Seasonal variability of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in a temperate lake system

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Abstract

Quantitative climate reconstructions are crucial for understanding the magnitude of and mechanisms behind natural and anthropogenic climate change, yet there are few proxies that can reliably reconstruct terrestrial temperature. Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial membrane lipids that are increasingly used to reconstruct paleotemperature from lake sediments, but despite their potential, we have a poor understanding of (1) autochthonous vs. allochthonous sources of brGDGTs in lakes and (2) the seasonality of and environmental controls on brGDGT production within lakes. To investigate these factors, we examined water column suspended particulate matter (SPM) and settling particles from a sediment trap collected on a bimonthly to monthly basis over a period of three years at Lower King Pond, a small kettle lake in northern Vermont, USA. We also compared the concentration and fractional abundances of brGDGTs in SPM and settling particles with those of catchment soils, river sediments, and lake surface sediments to constrain the relative importance of brGDGTs derived from the landscape versus brGDGTs produced within the lake itself. We find significant differences in concentrations and fractional abundances of brGDGTs between soil and river sediment samples from the catchment and lake sediments, indicating a mostly autochthonous source for lacustrine brGDGTs. BrGDGT concentrations, fluxes, and fractional abundances in SPM vary over the annual cycle, indicating that brGDGTs are produced throughout the year and respond to changes within the water column. The total annual flux of brGDGTs settling through the water column is comparable to the brGDGT accumulation rates in surface sediments, indicating that in this lake brGDGTs are mostly produced within the water column, not in the sediment itself. While brGDGTs are produced in all seasons within the water column, the flux to the sediments is highest during periods of spring and fall isothermal mixing, potentially biasing paleotemperature reconstructions towards mixing season temperature. Because the seasonal timing and frequency of lake mixing varies as a function of regional climate, lacustrine brGDGT calibrations should be regional in nature and comprise lakes with similar mixing regimes.

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1. INTRODUCTION

Quantitative paleotemperature records are needed to understand Earth system sensitivity to climate forcing (Shakun et al., 2012). The scarcity of quantitative proxies...
for continental paleotemperature therefore represents a fundamental limitation to paleoclimate research. The most widely used continental paleotemperature proxies, including tree-rings and pollen, are subject to the effects of confounding climate variables such as precipitation (e.g., Rochefort et al., 1994; Briffa et al., 2004). Organic geochemical-based proxies, including alkenone paleothermometry (Brassel et al., 1986) and the TEX\textsubscript{86} proxy (Schouten et al., 2002), have proven useful for past temperature reconstruction (e.g., Powers et al., 2005; Tierney et al., 2008; D’Andrea et al., 2011, 2012; Blaga et al., 2013) but their application is limited to a subset of lakes due to confounding factors such as allochthonous inputs, multi-species production, or the absence of the compounds of interest (e.g., Zink et al., 2001; Powers et al., 2010; Toney et al., 2012). Thus, new approaches are needed to quantify past temperature variability.

Branched glycerol dialkyl glycerol tetraethers (brGDGTs, Fig. 1) have been proposed as a universal continental paleothermometer, as they are ubiquitous in peats (e.g., Weijers et al., 2006; Huguet et al., 2010, 2013), soils (e.g., Weijers et al., 2007a; Peterse et al., 2012), lake sediments (e.g., Blaga et al., 2009, 2010; Tierney et al., 2010; Pearson et al., 2011; Loomis et al., 2012), and shallow marine sediments (e.g., Weijers et al., 2007b; Donders et al., 2009). BrGDGTs are microbial membrane lipids, likely produced by heterotrophic bacteria (Weijers et al., 2006, 2009; Sinninghe Damsté et al., 2011). The fractional abundances of nine brGDGTs vary in relation to mean annual air temperature (MAAT) and pH (Weijers et al., 2007a; Tierney et al., 2010), indicating that the bacteria that produce brGDGTs alter the lipid structure of their membranes to optimize membrane fluidity and permeability of ions and molecules in and out of the cell. However, the relationships between the fractional abundances of brGDGTs and temperature differ between soils and lake sediments on global (Sun et al., 2011), regional (Loomis et al., 2011) and watershed (Sinninghe Damsté et al., 2009; Tierney and Russell, 2009; Tierney et al., 2012; Wang et al., 2012) scales. This offset has led many workers to propose an in situ source of brGDGTs in lakes (e.g., Sinninghe Damsté et al., 2009; Tierney and Russell, 2009; Tierney et al., 2010, 2012; Loomis et al., 2011; Buckles et al., 2014) and to develop temperature calibrations for use in lacustrine sediment. While there is now strong evidence for production of brGDGTs in lacustrine environments, we lack fundamental information on when and where brGDGTs are produced in lakes, which severely limits the interpretation of the environmental signal recorded by brGDGTs in lake sediments.

