAs were calculated in the (A) Meth-Isom (MI), (B) Cyc-Isom (CI), and (C) Isom structural sets (Raberg et al., 2021) (Fig. S1 and Table S1). $R^2$ values for linear regressions ($p < 0.01$ for all) are provided. Samples with $FA = 1$ or $0$ (open circles) were excluded from statistical analyses ($n = 2359, 2203$, and $2129$ for A, B, and C, respectively). FAs are calculated as follows (Fig. S1 and Table S1): $fIaMI = Ia / (Ia + Ila + IIIa + Ila' + IIIa')$ and $fIbMI = Ib / (Ib + Iib + IIIb + Iib' + IIIb')$; $fIIaCI = IIa / (IIa + Iib + Iic + Ila' + IIb' + Iic')$ and $fIIICl = IIIa / (IIIa + IIIb + IIIc + IIIa' + IIIb' + IIIc')$; $fIIIIa'Iosm = IIa' / (IIa + Ila)$ and $fIIIIa'Iosm = IIa' / (IIa + IIIa')$.

While the basic functional forms of the relationships between brGDGTs, temperature, and pH are in good agreement, differences may be present in their details. An analysis of the fitting coefficients for each sample type revealed that they are often but not always within one standard error. However, challenges, such as a lack of standardized temperature measurements between sample types, limits the utility of this approach at this point. An expanded discussion of these challenges is available in the Supplementary Material (Section 8.3.1).
4.3 Discussion

Microbial community composition can be expected to vary markedly across the diverse media and environments of the compiled dataset. The fact that near-universal temperature and pH trends supersede these community differences is therefore remarkable and suggests that either 1) brGDGTs are produced by a small number of widespread source organisms or 2) brGDGTs play a fundamental physiological role for a wide range of bacterial life. In either case, these near-universal trends have important implications for brGDGT-based proxies. First, they encourage proxy development beyond the well-studied soil/peat and lake sediment datasets. In particular, they support recent work in marine sediments (Dearing Crampton-Flood et al., 2018; Gao et al., 2021; Liu et al., 2021), which exhibited the highest compound-specific temperature correlation in our dataset (flaMeth; \( R^2 = 0.77, p < 0.01 \)), as well as in bones (Zhao et al., 2020) and lacustrine SPM (Zhao et al., 2021). The widespread trends also suggest that it may be possible to construct modern calibrations that join multiple sample types in future studies. Currently, the sample types in the compiled dataset (necessarily) have different associated temperatures (e.g., air versus water; see Methods) and exhibit offsets in pH relationships (Figs. 4-2 and 8-19). While these incongruities preclude the construction of universal calibrations at this time, advances in brGDGT research, such as the growing body of in situ (Wang et al., 2020; Pérez-Angel et al., 2020) and/or more standardizable (Stefanescu et al., 2021) temperature measurements, may allow for such efforts to succeed in the future. Even without these mixed or universal calibrations, the common functional forms exhibited by different sample types lend confidence to qualitative trends in paleoclimate reconstructions from mixed-source archives (provided the relative contribution of each source can be constrained). Furthermore, the commonalities in brGDGT temperature and pH responses support the use of the proxies in paleoclimate reconstructions where modern analogs and details of the depositional setting may be unavailable.

Correlations between pairs of brGDGT FAs (Fig. 4-3) revealed relationships between lipids that spanned nearly every sample type examined. Such connections may provide insight into the biosynthesis and/or biochemical roles of brGDGTs in cells. For example, the correlated Isom set FAs of IIa' and IIIa' (Fig. 4-3c), may indicate that the same enzyme is responsible for methylating both compounds and/or that the two lipids perform related functions. Similarly, the fact that the FA of brGDGT-Ia (with respect to all uncyclized compounds) increases in tandem with the FA of brGDGT-Ib (with respect to all monocyclized compounds) indicates that methylation number can vary independently of cyclization number (Fig. 4-3a). This result may suggest that these two structural variations are able to perform their biological roles independently. New avenues of research based on such observations may provide rare insight into the biochemistry of brGDGTs.

Deviations from the trends described above are nearly as important as the trends themselves. Samples with low associated DO, such as groundwater and some lacustrine SPM, show anomalously high flaMeth (Fig. 4-1g), which would lead to underestimates of temperature in most brGDGT calibrations. These samples are also associated with narrow pH ranges yet vary widely in cyclization and isomer abundances (e.g., Fig. 4-2e). Additionally, the origin of the offset between the pH responses of terrestrial and lacustrine samples (e.g., Fig. 4-2j) is unknown. While it is possible that the discrepancy represents important differences between these sample media, it is also possible that bulk pH measurements do not accurately represent the chemical microenvironments experienced by brGDGT producing microbes (e.g., in soils (Baveye et al., 2018)). These or other possibilities warrant further investigation.
Finally, we stress that while clear trends emerge at the global scale, they do not always hold at the regional or site-specific level (e.g., ref. (Van Bree et al., 2020)), indicating that microbial community effects and/or environmental forcing outside of temperature and pH remain important controls.

In conclusion, a comparison of more than 2500 samples spanning a dozen sample types from across the globe revealed near-universal trends between brGDGTs, temperature, and pH. Five distinct relationships displayed R² values between 0.68 and 0.80 across 8-12 diverse sample media (Fig. 4-1a, Fig. 4-2d, and Fig. 4-3a-c). Other relationships exhibited weaker correlations but still possessed similar functional forms (e.g., Fig. 4-1d). Recent work has revealed that brGDGT distributions vary in tandem with bacterial communities (Weber et al., 2018; De Jonge et al., 2019; Van Bree et al., 2020; Martínez-Sosa et al., 2020a; Wu et al., 2021; De Jonge et al., 2021), connecting them only indirectly to pertinent variables such as temperature and pH. However, given the wide range of microbial life expected to inhabit the diverse environments of the compiled dataset, the coherent trends presented here suggest at least some physiological basis for the relationships between brGDGTs and their environment. This observation encourages the further development of brGDGT-based proxies, lends confidence to their continued application, and provides a new framework for investigating controls on brGDGT distributions in the future.

4.4 Materials and Methods

4.4.1 Experimental Design

The objective of this study was to compare how brGDGTs in different sample types relate to temperature, pH, and one another. While brGDGT FAs can be quantitatively compared without trouble, environmental parameters pose a challenge. First, temperatures associated with different sample types are often different (e.g., air versus water temperature), with seasonality effects further complicating temperature selection (e.g., summer versus mean annual). Furthermore, many commonly used measurements are themselves only proxies for the true environmental conditions experienced by brGDGT-producing microbes (e.g., air temperature instead of in situ soil temperature). As a result, it is not possible at this time to standardize environmental parameters such that datasets of different sample types can be fully quantitatively merged. However, qualitative and semi-quantitative comparisons of the ways in which brGDGTs relate to temperature and pH can still provide meaningful results with important implications and are worthy of pursuit.

To this end, we compiled the brGDGT FAs and, to the best of our ability, associated temperature and pH values from previously published datasets. We selected temperature parameters that were widely supported in the literature when possible. Where a consensus had yet to be reached (e.g., marine sediments), we selected standardizable and accessible parameters (e.g., sea surface temperatures). These selections are not intended to opine on these areas of research, only to allow for broad comparison with other sample types in this study.
4.4.2 Sample types and associated environmental parameters

Lipid and environmental data were compiled from previously published datasets of modern samples that used the most recent chromatographic methods that separate 5- and 6-methyl isomers (De Jonge et al., 2014a). The compiled dataset (n = 2535) consisted of bone (n = 191), groundwater (n = 14), lake water meso/microcosm (n = 36), lake surface sediment (n = 341), lake water SPM (n = 227, including sediment traps (n = 115) and water filtrates (n = 112)), low DO lake water SPM (n = 135, including sediment traps (n = 29) and water filtrates (n = 106)), authigenic carbonates from a marine methane cold seep (n = 13), marine surface sediment (n = 325, including deep ocean trench sediments (n = 31)), marine SPM (water filtrates, n = 25), peat (n = 96), riverine surface sediments (n = 52) and SPM (water filtrates, n = 85), and soil (n = 995, including permafrost active layer (n = 17)). Data from other sample media, including hot springs, speleothems, and hydrothermal vents, could not be included as these studies did not separate the 5- and 6-methyl isomers. All data and references are available in the dataset provided in the Appendix (Section 8.3.2). Fractional abundances (FAs) were calculated according to Figure 8-16 and Table 8-2 (see ref. (Raberg et al., 2021) for further details).

Temperatures associated with each sample type are as follows: mean air temperature of months above freezing (MAF; lake surface sediments), warmest month temperature (WMT; soils, peats, bones), in situ water temperature (lacustrine SPM (integrated summer water column where available for sediment traps, point measurement otherwise), meso/microcosm, and groundwater), sea surface temperature of the warmest month (SST, marine sediments above 72°N), and mean annual SST (all other marine sediments). These temperatures were primarily based on previous calibrations and data availability, with some adjustments as follows.

Lake sediments have recently been shown to correlate with the MAF warm-season air temperature index on a global scale (Raberg et al., 2021; Martinez-Sosa et al., 2021). Soil and peat brGDGTs have also been shown to correlate with MAF (Dearing Crampton-Flood et al., 2020). However, we used another warm-season temperature index, WMT, which provided comparable compound-specific correlations and better overlap with other sample types in this study. To bolster the soil dataset on the cold end, we generated monthly air temperature normals (1970-2000) for permafrost soil active layer samples (Kusch et al., 2019b) using the WorldClim database (Fick and Hijmans, 2017).

Though bones were initially calibrated against mean annual air temperature, a warm-season bias was observed in that study (Zhao et al., 2020). Due to this bias, and to match the soil and peat datasets, we used WMT values generated with the WorldClim database as above. Only bones with substantial in situ brGDGT production (Zhao et al., 2020) (isomer ratio > 50%) were included in temperature analyses.

Temperature calibrations for brGDGTs in marine settings are an active area of research. Thus far brGDGT production has been demonstrated in both deep (Liu et al., 2014) and shallow (Zhu et al., 2011) marine water columns and correlations have been found between marine surface sediment brGDGTs and both sea surface (Gao et al., 2021; Liu et al., 2021) and bottom water (Dearing Crampton-Flood et al., 2018) temperatures. Due to these uncertainties and data availability constraints, we used the SST temperature parameter for marine sediments in this study. Where published data were not available, we generated SSTs using the Extended Reconstructed Sea Surface Temperature database (Huang et al., 2017).
We used mean annual SST except for sites above 72°N, which experience substantial sea ice cover, for which we used the SST of the warmest month. To avoid marine sediments with terrestrial influence, we excluded those samples with a #ringSietra index (Sinninghe Damsté, 2016) and (IIIa + IIIa’)/(IIa + IIa’) < 0.92 from temperature analyses (Xiao et al., 2020; Liu et al., 2021).

A calibration has yet to be generated for lake water filtrates; however, point measurements of water temperature taken at the time of sampling are widely available and were thus used in this study. Integrated summer (June, July, August) water column temperatures were recently shown to correlate well with brGDGTs in sediment traps (Zhao et al., 2021) and were used where available; point measurements of in situ water temperature were used otherwise. “Low DO” was defined as DO < 1 mg/L. For the three lakes in this study without a published DO profile, samples below a reported redox transition zone were considered “Low DO” (Yao et al., 2019; Yao et al., 2020).

Where available, in situ pH values were used for all sample types except lake surface sediments, for which we used average water column pH.

### 4.4.3 Statistical Analysis

All statistical analyses were performed in R (Team R Development Core, 2021). We generated quadratic (Figs. 4-1d and g) or linear (all others) regressions for all relationships in the main text and figures. P values were < 0.01 for all regressions or else are denoted by an asterisk (Figs. 4-1 to 4-3, 8-18, and 8-20) or an open point (Figs. 8-17, 8-19, and 8-21). Due primarily to the low abundances of some compounds, some samples in Figures 4-1 and 4-3 displayed an FA = 0 or 1. These points, which are denoted by open circles in the plots, were clear outliers and were not included in statistical analyses (R² values, fitting coefficients, or plotted trend lines).

### 4.5 Acknowledgements

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5 CHAPTER V: Intact polar brGDGTs at Lake Qaupat, Baffin Island: implications for lipid sources and paleoclimate applications

Abstract. Paleotemperature histories derived from lake sediment archives can provide valuable context for modern climate changes and inform future trajectories. BrGDGT lipids have proven to be a valuable tool in such pursuits due to their empirical correlation with temperature and near ubiquity in nature. Despite their widespread application, however, the relative contributions of terrestrial and lacustrine sources of brGDGTs to lake sediment archives is site-dependent and difficult to constrain. Here, we explore the potential for intact polar brGDGTs – the lipids as they exist in living bacteria – to provide insight into the sources of brGDGTs on the landscape and their contributions to the sedimentary record. We examined the distributions of core and intact brGDGTs in soils, surface and downcore sediments, water filtrates, and sediment traps across five lake catchments in the Eastern Canadian Arctic, with an emphasis on Lake Qaupat (QPT), Baffin Island. We found soils to be dominated by brGDGTs with a monoglycosyl (1G) head group, while lacustrine samples displayed a far greater proportion of phosphohexose (PH) brGDGTs, providing evidence for in situ brGDGT production in both settings. We further found a heightened relative abundance of core- and PH-brGDGT-IIIa in sediments, implying an additional post-depositional source of in situ brGDGTs. A hierarchical clustering analysis of Lake QPT core and intact brGDGT distributions indicated that fossil core brGDGTs in lake sediments were largely lacustrine in origin, while 1G-brGDGTs were primarily soil derived. Additionally, we found evidence for substantial preservation of intact brGDGTs – especially 1G-brGDGTs – downcore on thousand-year timespans, though in situ production cannot be ruled out. In light of this apparent downcore recalcitrance, we explored the possibility of reconstructing 1G-brGDGT-derived soil temperatures and core-brGDGT derived lake temperatures in tandem from lake sediment archives.

5.1 Introduction

Over the past 50 years, the Arctic has experienced substantial changes in snow cover, vegetation greening, sea and land ice cover, and precipitation (Box et al., 2019 and references within). These changes are linked in large part to a marked rise in surface air temperatures, which have been increasing at more than twice the global average for the past two decades (Ballinger et al., 2020). As high latitude regions begin to diverge from historical analogs, the paleoclimate approach becomes an increasingly important tool for constraining their possible futures (Miller et al., 2010a; Tierney et al., 2020).

3 This chapter is in prep:
One tool for reconstructing high latitude paleotemperature records is a class of lipids called branched glycerol dialkyl glycerol tetraether lipids (brGDGTs). Structural variations in the alkyl-chain backbones of these bacterial membrane lipids were found to correlate with temperature and pH in soils (Weijers et al., 2007b). Analogous relationships were soon found in numerous other sample media including lake sediments (Tierney et al., 2010), allowing for the generation of global empirical temperature calibrations (Raberg et al., 2021; Martínez-Sosa et al., 2021) and the reconstruction of temperature histories from lacustrine archives across the high northern latitudes (Thomas et al., 2018; Crump et al., 2019; Harning et al., 2020; Lindberg et al., 2021).

Despite their widespread application, however, the sources of brGDGT lipids to sedimentary archives remain difficult to constrain. The seemingly ubiquitous compounds have been found in terrestrial (e.g., soil), freshwater, and marine environments around the world (see Chapter 4). BrGDGTs in these different environments may record substantially different temperatures (e.g., soil versus water temperature) and can contribute to sedimentary archives to varying degrees. For example, studies that tracked the abundance and distribution of brGDGTs in lakes (Tierney et al., 2012; Loomis et al., 2014b; De Jonge et al., 2015a) and river systems (De Jonge et al., 2014b; Hanna et al., 2016; Guo et al., 2020) have shown significant freshwater production and transport within a catchment. Furthermore, evidence has been found for brGDGT production within the lake sediments themselves (Tierney et al., 2012; Buckles et al., 2014b; Van Bree et al., 2020). As a result, brGDGTs in lake sediment archives can range from largely autochthonous (e.g., Loomis et al., 2014) to primarily soil-derived (e.g., Ning et al., 2019; Peterse et al., 2014). Some tools have been developed to disentangle sources of brGDGTs in marine settings (e.g., Sinninghe Damsté, 2016; Xiao et al., 2020) and even apply mixed calibrations (Dearing Crampton-Flood et al., 2018). However, while these techniques from the marine realm have recently been tested in lacustrine systems (Martin et al., 2019), methods to disentangle the various sources of brGDGTs in lake sediment archives remain in their nascent stages.

A second challenge to the interpretation of brGDGT-based paleotemperature records is the convoluting influence of dissolved oxygen levels on lipid distributions. The potential influence of oxygen depletion on brGDGT distributions was recognized early in the study of lacustrine brGDGTs (Tierney et al., 2012). Its effect has since been demonstrated in numerous lake systems (e.g., Van Bree et al., 2020; Colcord et al., 2017; Weber et al., 2018), and is often associated with an increasing proportion of hexamethylated brGDGTs. As these brGDGTs are also associated with colder temperatures, anoxic conditions in lake waters have the potential to artificially lower reconstructed temperatures. Apart from intensive studies of compound-specific isotopes (Colcord et al., 2017; Weber et al., 2018), tools for circumventing or even indicating an influence of dissolved oxygen on paleotemperature records are virtually non-existent.