Recent studies have examined concentrations and fluxes of brGDGTs in suspended particulate matter (SPM) and settling particles captured in sediment traps in temperate and tropical lakes. BrGDGT concentrations in SPM have been shown to increase below lake thermoclines (Sinninghe Damsté et al., 2009; Bechtel et al., 2010; Blaga et al., 2011; Woltering et al., 2012; Buckles et al., 2014), suggesting increased production of brGDGTs in the hypolimnion. Furthermore, brGDGT fluxes have been found to correlate with total sediment fluxes over the annual cycle (Sinninghe Damsté et al., 2009; Woltering et al., 2012). This could result from processes such as increased production of lacustrine brGDGTs during periods of increased sedimentation, potentially tied to increased primary production in the surface waters, increased fluxes of brGDGTs and sediment from the landscape, or resuspension of brGDGTs and sediments within the lake itself.

Whatever the cause, seasonal and spatial variability in brGDGT distributions could strongly influence paleotemperature reconstructions. However, there has been little investigation of spatiotemporal variability in the fractional abundances of brGDGTs within lacustrine systems. Here we examine the fractional abundances and concentrations of brGDGT core lipids in soil, river and lake sediments, settling particles, and SPM collected between 2009 and 2012 in Lower King Pond to evaluate the sources and seasonal variability of brGDGTs and their potential influences on the interpretation of paleotemperature reconstructions.

2. METHODS

2.1. Site information and field sampling

Lower King Pond is located in South Woodbury, VT, USA (44°24′43″N, 72°26′3″W; Fig. 2a). The small (1.6 ha), dimictic, mesotrophic lake is shallow (8 m maximum depth), sits in a limestone catchment, and contains organic carbon rich sediments (Ford, 1990).

We deployed a sediment trap at 6.5 m depth (~1 m above the lake floor; Fig. 2b) to collect settling particles on a biweekly to monthly basis in Lower King Pond from May 2009 to April 2012, with a brief period absent of sample collection during the winter of 2011 (Nov 2010–April 2011), resulting in 35 samples total. From April 2010 to April 2012, we collected 20 L of water from the surface (~0.5 m depth) and bottom (~6.5 m depth) of the water column on 21 separate occasions to analyze SPM on a semi-monthly to bi-monthly basis. We collected water samples simultaneously to analyze for nutrient (nitrate, nitrite, ammonium, and phosphate)
and redox-sensitive element concentrations (iron, manganese, and sulfate). Water was collected at the same location where the sediment trap was deployed (Fig. 2b).

Soils (10 samples), river sediments (6 samples), and littoral sediments (3 samples) were collected from the catchment to compare with lake surface sediment samples (0–2 cm; 8 samples) taken at 1 m depth intervals (Fig. 2b) in a transect across Lower King Pond. We also analyzed brGDGTs in eight samples from a 25 cm-long gravity core, located adjacent to the sediment trap (Fig. 2b) in May of 2009.

We deployed Onset® HOBO® U21-001 TidbiT temperature loggers in the surface and bottom waters of Lower King Pond to measure lake temperature at hourly intervals over the study period. Air temperature data at the site is mainly derived from loggers deployed in the catchment of Lower King Pond; however, due to logger failure June 2010–April 2011 and November 2011–April 2012, we also utilized air temperature data from the National Oceanic and Atmospheric Administration’s (NOAA) National Climatic Data Center (NCDC) Global Summary of the Day (GSOD) from Knapp Airport in Berlin, VT (44°12’N, 72°34’W), located 33 km from the lake. Due to the high correlation between mean daily air temperature at Lower King Pond and the GSOD station ($r = 0.96, p < 0.0001$), we created a linear transfer function to relate GSOD air temperature data to that of Lower King Pond to fill in the missing data. Precipitation data are derived from the same GSOD dataset. We measured lake water temperature, pH, and dissolved oxygen (DO) at 0.5 m depth intervals on a semi-monthly to bi-monthly basis over the study period using a YSI 6920 Data Sonde.

### 2.2. Laboratory and statistical methods

All samples from Lower King Pond were transported to Brown University within 24 h of collection. The 20 L water samples were immediately filtered through a 293 mm Pall glass fiber filter with a pore size of 1 μm. The filters were frozen along with soil, sediment and water samples until analysis.

Water samples were analyzed for nutrients, cations, and anions using a Westco Smartchem 200 Discrete Analyzer, a JY2000 Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), and a Dionex Modular DX500 Ion Chromatography System, respectively.

Sediment and soil samples were freeze-dried, homogenized with a mortar and pestle, and split into aliquots for pH measurements (soil only), carbon/nitrogen analysis, biogenic opal quantification (sediment trap samples only), and brGDGT analysis. The ~5 mL soil pH aliquots were mixed with distilled water (1:2 by weight), and pH was measured using an Accumet AP 115 pH meter (Loomis et al., 2011). Total organic carbon (TOC) and total organic nitrogen (TON) were measured using an NC2100 elemental analyzer. Biogenic opal was analyzed using a modified Mortlock and Froelich (1989) method.

We chose not to examine intact polar lipids and focus only on core brGDGTs since the spatial and temporal patterns of core and intact polar brGDGT variability in lakes have been shown to be similar in settling particles and SPM (Buckles et al., 2014), and the validity of using intact polar lipids as a “living” tracer is still debated (e.g., Bauersach et al., 2010; Schouten et al., 2010, 2012; Lengger et al., 2012a). Because the Bligh–Dyer method (Bligh and Dyer, 1959) and an accelerated solvent extractor (ASE) result in similar yields of core lipids (Lengger et al., 2012b), lipids were extracted from all samples using a Dionex 350 ASE using dichloromethane (DCM):methanol (MeOH) 9:1 (v:v). A synthetic C$_{46}$ standard was added to the samples for brGDGT quantification (Huguet et al., 2006). An Al$_{2}$O$_{3}$ column was used to isolate the apolar and polar fractions of the samples using n-hexane:DCM (9:1, v:v) and DCM:MeOH (1:1, v:v) as eluents. The polar fractions containing brGDGTs were analyzed using high performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS) at the Royal Netherlands Institute for Sea Research (NIOZ) using instrument protocols described by Peterse et al. (2012). Peak areas were used to calculate fractional abundances and concentrations of brGDGTs. Concentrations were corrected for the relative response factor between the C$_{46}$ internal standard and crenarchaeol, assuming the same response factor for crenarchaeol and brGDGTs. Concentrations were normalized to TOC for sediment samples and to water volume for SPM samples. Methylation and cyclization of...
the brGDGTs were quantified using the MBT and CBT indices (Weijers et al., 2007a):

$$\text{MBT} = \frac{[\text{Ia} + \text{Ib} + \text{Ic}]}{[\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa} + \text{IIIb} + \text{IIIC}]}$$  \hspace{1cm} (1)

$$\text{CBT} = -\log([\text{Ib} + \text{IIb}] / [\text{Ia} + \text{IIa}])$$  \hspace{1cm} (2)

To explore the potential for brGDGTs to reconstruct temperature, we applied the Sun et al. (2011) global lakes calibration:

$$\text{MAAT} = 37.09 \times \text{MBT} - 7.06 \times \text{CBT} + 6.80$$  \hspace{1cm} (3)

to the fractional abundances of brGDGTs in all lake derived samples. We chose to use this calibration because it contains many temperate-zone lakes with similar seasonal temperature changes to that of Lower King Pond.