The vast majority of studies on brGDGTs have relied on measurements of the recalcitrant “core” skeletons of the brGDGT lipids (c-brGDGTs). However, much additional information is contained in the more diverse pool of intact polar brGDGTs (i-brGDGTs), i.e., the lipids as they exist in living cell membranes. Due to analytical challenges, these intact lipids have been primarily analyzed by hydrolyzing them to their core skeletons and measuring the resulting c-brGDGT distributions. This method has allowed access to a putative “living” pool of brGDGTs. It has provided much information on the production rates and locations of brGDGTs (e.g., Huguet et al., 2017; Zell et al., 2013), but it sacrifices the information contained in the full suite of intact lipids.
In recent years, chromatographic methods have been developed to analyze brGDGTs in their full, intact form (e.g., Wörmer et al., 2013), though their application in the literature is limited (Liu et al., 2010; Peterse et al., 2011). These techniques expand the number of measurable brGDGTs from fifteen to over sixty, allowing for the resolution of more subtle distinctions between brGDGT distributions. Studies employing these methods have provided evidence for enhanced productivity of brGDGTs in anoxic zones of peats (Liu et al., 2010; Peterse et al., 2011). However, the distributions of i-brGDGTs across the terrestrial landscape and in lacustrine systems remain largely unexplored, and to our knowledge, no study has yet examined them in the Arctic.

Here we investigate the potential for i-brGDGTs to distinguish between sources of brGDGTs on the landscape in modern lake catchments from the Eastern Canadian Arctic, with an emphasis on Baffin Island. We measure brGDGTs in both their core and intact forms in soils, surface and downcore lake sediments, and aqueous environments across five lake sites, with a primary focus on Lake Qaupat. We further explore whether our findings can be used to inform c-brGDGT applications downcore and/or develop new i-brGDGT-based proxies.

### 5.2 Methods

#### 5.2.1 Study sites and sample collection

Soil, water filtrate (WF), sediment trap (ST), and surface sediment (SS) samples were collected from five lake sites across Baffin Island (Clyde Forelands Lake 8 (CF8), Clyde Forelands Lake 3 (CF3), Brother of Fog Lake (BRO), and Lake Qaupat (QPT), informal names) and northern Québec (3 Lakes North (3LN)) during the summers of 2017 and 2018 (Fig. 5-1 inset). Additionally, three samples from sediment core QPT-2A, collected in 2016 and described by Crump et al. (2019) were re-extracted for this study, as well as downcore sediment from a suboxic interval of Early Holocene sediment collected from BRO Lake in 2017. We analyzed 53 samples in total, including 25 samples from our primary study site, Lake QPT (Fig. 5-1).
Soils were sampled from beneath the dominant plant communities in the lake catchment (Martha K. Raynolds, personal communication in the field) at a depth interval of 0-10 cm, except for one (QPT P9) which was point sampled at 10 cm. Surface (0 m) and bottom (1-2 m above lakebed) waters were collected from lake centers and filtered through 0.3μm glass fiber filters (Advantec). Sediment traps at deep (2 m above the lakebed) and shallow (2 m below the surface) positions at Lake QPT were deployed from August 2017-July 2018 (ST Upper and ST Lower). After collection, these sediment traps were redeployed for the peak summer month, July 2018-August 2018 (ST Upper Sum and ST Lower Sum). A deep ST was also deployed from May-August 2018 at BRO Lake. Temperature, pH, conductivity, and dissolved oxygen concentration of the lake water columns were measured using a multiparameter sonde (HydroLab HL4, OTT HydroMet) at the time of deployment and collection of STs. Lake water samples were collected at the same time and analyzed for total phosphorus at the Arikaree Lab at the University of Colorado at Boulder. Drone imagery was collected at 6 cm resolution with a Mavic Pro (DJI) drone on July 22, 2018. A composite (Fig. 5-1) was generated using MapsMadeEasy.
5.2.2 Lipid extraction and analysis

After collection, samples were kept as cool as possible in the field and frozen upon returning to Colorado. All samples were freeze-dried and extracted using a modified Bligh & Dyer (BD) extraction method (Bligh and Dyer, 1959; Wörmer et al., 2013; Raberg et al., 2021). We extracted ~1g sediment, ~5g soil, glass fiber filters (2.5-12 L water filtered total), and all available material from sediment traps. Samples were first vortexed and sonicated in solvent Mix A, consisting of dichloromethane (DCM):methanol (MeOH):50 mM phosphate buffer (aq., pH 7.4) [1:2:0.8, v:v:v]. After centrifugation (3000 rpm and 10°C for 10 min), the supernatant was poured or pipetted into a glass separatory funnel. The process was performed twice with Mix A, twice with Mix B (DCM:MeOH:5% trichloroacetic acid buffer (aq., pH 2) [1:2:0.8, v:v:v]), and once with Mix C (DCM:MeOH [1:5, v:v]). Separation of aqueous and organic fractions was induced by adding equal volumes of HPLC-grade water and DCM to the separatory funnel. The organic (bottom) fraction was collected and dried under a nitrogen stream. DCM was then added to the aqueous phase, shaken, and allowed to separate to remove any residual lipids. The resulting organic fraction was then added to the extract and dried. Aliquots of the resulting total lipid extract (TLE) were redissolved in 99:1 (v:v) hexane:isopropanol (c-brGDGTs) or 9:1 (v:v) DCM:MeOH (i-brGDGTs) and filtered (0.45 μm, PTFE) before analysis. Six samples were additionally extracted using a standard Accelerated Solvent Extraction (ASE) method, as described previously (Raberg et al., 2021).

Core and intact brGDGTs were analyzed using a Thermo Scientific UltiMate 3000 high-performance liquid chromatography instrument coupled to a Q Exactive Focus Orbitrap-Quadrupole high-resolution mass spectrometer (HPLC-MS). Core brGDGTs were analyzed using a slightly modified version (Raberg et al., 2021) of the methods of Hopmans et al. (2016). Separation of i-brGDGTs was achieved using the chromatographic conditions described by Wörmer et al. (2013) and Cantarero et al. (2020). Briefly, two mobile phases (Phase A: 0.01% formic acid and 0.01% NH₄OH in acetonitrile:DCM [75:25, v:v]; Phase B; 0.4% formic acid and 0.4% NH₄OH in MeOH:H₂O [50:50, v:v]) were combined in a gradient from 1% B to 40% B. Intact brGDGTs were ionized via electrospray ionization and identified by exact masses (± 5 ppm) and retention times. Due to the lack of a suitable standard, we did not quantify absolute i-brGDGT abundances. Instead, we relied on fractional abundances of integrated peak areas as described below.

5.2.3 Index calculation and terminology

We defined multiple fractional abundances (FAs) for our analysis of both c- and i-brGDGTs. First, we calculated the standard Full Set FAs (Raberg et al., 2021) of c-brGDGTs,

\[
f_{x,\text{Full}} = \frac{x}{I(a + Ic + IIa + IIb + IIc + IIIa + IIIb + IIIc + Ila' + IIb' + IIIc')}
\]

where \(x\) is the integrated peak area of any compound in the denominator. An analogous FA cannot unfortunately be calculated for i-brGDGTs as it is currently unknown whether the analytical method can achieve separation of the 5- and 6-methyl isomers. We therefore calculated FAs of brGDGTs for each head group using the 9 compounds that are commonly measured without isomer separation and denote the difference with an asterisk:
\[ f_x - HG_{\text{Full}*} = \frac{x}{(HG-1a + HG-1b + HG-1c + HG-11a_C + HG-11b_C + HG-11c_C + HG-111a_C + HG-111b_C + HG-111c_C) - 2} \]

For each head group, HG, \( x \) is any compound in the denominator and all penta- and hexamethylated compounds represent the combined sum of 5- and 6-methyl isomers, as denoted by the subscript “C”.

\[ f_x - HG_{\text{Meth}*} = \frac{x}{(HG-1a + HG-11a_C + HG-111a_C)} \]  \hspace{1cm} (5 - 3)

and MBT’ index (Peterse et al., 2012),

\[ MBT'_{HG} = \frac{(HG-1a + HG-1b + HG-1c)}{(HG-1a + HG-1b + HG-1c + HG-11a_C + HG-11b_C + HG-11c_C + HG-111a_C) - 4} \]  \hspace{1cm} (5)

again using the sums of penta- and hexamethylated compounds. Finally, we calculated FAs within the entire pool of i-brGDGTs,

\[ f_x - HG_{\text{Full*-IPLs}} = \frac{x}{\sum i-brGDGTs} \]  \hspace{1cm} (5 - 5)

and the FA of each HG,

\[ fHG = \sum HG - brGDGTs / \sum i-brGDGTs \]  \hspace{1cm} (5 - 6)

### 5.2.4 Statistical methods

We used principal component analyses (PCAs) and hierarchical clustering to compare similarities in brGDGT distributions. PCAs were performed using the FactoMineR package in R (Lê et al., 2008; Team R Development Core, 2021). We used Full Set FAs (Eq. 5-1) for c-brGDGTs and Full*-IPLs Set FAs (Eq. 5-5) for i-brGDGTs. We chose the Euclidean distance measure to produce hierarchical clustering of Full* Set FAs (Eq. 5-2). Unweighted Pair Group Method with Arithmetic Mean (UPGMA; average-linkage) agglomerative clustering method was used according to Ramos Emmendorfer and de Paula Canuto (2021). We calculated approximately unbiased p-values (AU) and bootstrap probability values (BP). The bootstrap supports were based on 10,000 replicates and clusters with AU ≥ 95% confidence were highlighted for this study (Suzuki and Shimodaira, 2006).

### 5.3 Results

#### 5.3.1 Source-dependent i-brGDGT distributions

BrGDGTs were detected both as core (HG = H) and intact polar lipids with monoglycosyl (1G) or phosphohexose (PH) head groups (Fig. 5-2). We used a chromatographic method
capable of separating the 5- and 6-methyl brGDGT isomers (solid and dashed colored methylations in Fig. 5-2a, respectively) for c-brGDGTs (see Methods). The multifaceted peaks present for many i-brGDGTs (e.g., PH-IIa; Fig. 5-2c) suggest the existence of isomers for these compounds as well. However, as the method used for i-brGDGTs separates compounds based on their polar head groups rather than their alkyl-chain moieties, it is unlikely that these peaks represent 5- and 6-methyl i-brGDGTs. We did not attempt to integrate these isomers separately in this study; however, they may reflect meaningful differences in head group position and their existence warrants further investigation.
Both 1G- and PH-brGDGTs were detected in most samples (Fig. 5-3). Clear differences in the relative proportions of these two HGs existed between sample types. Soils were dominated by 1G-brGDGTs at all sites, with a mean fractional abundance of 1G versus PH (f1G; Eq. 5-6) of 0.89 ± 0.10 (Figs. 5-3 and 5-4). Surface sediments had a consistently lower proportion of 1G-brGDGTs (f1G = 0.34 ± 0.21) but ranged substantially across lakes (f1G = 0.20-0.68; Figs. 5-3 and 5-4). Water column samples (water filtrates and sediment traps) displayed both the largest range and within-site variance (Figs. 5-3 and 5-4), likely due at least in part to low lipid concentrations in the water filtrates that may have left many compounds below the detection limit.
Figure 5-3: Distributions of i-brGDGTs. Fractional abundances (calculated using Eq. 5 of the main text) of 1G- and PH-brGDGTs in Canadian soil, surface sediment (SS), sediment trap (ST), and water filtrate (WF) samples. QPT samples marked with an asterisk were excluded from principal component and clustering analyses due to low compound abundances.
Finally, we examined the relationship between lake water total phosphorous (TP) and the fractional abundance of PH-brGDGTs (fPH; Eq. 5-6) in surface sediments. While we found a weak positive correlation (adjusted $R^2 = 0.39$), it was not significant ($p > 0.2$; Fig. 8-22).

### 5.3.2 Connections between c- and i-brGDGT distributions

To elucidate connections between c- and i-brGDGTs, we compared the distributions of brGDGTs within each head group (H (i.e., c-brGDGTs), 1G, and PH) at Lake QPT. We performed principal component analyses (PCAs) on the resulting c- and i-brGDGT FAs (Figs. 5-5a and 5-5b, respectively). In the c-brGDGT PCA, uncyclized compounds (Ia, IIa/Ila’, and IIIa) were the primary drivers of principal component 1 (PC1), which explained 77.2% of the variance and separated soils from lacustrine samples (Fig. 5-5a). Uncyclized i-brGDGTs (especially PH-IIIa, 1G-IIa, PH-IIa, and PH-Ia) drove a similar separation of these sample types (Fig. 5-5b). In the c-brGDGT PCA, lake surface sediments were separated from water column samples by a heightened proportion of brGDGT-IIIa (Fig. 5-5a). Surface sediments were separated in a similar manner from sediment traps, but not water filtrates, by PH-IIIa in the i-brGDGT PCA (Fig. 5-5b). As suggested by the c- and i-brGDGT PCAs, the Meth* set FAs of brGDGTs IIIa and PH-IIIa displayed a strong one-to-one correlation (adjusted $R^2 = 0.80$, $p < 2.2e$-16) across all sample types, including soils (Fig. 5-6a). In contrast, the analogous FAs of IIIa and 1G-IIIa were only weakly related (Fig. 5-6b, adjusted $R^2 = 0.27$, $p = 0.0003$).
Figure 5-5: Principal component analyses (PCAs) of fractional abundances (FAs) of Lake QPT A) core and B) intact brGDGTs. FAs of c-brGDGTs were calculated according to Eq. 1 (i.e., with 5- and 6-methyl isomers separated); those of i-brGDGTs were calculated with Eq. 5-5. Compound loadings are plotted in black text (scaled by 1.8 for clarity).

Figure 5-6: Fractional abundances of core and intact brGDGT-IIIa. Relationship between the fractional abundance (FA) of IIIa in the Meth* set (Eq. 5-3) for the core and A) PH- and B) 1G-brGDGTs. Samples with FA = 0 or 1 were excluded. A one-to-one relationship is plotted with a dashed line. The three samples influenced by suboxic conditions (BRO Spr. Bottom WF, Lower ST, and Early Holocene downcore sediment) are marked in A).
To further compare the similarities of c- and i-brGDGT distributions, we performed a hierarchical cluster analysis, again using FAs calculated within each HG independently (Eq. 5-2). The analysis produced three statistically significant groupings (red boxes in Fig. 5-7; see Methods). Group 1 clustered all lacustrine core (H, light blue) and PH (orange) brGDGTs. Group 2 contained all 1G-brGDGT distributions, including soils and lacustrine samples. Group 3 was comprised of soil core and PH distributions. Groups 2 and 3 were more similar to each other than to Group 1.
Figure 5-7: Clustering dendrogram of all samples at Lake QPT. Fractional abundances of core lipid backbones were calculated independently for head group (HG = H (core), PH, and 1G) accord to Eq. 5. Approximately unbiased p-values (au; blue) and bootstrap probability values (bp; orange) are provided. Clusters with au ≥ 95% confidence are highlighted in red boxes.

5.3.3 Potential for downcore applications

In addition to using i-brGDGTs to distinguish lipid sources in modern lake catchments, we examined their potential for applications in sedimentary archives. To test the preservation potential of i-brGDGTs, we extracted three downcore samples from Lake QPT (0, 50, and 100 cm; ~0, 4.3, and 6.3 ka (Crump et al., 2019)) and one from BRO Lake (~50–60 cm, Early Holocene in age) using the BD extraction method. Both 1G- and PH-brGDGTs were detected in all four samples (Fig. 5-8a). However, downcore sediments are generally extracted using the ASE rather than the BD method. We therefore extracted six samples in parallel with the ASE and BD methods. We found both 1G- and PH- head groups in the BD-extracted samples, but only 1G after extracting with ASE (Fig. 5-8b).

Figure 5-8: Potential for downcore applications of intact brGDGTs. A) 1G- and PH-brGDGTs present in downcore sediments extracted using the Bligh and Dyer (BD) method. B) Samples extracted in parallel using the ASE and BD methods. From top to bottom, surface sediments in B) are QPT-SS1B 2.5cm, 1.5cm, and 0cm and soils are QPT Soil 3, QPT Soil 1, and CF3 Soil 2 – see also Fig. 1.

To test whether i-brGDGTs could serve as proxies for environmental conditions in the same manner as c-brGDGTs, we calculated MBT’, fIIIaMeth*, and mean annual air temperature (MAAT) on c- and i-brGDGTs at Lake QPT. The MBT’ index was similar for core, PH-, and 1G-brGDGTs for lacustrine samples, but varied widely for soils (Fig. 5-9a). The FA of IIIa in the Meth* set (Eq. 5-3) was in good agreement across HGs for soils (Fig. 5-9b).
In contrast, the values of fIIIa-PH\textsuperscript{Meth*} and fIIIa\textsubscript{Me} were similar for lacustrine samples, but fIIIa-1G\textsubscript{Meth*} displayed substantially lower values that were in closer agreement with those of the soils (Fig. 5-9b). Finally, we applied a transfer function (Eq. 12 of Loomis et al. (2012)) to calculate MAAT from core, PH-, and 1G-brGDGTs. Again, soils showed good agreement of MAAT values across head groups (Fig. 5-9c). For lacustrine samples, MAATs calculated from 1G-brGDGTs broadly agreed with soil MAATs, while those from core and PH-brGDGTs were substantially lower (Fig. 5-9c). Temperatures reconstructed using two other published transfer functions (Pearson et al., 2011; Foster et al., 2016) did not show the same agreements (not shown).

5.4 Discussion

5.4.1 Intact brGDGTs as a tool for distinguishing lipid sources

An analysis of c- and i-brGDGT distributions revealed in situ production of brGDGTs in the soil, water column, and surface sediment at Lake QPT. Soils were distinguished from lacustrine samples by a stark difference in the relative abundances of 1G-brGDGTs (Figs. 5-3 and 5-4). Within the lake, surface sediments were further distinguished from water column samples by a heightened relative abundance of hexamethylated brGDGTs, particularly IIIa and PH-IIIa (Figs. 5-5 and 5-6). These results support the conclusions of previous work using core lipids, which suggested that the production of brGDGTs is commonplace in both terrestrial and lacustrine environments (e.g., Buckles et al., 2014; Guo et al., 2020; Peterse et al., 2014; Tierney et al., 2012).