We performed ANOVA to test the difference between sample types (e.g., soil vs. lake sediment) and to determine the similarity between the fractional abundances of brGDGT in these environments. We also examined Pearson correlation coefficients (r) to evaluate environmental controls on the brGDGT data for trap and SPM samples. SPM samples were correlated to environmental data gathered on the date of sample collection. Sediment trap data were correlated to mean environmental data that encompassed the entire period of sample collection (e.g., mean air and water temperatures from the in situ temperature loggers, mean YSI measurements taken from the day of sample deployment though the day of the next sample deployment). Correlations and ANOVA tests are considered significant for p < 0.05.

We used surface sediments from the deepest part of the lake to estimate accumulation rates (A) using the following equation:

$$A = (1 - w) \times [\text{brGDGT}] \times \rho \times R$$  \hspace{1cm} (4)

where w is the water content of the surface sediments, [brGDGT] is the average concentration of brGDGTs in the surface sediments normalized to sediment weight, \( \rho \) is the average dry bulk density of the surface sediments, and R is the sedimentation rate. Dry bulk density was estimated from organic matter content in the surface sediments (Boyd, 1995), and we assumed a water content of 95%. Sedimentation rates were derived from stratigraphic correlation of our gravity core to a core from a previously published study from Lower King Pond (Ford, 1990). The Ford (1990) age model is derived from bulk radiocarbon dates and a palynologically-determined historical sediment horizon near the top of the core. This stratigraphic correlation puts sedimentation rates for our gravity core at 8.6 ± 1.6 years/cm. Errors on accumulation rates were calculated using the standard deviation of the density and brGDGT concentration data and the error on the age model for the gravity core.

### 3. RESULTS

#### 3.1. Environmental variations

Over the duration of the three year study, air temperature loggers at Lower King Pond recorded an MAAT of 6 °C at Lower King Pond, with average monthly air temperatures ranging from –6 to 18 °C (Supplementary Material, Fig. S1). Water column temperature profiles follow a pattern typical of north temperate dimictic lakes, with normal stratification in summer, inverse stratification during the period of ice cover, and isothermal mixing in spring and fall (Supplementary Material, Figs. S1, S2a). A shallow thermocline is established early after ice-off in April, deepens during the spring and summer, and is eroded during the longer autumnal isothermal mixing. Lake surface waters track daytime air temperatures in the summer and range from 25 °C in summer to 2 °C in winter (mean = 11.6 °C; Table 1), while average monthly bottom water temperatures vary between 14 and 5 °C (mean = 7.9 °C; Table 1). Average dissolved oxygen (DO) concentrations are 8.3 mg/L in the surface waters and 1.48 in the bottom waters (Table 1). Maximum DO concentrations in summer occur between 2.5 and 4.5 m, reaching supersaturated levels up to 15.1 mg/L (Supplementary Material, Fig. S2b). The bottom 0.5 m of the water column is perennially hypoxic to anoxic, even during isothermal mixing. Nutrient and redox sensitive element concentrations were generally higher in the bottom waters compared to the surface waters (Table 1; Supplementary Material, Figs. S3–S4). pH is circumneutral to slightly alkaline (6.5–8.6) throughout the course of the year (Supplementary Material, Fig. S2c), but is generally highest in the surface water (mean = 8.0; Table 1) and lowest in the bottom of the water column (mean = 7.5; Table 1) during the ice-free season. Average water column temperature, DO, and pH are all significantly different from organic matter content in the surface sediments (Boyd, 1995), and we assumed a water content of 95%. Sedimentation rates were derived from stratigraphic correlation of our gravity core to a core from a previously published study from Lower King Pond (Ford, 1990). The Ford (1990) age model is derived from bulk radiocarbon dates and a palynologically-determined historical sediment horizon near the top of the core. This stratigraphic correlation puts sedimentation rates for our gravity core at 8.6 ± 1.6 years/cm. Errors on accumulation rates were calculated using the standard deviation of the density and brGDGT concentration data and the error on the age model for the gravity core.