Widespread production poses a challenge for downcore applications, where a mixture of brGDGTs derived from multiple sources can complicate interpretations (c.f., Blaga et al., 2010). Promisingly, our results from Lake QPT suggest that 1G-brGDGTs in lacustrine samples may be primarily soil-derived and may therefore serve as a tool for disentangling...
lipid sources at this site. Soils at Lake QPT (and across the Canadian Arctic) were characterized by high f1G values (Fig. 5-3 and 5-4). Furthermore, 1G-brGDGTs in lacustrine samples were found to have similar distributions to those in the soils, grouping together in a cluster analysis (Fig. 5-7). These patterns suggest either that lacustrine 1G-brGDGTs are largely soil derived or that 1G-brGDGTs are produced in similar distributions regardless of environmental setting. The fact that the cluster of 1G-brGDGTs (Group 2) is more similar to soil (Group 3) than lacustrine (Group 1) core and PH-brGDGTs provides evidence for the former (Fig. 5-7), though the latter cannot be ruled out. Finally, it is important to note that while the 1G-brGDGT distributions group together in the Lake QPT cluster analysis, different results may emerge with a larger sample set or at other lake sites. For example, surface sediments at Lakes CF3, CF8, and BRO displayed higher relative abundances of 1G-IIIa than almost all soils in this study (Figs. 5-3 and 5-6b). While it is possible that soils with distributions similar to CF3 Soil 1 (Figs. 5-3 and 5-6b) contribute an outsized proportion of 1G-brGDGTs to these lakes, it seems likely that at least some of the 1G-brGDGTs were produced in the lacustrine environment at these sites, perhaps driven by lower available phosphorous (Fig. 8-22), which has been shown to lead to HG remodeling (Van Mooy et al., 2009). Despite these unknowns in other lake systems, our detailed study at Lake QPT suggests that 1G-brGDGTs throughout the Lake QPT study site are primarily soil-derived.

Despite the presence of soil-derived 1G-brGDGTs in lacustrine samples, our results suggest that the fossil lipid pool is primarily derived from PH-brGDGTs produced in situ. A cluster analysis grouped lacustrine core and PH-brGDGTs separately from soil and 1G-brGDGTs (Fig. 5-7). Furthermore, the MBT' index, fIIIaMeth*, and MAAT_Loomis of core brGDGTs agreed better with those derived from PH-brGDGTs than 1G-brGDGTs (Fig. 5-9). Finally, the strong correlation between the Meth* set FAs of IIIa and PH-IIIa (Fig. 5-6) suggest that the phospho- rather than glycolipid is the primary precursor of the core brGDGT-IIIa. This last connection is of particular interest for two reasons. First, a heightened relative abundance of core brGDGT-IIIa was the distinguishing marker of in situ surface sediment production at Lake QPT. Second, this same heightened abundance has been observed in multiple locations (e.g., Peterse et al., 2014; Tierney et al., 2012) and has been identified as the cause of an artificial “core-top cooling” signal in paleotemperature reconstructions (e.g., Miller et al., 2018; Zhao et al., 2021). The tight correlation between IIIa and PH-IIIa in our study suggests that this signal may be driven by the production of hexamethylated phosphohexose i-brGDGTs in the lake surface sediment.

Interestingly, the connection between core and PH-brGDGTs was apparent even for soils, despite their high f1G values. One possible explanation for the stronger relationship between PH- and c-brGDGTs is the higher lability of the phosphohexose head group (Harvey et al., 1986), which could lead to a greater turnover rate and a disproportionate contribution to the fossil lipid pool. Under this hypothesis, distributions of PH-brGDGTs may respond more rapidly to changes in environmental conditions and could prove useful in studies of shorter-term (e.g., seasonal) trends in brGDGTs.

### 5.4.2 Potential applications of i-brGDGTs in sedimentary archives

Both PH- and 1G-brGDGTs were detected in sediments up to 1 m below the lake floor and Early Holocene in age (Fig. 5-8a). While these compounds represent the first i-brGDGTs measured in their intact forms downcore to our knowledge, IPL-derived c-brGDGTs have been studied in lake sediment archives (e.g., Tierney et al., 2012) and marine sediments >140
ka old (Lengger et al., 2013). Whether these downcore i-brGDGTs are produced in situ, preserved over geologic timescales, or a combination of both is not clear, however. Some studies have found evidence for in situ production of intact branched (Lengger et al., 2013) and isoprenoidal (Liu et al., 2011) GDGTs in marine sediments. However, these IPLs have also demonstrated a remarkable recalcitrance. Tetraethers with phosphate-based head groups have been shown to survive over millennial timescales in marine sediments (Lengger et al., 2014). Glycosidic GDGTs are even more recalcitrant, with a degradation rate on the order of millions of years (Lengger et al., 2014). Furthermore, both br- (Fig. 5-8b; this study) and iso- (Lengger et al., 2012) GDGTs with 1G head groups have been shown to survive the high temperatures and pressures of ASE extractions. Finally, soil-derived 1G-brGDGTs appear to survive transport, deposition, and burial without significant alteration to their distributions at Lake QPT (Fig. 5-7). Therefore, while further study is needed to determine whether a significant in situ contribution exists, it is likely that the pool of downcore i-brGDGTs – and especially 1G-brGDGTs – at Lake QPT is ancient in origin.

The preservation of uniquely soil-derived 1G-brGDGTs into the geologic record could open the door for the development of qualitatively new proxies. BrGDGTs in lacustrine systems have been increasingly shown to be influenced by lake water chemistry, especially dissolved oxygen (e.g., Loomis et al., 2014a; Raberg et al., 2021; Weber et al., 2018). This influence affects brGDGT-derived temperatures and poses a major challenge for obtaining accurate paleotemperature reconstructions. Temperatures calculated from soil-derived compounds would circumvent this issue. Furthermore, as 1G-brGDGTs survive the ASE extraction process, which is commonly used for downcore sediments, samples may not need to be (re-)extracted with the more time-consuming BD method to apply 1G-brGDGT-based proxies.

To test whether i-brGDGTs display similar temperatures relationships to c-brGDGTs, we calculated and compared temperatures and temperature-related indices derived from PH, 1G, and core brGDGTs. The MBT’ index has a positive correlation with temperature (Peterse et al., 2012). At Lake QPT, we find a general agreement in the MBT’ values of lacustrine samples from PH-, 1G-, and c-brGDGTs and higher and more variable values in soils. However, the MBT’ index relies on several compounds (e.g., IIc) that were rare or absent from our i-brGDGT distributions, perhaps due to lower compound abundances overall, and may therefore not be appropriate for use here. A better index for this study is fIIIaMeth*. This fractional abundance relies only on the three most abundant brGDGTs (Ia, IIaC, and IIIaC) and can be expected to have a robust negative relationship with temperature (Raberg et al., 2021). As shown in Figure 5-9b, all three values of fIIIaMeth* agree well in the soils, indicating that 1G- and PH-brGDGTs have similar temperature sensitivities and that the core pool may be primarily derived from these IPLs. In contrast, lacustrine samples show good agreement between core- and PH-derived values, but a strong warm bias in those recorded by the 1G-brGDGTs (Fig. 5-9). Furthermore, these 1G-derived fIIIaMeth* values generally agree with those recorded in the soils. This discrepancy can be explained if the PH-brGDGTs in the lake are primarily autochthonous, recording lake water temperatures, while the 1G-brGDGTs are largely allochthonous and record the temperatures of catchment soils. Under this hypothesis, a single lake sediment sample can record both soil and lake water temperatures independently. Considering that both sets of IPLs can be measured downcore, these results present the potential for independent soil and lake temperature reconstructions from the same sample extracts. Indeed, temperatures calculated using the Loomis et al. (2012) calibration appear to do just this (Fig. 5-9c). However, other calibrations (Foster et
al., 2016; Pearson et al., 2011; not shown) produce qualitatively different results, indicating that IPL-specific calibrations are likely to be necessary for downcore applications.

5.5 Conclusions

Intact brGDGTs at Lake Qaupat and across the Eastern Canadian Arctic provided new insight into the sources of brGDGTs in terrestrial and lacustrine environments. We found a stark distinction between the i-brGDGT composition of soils and lacustrine samples, with 1G-brGDGTs dominating in the soils and PH-brGDGTs in the lakes. We further found heightened relative abundances of IIIa and PH-IIIa in the lake sediments, suggesting post-depositional in situ production. 1G-brGDGTs in lacustrine samples at Lake QPT were likely soil derived, showed a high preservation potential, and survived the ASE extraction procedure, opening the door for the development of soil-specific proxies that can be measured in lake sediment archives. In contrast, core brGDGTs in the lacustrine environment were primarily derived from PH-brGDGTs produced in situ at Lake QPT. A single downcore sediment sample extracted using standard ASE methods can therefore theoretically be used to reconstruct both soil and lake water temperatures at this site. However, further work is needed to calibrate, test, and refine 1G-brGDGT-based proxies and to expand our understanding of i-brGDGT distributions to other lake sites.
6 CHAPTER VI: Conclusions

6.1 Key results of the thesis

In this thesis, I have presented research focusing on three of the major challenges facing the development and application of brGDGT-based proxies in high latitude regions. My first study (Chapter 3) examined the effects of spurious environmental parameters such as seasonality, conductivity, and dissolved oxygen on brGDGT temperature and pH calibrations. My second study (Chapter 4) addressed the issue of a physiological versus community-based origin for the relationships between brGDGTs and the environment. My third study (Chapter 5) aimed to fingerprint sources of brGDGTs in terrestrial and lacustrine settings and determine their relative contributions to the sedimentary pool. Characterization of the study sites (Chapter 2) provided valuable information on lake catchment morphology, lake water chemistry, and in situ temperatures which informed each of these three studies. Key takeaways include:

- Drone surveys, water chemistry measurements, and in situ temperature monitoring provide valuable context and baseline data for a variety of studies.
- Structurally based groupings of brGDGTs can deconvolve the influences of environmental parameters on brGDGT distributions.
- Through methylation number alone, brGDGTs in lake sediments record the mean temperature of the months above freezing.
- Cyclization numbers and isomer abundances of lake sediment brGDGTs are related to lake water pH and conductivity.
- Near-universal trends across a dozen disparate sample types suggest a physiological origin for the relationships between brGDGTs and the environment.
- Intact polar brGDGTs can be source specific.
- The preservation of i-brGDGTs downcore – especially the soil-derived 1G-brGDGTs – offers a potential new avenue for soil- and lake-specific temperature reconstructions from the same sample extracts.

The studies in this thesis were undertaken with the intent of furthering paleoclimate efforts focused on Quaternary lake sediment archives in high latitude regions. Additional key takeaways in this regard include:

- The brGDGT paleothermometer will reconstruct warm-season temperatures.
- Core brGDGTs in lacustrine settings (including sediments) in the Eastern Canadian Arctic are primarily produced in situ.
• Samples associated with low dissolved oxygen deviated from global trends (Chapter 4). Considering that lake water dissolved oxygen generally becomes depleted under ice in the ECA and Iceland (Chapter 2), current calibrations may not produce accurate temperature from anoxic lake sediments in these locations.

• The development of soil-specific 1G-brGDGT calibrations may allow for soil temperature reconstructions from lake sediments, avoiding the complications of lake water anoxia.

6.2 Important remaining challenges

While the results of this thesis have made progress in our understanding of brGDGTs, important challenges remain. First, the influence of oxygen availability on brGDGT distributions must be constrained. While grouping brGDGTs into structural sets deconvolved temperature and pH/conductivity effects, it was unable to characterize oxygen effects. This was likely due to the sparse dataset of oxygen measurements, which often consisted of a single point measurement taken at the time of sampling. A long-term and large-scale study including continuous monitoring of dissolved oxygen levels may be necessary for determining its role in natural settings. Next, though the results of Chapter 4 supported a physiological basis for the environmental relationships of brGDGTs at the global scale, bacterial community composition may still have an important influence on the regional or site-specific level. Such an influence would have important implications for the fidelity of the brGDGT paleothermometer. Finally, the decoupling of lake water and soil temperatures from air temperatures in our high latitude study sites (Chapter 2) highlights the importance of in situ temperature monitoring for calibrations studies.

6.3 Future directions

The studies I presented in this thesis have inspired ongoing and future research, some of which I will highlight here. First, the extensive datasets generated as part of this thesis can continue to lend themselves to studies exploring modern relationships between lipid distributions and environmental parameters. For example, in situ soil and water temperatures can be used to further improve brGDGT calibrations. Similarly, water (and in the future, soil) chemistry measurements (e.g., total phosphorous) may reveal other parameters that are important to brGDGT distributions. This extensive set of modern environmental data can also be used in conjunction with other lipid classes, such as i-brGDGTs or iso-GDGTs, while the compiled dataset of Chapter 4 may prove useful for examining additional global trends. Finally, in addition to these research questions in the modern environment, I also aim to investigate the paleoclimate questions central to the PACEMAP and ILLUME goals by generating brGDGT-based proxy reconstructions from Icelandic and Canadian sedimentary archives.
7 References

References


Cantarero S. I., Henríquez-Castillo C., Dildar N., Vargas C. A., von


Hutchinson G. E. (1938) On the Relation between the Oxygen Deficit and


92


Tierney J. E., Schouten S., Pitcher A., Hopmans E. C. and Sinninghe


8 Appendices

8.1 Appendix 1: Chapter 2 Supplemental Information

8.1.1 True color and bathymetric maps

True color and bathymetric maps are provided below in alphabetical order for all lakes surveyed. A drone survey was not performed for Lake ARQ. However, an approximate bathymetric map based off satellite imagery is provided. Geospatial support for the ARQ imagery was provided by the Polar Geospatial Center under NSF-OPP awards 1043681 and 1559691. Full resolution files from drone surveys are available in an online data repository (Section 8.1.2).
Figure 8-1: Lake 3LN drone imagery. Imagery collected August 23, 2019.
Figure 8-2: Lake 3LN approximate bathymetry. Figure courtesy of Martha Raynolds.
Figure 8-3: Lake AFR drone imagery. Imagery collected on August 14, 2019.
Figure 8-4: Lake AFR approximate bathymetry. Points show manually measured depths in meters. Figure courtesy of Martha Raynolds.
Figure 8-5: Lake ARQ satellite imagery. Points in show manually measured depths in meters. (Yellow points represent vegetation plots.) Figure courtesy of Martha Raynolds. Imagery (summer of 2020) was provided by the Polar Geospatial Center.
Figure 8-6: Lake ARQ very approximate bathymetry. Figure courtesy of Martha Raynolds.
Figure 8-7: Lake BIR drone imagery. Imagery collected on July 30, 2018.
Figure 8-8: Lake BIR approximate bathymetry. Points show manually measured depths in meters. Figure courtesy of Martha Raynolds.
Figure 8-9: Lake BRO drone imagery. Imagery collected on August 16, 2018.
Figure 8-10: Lake BRO approximate bathymetry. Points show manually measured depths in meters. Figure courtesy of Martha Raynolds.
Figure 8-11: Lake CF8 drone imagery. Imagery collected on August 1, 2019.
Figure 8-12: Lake CF8 approximate bathymetry. Points show manually measured depths in meters. Figure courtesy of Martha Raynolds.
Figure 8-13: Lake QPT drone imagery. Imagery collected on July 22, 2018.
8.1.2 Supplementary data

All drone imagery is available at https://doi.org/10.18739/A2FN10T2V. Water chemistry measurements are available at https://doi.org/10.18739/A29Z90D0D (PACEMAP project), https://doi.org/10.18739/A26688K7F (ILLUME project). Temperature logger data is available at https://doi.org/10.18739/A22F7JS2T (PACEMAP project) and https://doi.org/10.18739/A2XP6V46R (ILLUME project), which will be updated periodically as new data is collected.

8.2 Appendix 2: Chapter 3 Supplemental Information

8.2.1 Appendix to the main text

Full and schematic structures of the 15 commonly measured brGDGTs are provided in Fig. 8-15. Table 8-1 details equations for calculating FAs within the structural sets.

Figure 8-14: Lake QPT approximate bathymetry. Points show manually measured depths in meters. Figure courtesy of Martha Raynolds.
Figure 8-15: Structures of the 15 commonly measured brGDGTs. Full structures and schematic representations are provided, with C6 methylations denoted in red in the schematics.
Table 8-1: Equations for calculating FAs within brGDGT subsets. The variables $f_{xy}$ and $x_y$ are the fractional and absolute abundances of the 5- or 6-methyl brGDGT with Roman numeral $x$ (I, II, or III) and alphabet letter $y$ (a, b, or c).