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correlated with depth and with each other (|r| > 0.93, p < 0.0001 for all). Soils are generally more acidic than the water column, with pH values of 4.3–6.8 (Supplementary Material, Table S1). Average mean monthly precipitation over the sampling period ranged from 6.8 cm in January to 11.7 cm in July, with large precipitation peaks coinciding with major flooding in May of 2011 and Hurricane Irene in August of 2011.

3.2. BrGDGTs in soils, river sediments, and lake sediments

Soil brGDGT concentrations range from 6 to 21 µg/g TOC (Fig. 3a; Supplementary Material, Table S1). Soils are dominated by brGDGTs without cyclopentyl rings (Ia, IIa, and IIIa; mean fractional abundance = 0.76), with smaller contributions from brGDGTs with one (Ib, IIb, and IIIb) and two (Ic, IIc, and IIIc) cyclopentane moieties (mean fractional abundances of 0.20 and 0.04, respectively). On average, brGDGTs Ia and IIa are most abundant (mean fractional abundance = 0.34; two rings mean = 0.07), but much lower abundances of Ia than soils (mean = 0.08) and cyclopentane-bearing brGDGTs (one ring mean = 0.32; two rings mean = 0.07) than soils (Fig. 3d). Littoral sediments have MBT values ranging from 0.35 to 0.41 (Fig. 3b) and CBT values ranging from 0.20 to 0.27 (Fig. 3c).

BrGDGT concentrations in lake surface sediments range from 69 to 110 µg/g TOC (with one outlier of 132 µg/g TOC; Fig. 3a; Supplementary Material, Table S2), which is significantly higher than brGDGT concentrations in soil (p < 0.0001) and river sediments (p < 0.0001) but not significantly different than those of the littoral sediments (p = 0.38; Table 2). The accumulation rate of brGDGTs in the surface sediments is 2.7 ± 0.4 mg/m²/yr. The fractional abundance of IIIa (mean = 0.18) is higher in lake surface sediments than in any other type of sample (Fig. 3d), and the fractional abundances of cyclopentane-bearing brGDGTs (one ring mean = 0.23; two ring mean = 0.03) are lower than river or littoral sediments. Lake sediment MBT values are the lowest for all sample types, with values between 0.28 and 0.32 (Fig. 3b), and CBT values range from 0.37 to 0.46 (Fig. 3c). Reconstructed MAAT in lake surface sediments ranges from 14.4 to 15.6 °C (Fig. 4d). BrGDGT concentrations are not correlated to DO concentrations at collection depth (r = 0.27, p = 0.52). CBT is not significantly correlated to water depth (r = −0.30, p = 0.47), nor is it correlated to measured water column temperature, pH, conductivity, or DO (|r| ≤ 0.40, p > 0.31). The fractional abundance of IIIa, MBT, and reconstructed MAAT are all significantly correlated with depth (r = 0.90, p = 0.003 for all).

![Figure 3](image-url)  
Fig. 3. Comparison of brGDGTs within the lake and catchment at Lower King Pond. Box and whisker plots showing the median, maximum, minimum, and interquartile range values of (a) brGDGT concentration, (b) MBT, and (c) CBT for soils (n = 10), river sediments (n = 6), littoral sediments (n = 3), and lake surface sediments (n = 8). Dots represent outliers that are more than 1.5 times outside the interquartile range. Averages and standard deviations of the fractional abundances of the nine brGDGTs for each sample type are shown in (d).
for IIIa; \( r = -0.84, \ p = 0.009 \) for MBT; \( r = -0.89, \ p = 0.003 \) for reconstructed MAAT), and reconstructed MAAT is strongly correlated to mean annual water column temperatures at the depth of sample collection (\( r = 0.91, \ p = 0.002 \)).