<table>
<thead>
<tr>
<th>(Sub)set name</th>
<th>(Sub)set compounds</th>
<th>Fractional abundance equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>$S = {I_{a}, I_{b}, I_{c}, I_{Ia}, I_{Ib}, I_{Ic}, II_{a}, II_{b}, II_{c}, III_{a}, III_{b}, III_{c}}$</td>
<td>$f_{xy}<em>{(sub\set)} = x</em>{y}/\sum S_{y}$</td>
</tr>
<tr>
<td>MC-5Me+</td>
<td>$S = {I_{a}, I_{b}, I_{c}, I_{Ia}, I_{Ib}, I_{Ic}, II_{a}^{r}, II_{b}^{r}, II_{c}^{r}, III_{a}, III_{b}, III_{c}}$</td>
<td></td>
</tr>
<tr>
<td>MC-6Me+</td>
<td>$S = {I_{a}, I_{b}, I_{c}, I_{Ia}, I_{Ib}, I_{Ic}, II_{a}^{r}, II_{b}^{r}, II_{c}^{r}, III_{a}^{r}, III_{b}^{r}, III_{c}^{r}}$</td>
<td></td>
</tr>
<tr>
<td>MC-5Me</td>
<td>$S = {II_{a}, II_{b}, II_{c}, III_{a}, III_{b}, III_{c}}$</td>
<td></td>
</tr>
<tr>
<td>MC-6Me</td>
<td>$S = {II_{a}^{r}, II_{b}^{r}, II_{c}^{r}, III_{a}^{r}, III_{b}^{r}, III_{c}^{r}}$</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>Use MC-5Me+ and MC-6Me FAs</td>
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</tr>
<tr>
<td>MI</td>
<td>$S_a = {I_{a}, I_{Ia}}$</td>
<td>$f_{xy}<em>{(sub\set)} = x</em>{y}/\sum S_{x}$</td>
</tr>
<tr>
<td></td>
<td>$S_b = {I_{b}, I_{Ib}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_c = {I_{c}, I_{Ic}}$</td>
<td></td>
</tr>
<tr>
<td>Meth-5Me+</td>
<td>$S_a = {I_{a}, I_{Ia}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_b = {I_{b}, I_{Ib}}$</td>
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</tr>
<tr>
<td></td>
<td>$S_c = {I_{c}, I_{Ic}}$</td>
<td></td>
</tr>
<tr>
<td>Meth-6Me+</td>
<td>$S_a = {I_{a}, II_{a}, III_{a}}$</td>
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<tr>
<td></td>
<td>$S_b = {I_{b}, II_{b}, III_{b}}$</td>
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<td></td>
<td>$S_c = {I_{c}, II_{c}, III_{c}}$</td>
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<tr>
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<tr>
<td></td>
<td>$S_b = {II_{b}, III_{b}}$</td>
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<td></td>
<td>$S_c = {II_{c}, III_{c}}$</td>
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<tr>
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<td>$S_a = {II_{a}, II_{Ia}}$</td>
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<td>$S_b = {II_{b}, II_{Ib}}$</td>
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<td>$S_c = {II_{c}, II_{Ic}}$</td>
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<tr>
<td>CI</td>
<td>$S_{Ia} = {I_{a}, I_{b}, I_{c}}$</td>
<td>$f_{xy}<em>{(sub\set)} = x</em>{y}/\sum S_{xy}$</td>
</tr>
<tr>
<td></td>
<td>$S_{Ib} = {I_{b}, I_{Ib}, I_{Ic}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{Ib} = {I_{Ia}, III_{a}, III_{b}, III_{c}}$</td>
<td></td>
</tr>
<tr>
<td>Cyc-5Me</td>
<td>$S_{I} = {I_{a}, I_{b}, I_{c}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{II} = {II_{a}, II_{b}, II_{c}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{III} = {III_{a}, III_{b}, III_{c}}$</td>
<td></td>
</tr>
<tr>
<td>Cyc-6Me</td>
<td>$S_{I} = {I_{a}, I_{b}, I_{c}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{II} = {II_{a}, II_{b}, II_{c}}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{III} = {III_{a}, III_{b}, III_{c}}$</td>
<td></td>
</tr>
<tr>
<td>Cyc</td>
<td>Use Cyc-5Me and Cyc-6Me FAs</td>
<td></td>
</tr>
<tr>
<td>Isom</td>
<td>$S_{IIa} = [II_{a}, III_{a}]; S_{IIIa} = [III_{a}, III_{a}]$</td>
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</tr>
<tr>
<td></td>
<td>$S_{IIb} = [II_{b}, III_{b}]; S_{IIIb} = [III_{b}, III_{b}]$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{IIc} = [II_{c}, III_{c}]; S_{IIIc} = [III_{c}, III_{c}]$</td>
<td></td>
</tr>
</tbody>
</table>

We used the following previously-defined brGDGT indices in this study: CBT (Weijers et al., 2007b); MBT’ (Peterse et al., 2012); MBT5Me, MBT6Me, CBT5Me, CBT’, and Index1 (De Jonge et al., 2014a); IR6Me (Dang et al., 2016); #rings_tetra, #rings_penta_5Me, and #rings_penta_6Me.
\( CBT = -\log \left( \frac{lb + lIb + IIb'}{Ia + IIa + IIa'} \right) \) \hspace{1cm} (A1)

\[ MBT' = \frac{(Ia + lb + lIc)}{(Ia + lb + lIc + IIa + IIb + IIc + IIa' + IIb' + IIc' + IIIa')} \] \hspace{1cm} (A2)

\[ MBT'_{5Me} = \frac{(Ia + lb + lIc)}{(Ia + lb + lIc + IIa + IIb + IIc + IIIa')} \] \hspace{1cm} (A3)

\[ MBT'_{6Me} = \frac{(Ia + lb + lIc)}{(Ia + lb + lIc + IIa' + IIb' + IIc' + IIIa')} \] \hspace{1cm} (A4)

\[ CBT_{5Me} = -\log \left( \frac{lb + IIb}{Ia + IIa} \right) \] \hspace{1cm} (A5)

\[ CBT' = -\log \left( \frac{lc + IIa' + IIb' + IIc' + IIlb + IIla' + IIb' + IIc'}{Ia + IIa + IIIa} \right) \] \hspace{1cm} (A6)

\[ Index1 = \log \left( \frac{Ia + lb + lIc + IIa' + IIIa'}{Ic + IIa + IIc + IIIa + IIIa'} \right) \] \hspace{1cm} (A7)

\[ IR_{6Me} = \frac{(IIa' + IIb' + IIc' + IIIa' + IIIb' + IIIc')}{(IIa' + IIb' + IIc' + IIIa' + IIIb' + IIIc' + IIa + IIb + IIc + IIIa + IIIb + IIIc)} \] \hspace{1cm} (A8)

\[ \#rngs_{tetra} = \frac{(lb + 2 * Ic)}{(Ia + lb + lIc)} \] \hspace{1cm} (A9)

\[ \#rngs_{penta\,5Me} = \frac{(IIb + 2 * IIc)}{(IIa + IIb + IIc)} \] \hspace{1cm} (A10)

\[ \#rngs_{penta\,6Me} = \frac{(IIb' + 2 * IIc')}{(IIa' + IIb' + IIc')} \] \hspace{1cm} (A11)

\[ DC = \frac{(lb + 2 * Ic + IIb + IIb')}{(Ia + lb + lIc + IIa + IIa' + IIb + IIb')} \] \hspace{1cm} (A12)

\[ HP5 = \frac{IIIa}{(IIa + IIIa)} \] \hspace{1cm} (A13)

\[ IBT = -\log \left( \frac{IIa' + IIIa'}{IIa + IIIa} \right) \] \hspace{1cm} (A14)
\[ CI = \frac{la}{(la + IIa + IIIa)} \quad (= fIa_{Meth}) \quad (A15) \]

### 8.2.2 Supplementary information

For all supplemental text, figures, and data, which have the prefix “S” in the main text (e.g., “Fig. S1”), the reader is referred to the online version of the published article (Raberg et al., 2021).
8.3 Appendix 3: Chapter 4 Supplemental Information

8.3.1 Comparison of fitting coefficients

**brGDGT Structural Sets**

<table>
<thead>
<tr>
<th>a) Methylation</th>
<th>b) Cyclization</th>
<th>c) Isomer</th>
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<tr>
<td><img src="image" alt="Meth_a" /> <img src="image" alt="Meth_b" /> <img src="image" alt="Meth_c" /></td>
<td><img src="image" alt="Cyc_a" /> <img src="image" alt="Cyc_b" /> <img src="image" alt="Cyc_c" /></td>
<td><img src="image" alt="Isomer" /></td>
</tr>
</tbody>
</table>

Example FA calculation:

\[
\text{flla}_{\text{Meth}} = \frac{\text{IIa}}{(\text{Ia} + \text{IIa} + \text{IIla})} \\
\text{flla'}_{\text{Meth}} = \frac{\text{IIa'}}{(\text{IIa'} + \text{IIla'})}
\]

**d) Meth-Isom**

| ![Meth_a](image) ![Meth_b](image) ![Meth_c](image) |
| ![Meth_a](image) ![Meth_b](image) ![Meth_c](image) |
| ![Meth_a](image) ![Meth_b](image) ![Meth_c](image) |

**e) Cyc-Isom**

| ![Cyc_a](image) ![Cyc_b](image) ![Cyc_c](image) |
| ![Cyc_a](image) ![Cyc_b](image) ![Cyc_c](image) |
| ![Cyc_a](image) ![Cyc_b](image) ![Cyc_c](image) |

**f) Meth-Cyc**

| ![Meth-Cyc](image) |
| ![Meth-Cyc](image) |
| ![Meth-Cyc](image) |

**g) Full**

| ![Full](image) |
| ![Full](image) |
| ![Full](image) |

Figure 8-16: Schematic of the basic (a-c) and combined (d-g) brGDGT structural sets. Fractional abundances are calculated within each boxed group independently (Table S1). Schematic structures highlight the defining alkyl-chain moieties, with cyclopentane rings filled in for emphasis and C6 methylations denoted in red. (Reproduced from ref. (Raberg et al., 2021).)
Figure 8-17: Coefficients of temperature fits. Fitting coefficients for quadratic (IIa, IIIa) and linear (all other compounds) regressions between brGDGT Meth Set FAs and Temperature, as plotted in Figure 1 of the main text, are provided. Error bars represent one standard error. Coefficients with $p$ values $\geq 0.01$ are plotted as open circles.
Figure 8-18: Relationships between temperature and brGDGT fractional abundances (FAs) calculated within the Full set. FAs were calculated using all compounds (Fig. 8-16 and Table 8-2). *Temperatures are the same as in Figure 1. Best fit curves (quadratic for IIa and IIIa; linear for all other compounds) and are plotted for each compound, with $R^2$ values provided. P values were < 0.01 except where marked with an asterisk. Open circles denote points with FA = 0 or 1. These points were excluded from statistical analyses to match the analysis done with the Meth set (Fig. 4-1).