Samples from the gravity core, which cover \( \sim 200 \) years, show little variability in brGDGT concentrations or fractional abundances (Supplementary Material, Table S2). Concentrations range from 69 to 81 \( \mu g/g \) TOC (Fig. 4a), and do not vary significantly with sediment depth (\( p = 0.52 \)). There is little to no variability in brGDGT distributions throughout the core, as MBT values are 0.30–0.31 (Fig. 4b), CBT values are 0.40 (Fig. 4c), and reconstructed MAAT is 14.9–15.3 \( ^\circ C \) (Fig. 4d). The lack of variability is likely not due homogenization due to bioturbation, as the bottom 0.5 m of the water column are perennially anoxic (Supplementary Material, Fig. S2b), and the down core homogeneity of TOC (21–23\% likely indicates that hypoxia has persisted throughout the time covered by the gravity core.
3.3. BrGDGTs in water column SPM

BrGDGT concentrations in SPM range from 0.6 to 11 ng/L (Fig. 4a; Supplementary Material, Table S3). In spring and early summer, concentrations of brGDGTs are highest in the surface waters and lowest in the bottom waters (Fig. 5d). In mid-summer through fall mixing, this relationship reverses, and concentrations are highest in the bottom waters and lowest in the surface waters. Under ice, the water column tends to have a lower concentration of brGDGTs than in other seasons (with the exception of one surface water sample in February 2011).

The fractional abundances of brGDGTs in SPM vary from 0.63 to 0.82 for brGDGTs without cyclopentane rings, 0.15–0.27 for brGDGTs with one cyclopentane ring, and below detection limit to 0.16 for brGDGTs with two cyclopentane rings (Fig. 4e). SPM MBT values vary between 0.27 and 0.47 (Fig. 4b), CBT values vary between 0.39 and 0.68 (Fig. 4e), and reconstructed MAAT varies between 13.5 and 21.2 °C (Fig. 4d). Measured water temperature is significantly correlated with the fractional abundance of brGDGT IIIa (r = −0.55, p < 0.001), IIIb (r = 0.45, p = 0.007), IIc (r = −0.67, p < 0.001), and IIc (r = −0.60, p < 0.001) (Supplementary Material, Table S4). pH is positively correlated with brGDGT concentration (r = −0.40, p = 0.02). Nitrate concentration is significantly correlated with brGDGT concentrations (r = 0.47; p = 0.01).

3.4. BrGDGTs in settling particles

The fractional abundances of brGDGTs in settling particles show little intra- or inter-annual variability over the three years of sampling (Supplementary Material, Table S5). The summed fractional abundances of brGDGTs without cyclopentane rings range from 0.71 to 0.74, and those of brGDGTs with one and two cyclopentane moieties range from 0.23 to 0.27 and 0.03 to 0.04, respectively (Supplementary Material, Table S5). MBT values range from 0.27 to 0.34 (Fig. 4b), CBT values range from 0.36 to 0.43 (Fig. 4c), and reconstructed MAAT ranges from 14.1 to 16.5 °C (Fig. 4d).

The fractional abundances of IIIa, IIIc, and IIa are significantly correlated to mean air temperature (r = −0.35, p = 0.04 for IIIa; r = −0.37, p = 0.03 for IIIc; r = 0.37, p = 0.03 for IIa) and surface water temperature (r = −0.43, p = 0.009 for IIIa; r = −0.34, p = 0.05 for IIIc; r = 0.42, p = 0.01 for IIa) (Supplementary Material, Table S6). CBT does not correlate to integrated water column pH or pH at the depth of the sediment trap.

While there is little variability in the fractional abundances of brGDGTs in settling particles, their fluxes vary by more than two orders of magnitude (Fig. 6b). The highest fluxes of brGDGTs occur during the periods of fall and spring mixing and are strongly positively correlated to total sediment, organic carbon, and biogenic opal fluxes (r > 0.78, p < 0.0001 for all). The mean annual flux of brGDGTs in settling particles was 3.6 mg/m²/yr, which is slightly higher than the accumulation rates observed in surface sediments.