Figure 8-19: Fitting coefficients for linear regressions between brGDGT Cyc-Isom Set FAs and pH. Error bars represent one standard error. Coefficients with p values ≥ 0.01 are plotted as open circles.
Figure 8-20: Relationships between pH and brGDGT fractional abundances (FAs) calculated within the Full set. FAs were calculated using all compounds (Fig. 8-16 and Table 8-2). Linear fits are plotted for each compound, with $R^2$ values provided. $P$ values were < 0.01 except where marked with an asterisk. $N = 1295$ for all subplots.

Figure 8-21: Fitting coefficients for linear regressions between the brGDGT compound pairs. Pairs are as plotted in Figure 4-3 of the main text. (Compound Pair A refers to Figure 4-3A, for example.) Error bars represent one standard error. Coefficients with $p$ values $\geq 0.01$ are plotted as open circles.
Table 8-2: Equations for calculating FAs within brGDGT structural sets used in this study. The terms $f_{xy}$ and $xy$ are the fractional and absolute abundances of the 5- or 6-methyl brGDGT with Roman numeral $x$ (I, II, or III) and alphabet letter $y$ (a, b, or c).

<table>
<thead>
<tr>
<th>Structural set name</th>
<th>Compound Groupings</th>
<th>Fractional abundance equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>$S = {Ia, Ib, Ic, IIa, IIb, IIc, IIIa, IIIb, IIIc, IIa', IIb', IIc', IIIa', IIIb', IIIc'}$</td>
<td>( f_{xy,full} = \frac{xy}{\sum S} )</td>
</tr>
<tr>
<td>Meth</td>
<td>$S_a = {Ia, IIa, IIIa}; S_a' = {IIa', IIIa'}$; $S_b = {Ib, IIb, IIIb}; S_b' = {IIb', IIIb'}$; $S_c = {Ic, IIc, IIIc}; S_c' = {IIc', IIIc'}$</td>
<td>( f_{xy,meth} = \frac{xy}{\sum S_y} )</td>
</tr>
<tr>
<td>Meth-Isom</td>
<td>$S_a = {Ia, IIa, IIIa, IIa', IIIa'}$; $S_b = {Ib, IIb, IIIb, IIb', IIIb'}$; $S_c = {Ic, IIc, IIIc, IIc', IIIc'}$</td>
<td>( f_{xy,mI} = \frac{xy}{\sum S_y} )</td>
</tr>
<tr>
<td>Cyc-Isom</td>
<td>$S_I = {Ia, Ib, Ic}$; $S_{II} = {IIa, IIb, IIc, IIa', IIb', IIc'}$; $S_{III} = {IIIa, IIIb, IIIc, IIIa', IIIb', IIIc'}$</td>
<td>( f_{xy,CI} = \frac{xy}{\sum S_x} )</td>
</tr>
<tr>
<td>Isom</td>
<td>$S_{IIa} = {IIa, IIa'}; S_{IIIa} = {IIIa, IIIa'}$; $S_{IIb} = {IIb, IIb'}; S_{IIIb} = {IIIb, IIIb'}$; $S_{IIc} = {IIc, IIc'}; S_{IIIc} = {IIIc, IIIc'}$</td>
<td>( f_{xy,Isom} = \frac{xy}{\sum S_{xy}} )</td>
</tr>
</tbody>
</table>

The qualitative similarities in Figures 4-1 to 4-3 can to some extent be quantified by comparing the fitting coefficients for individual sample types. Such an analysis can be informative but has important limitations that we list below.

A comparison of fitting coefficients reveals sample-type-specific slopes and intercepts that are often within error (Figs. 8-17, 8-19, and 8-21). For soils and peats, this overlap may be expected as these sample types were shown to have statistically indistinguishable relationships between MBT'$_{5Me}$ and temperature (Dearing Crampton-Flood et al., 2020). However, no other datasets have been successfully merged in this manner. It is therefore remarkable that even such dissimilar sample media as soil and marine sediment (e.g., IIb in Fig. 8-17), peat and lake sediment (e.g., Ia in Fig. 8-19), or groundwater and bone (e.g., pair C in Fig. 8-21), display compound-specific relationships with environmental parameters that are within error of one another.

In several cases, however, fitting coefficients for different sample types do not overlap. One notable example is that of soils and lake sediments. While these sample types display undeniably similar relationships with temperature (e.g., Fig. 4-1e) and pH (e.g., Fig. 4-2a), offsets consistently appear in their slopes and intercepts (Fig. 8-17 and 8-19). This disparity agrees with previous work showing that the relationships between MBT'$_{5Me}$ and temperature were distinct for lake sediments versus soils and peats (Martínez-Sosa et al., 2021). Even for sample types that might be more likely to overlap, such as lacustrine sediments and SPM, offsets in slope and intercept can appear (e.g., Fig. 8-17).

The disagreements in fitting coefficients could be evidence of important differences between sample types. However, several uncertainties exist that make such a conclusion beyond the resolution and scope of our approach with the existing data at this time. First, while we have
excluded samples with multiple sources of brGDGTs as much as possible (e.g., marine sediments with clear soil input), it is likely that some still exist in our dataset. This issue could be alleviated in future studies as further methods are developed to fingerprint mixed sources or by making use of intact polar brGDGTs, which are more likely to be produced in situ. Second, while grouping brGDGTs into the Meth and Cyc-Isom Sets aided in isolating the effects of temperature and pH for lake sediments (Raberg et al., 2021), other environmental parameters can still influence brGDGT FAs (e.g., low DO lacustrine SPM in Fig. 4-1). Third, samples from a single sample type sometimes do not span the full range of values in the dataset (e.g., marine sediments in Fig. 4-3c) or are biased to one end (e.g., lacustrine SPM in Fig. 4-2), making it difficult to infer fitting coefficients across the full dataset range. Finally, and perhaps most simply, the environmental variables associated with each sample type are often (necessarily) different (see Methods). For example, air temperatures were used for the lake sediment dataset, while water temperature was used for lacustrine SPM. While these variables can be standardized to a certain extent (e.g., by using WMT for all terrestrial sample types), most of the available temperature and pH measurements are themselves imperfect proxies for the true physical or chemical conditions that brGDGT-producing organisms may experience (e.g., air temperature instead of in situ soil or water temperature). When in situ temperature values are available, likely biases in biological activity towards more favorable growing conditions (e.g., warmer temperatures (Cao et al., 2020) or higher nutrient availability (Loomis et al., 2014a)) can further complicate the selection of an appropriate temperature index. Even measurements of pH are imperfect to some extent as bulk properties may not accurately represent the chemical microenvironments in which microbes operate (e.g. in soils (Baveye et al., 2018)).

As a result of these challenges, we are unable to determine at this time whether the discrepancies in the precise relationships between brGDGTs, temperature, and pH are due to true differences between sample types or not. Advances in brGDGT research, such as the growing body of in situ temperature measurements (Wang et al., 2020; Pérez-Angel et al., 2020) or more advanced ways of approximating them (Stefanescu et al., 2021), may allow for this question to be addressed with more confidence in the future. However, despite these lingering uncertainties, we can still conclude that remarkable similarities exist in the relationships between brGDGTs, temperature, and pH across nearly all sample types examined and explore the implications of these broader agreements.

### 8.3.2 Supplementary Data

Supplementary data for Chapter 4 will be available with the published article or upon request.
8.4 Appendix 4: Chapter 5 Supplemental Information

Figure 8-22: Average surface sediment fPH (Eq. 6) versus total phosphorous (µg/L) of lake water. Uncertainties (one standard deviation of repeat measurements; dashed gray lines) are shown where available. Data for Lake CF3 and some data for Lake CF8 were provided by Neil (unpublished; Michelutti et al., 2007).

8.5 Appendix 5: Glossary of terms

1G: monoglycosyl head group

brGDGT: branched glycerol dialkyl glycerol tetraether

c-brGDGT: core brGDGT

DO: dissolved oxygen

ECA: Eastern Canadian Arctic

FA: fractional abundance

H: hydrogen (the “head group” for a core brGDGT)

HG: head group

i-brGDGT: intact brGDGT

MAF: mean temperature of months above freezing

MAT/MAAT: mean annual (air) temperature

PH: phosphohexose head group