4. DISCUSSION

4.1. In situ production of lacustrine brGDGTs in Lower King Pond

Several lines of evidence suggest that brGDGTs deposited in Lower King Pond sediments are derived primarily from production within the lake. Firstly, we observe significant differences between the fractional abundances and concentrations of brGDGTs in soils, river sediments, and lake sediments at Lower King Pond (Table 2; Fig. 3), indicating in situ production of brGDGTs in soil, riverine, and lacustrine environments. The degree of cyclization in brGDGTs is significantly (p = 0.01) higher in river and lake sediments (mean CBT = 0.30) than it is in soils (mean CBT = 0.71; Table 2), and brGDGTs are significantly more methylated (p = 0.0006) in river and lake sediments (MBT = 0.33) as well. This finding is in agreement with differences in brGDGT distributions observed in other temperate (Tierney et al., 2012; Wang et al., 2012) and tropical (Sinninghe Damsté et al., 2009; Tierney and Russell, 2009; Loomis et al., 2011; Buckles et al., 2014) lakes and watershed soils, and strongly suggests in situ production of relatively cyclized and methylated brGDGTs within lakes.

Second, biweekly to monthly variations in the fluxes of brGDGTs in settling particles indicate that lacustrine brGDGTs are primarily derived from an in situ source. Fluxes of brGDGTs are not correlated to precipitation (p = 0.67), even during extreme precipitation events such as Hurricane Irene in which we would expect increased soil runoff and/or riverine delivery of terrestrially-derived brGDGTs. Instead, brGDGT fluxes are highest – by several orders of magnitude – during periods of isothermal lake mixing (Fig. 6b). This suggests that brGDGTs are most likely produced within the lake itself (see Section 4.4 for further discussion).

Lastly, variations in the distributions of brGDGTs from SPM indicate that brGDGTs are produced throughout the water column and are not primarily derived from soil runoff. Fractional abundances of brGDGTs in SPM vary throughout the course of the year (Figs. 5b–d and 6b), indicating a seasonal, in-situ influence on brGDGT production. The distributions of brGDGTs in soils do not vary over the annual cycle due to a large pool of residual GDGTs (Weijers et al., 2011); hence the variability in SPM likely derives from production within the lake. Furthermore, measured spatial gradients in temperature measured within the water column (i.e., warmer temperatures in the surface waters, cooler temperatures in the bottom waters; Fig. 5a) match variability in the fractional abundance of brGDGT IIIa (Fig. 5b), which is strongly correlated to temperature in lacustrine settings (Tierney et al., 2010; Pearson et al., 2011; Loomis et al., 2012, 2014), and reconstructed MAAT (Fig. 5c), demonstrating that brGDGTs in SPM are likely recording water temperatures at different depths. Variability in the fractional abundances of brGDGTs in SPM, combined with the other evidence outlined above, indicate that lacustrine brGDGTs at Lower King Pond are primarily produced within the lake.
4.2. Influence of chemistry and depositional environment on brGDGT distribution

While there is substantial agreement for an in situ source for lacustrine brGDGTs and clear evidence that the relationship between brGDGT distributions and temperature differ between soils and lake environments (e.g., Tierney and Russell, 2009; Tierney et al., 2010; Loomis et al., 2011; Sun et al., 2011), there is little understanding of the mechanisms that cause these relationships to vary. We note that in Lower King Pond, brGDGT concentrations are lowest in soils, significantly higher in river sediments, and...
Several studies have noted increased brGDGT concentrations in anaerobic environments (Weijers et al., 2006, 2009; Huguet et al., 2010; Peterse et al., 2011), so this trend could result from anaerobic conditions in the water column favoring brGDGT producers. However, culture experiments have identified two aerobic Acidobacteria that produce brGDGT Ia (Sinninghe Damsté et al., 2011), and brGDGT concentrations in Lower King Pond surface sediments are not significantly correlated to average water column DO concentrations at collection depth, indicating that oxygenation may not be the cause of increased brGDGT concentrations in lake sediments. This trend may instead be related to other environmental factors that influence the growth conditions of brGDGT producers, such as the availability of electron acceptors or the type of organic matter found in the soils vs. lake sediments. In general, soils tend to contain relatively more recalcitrant organic matter, while organic matter in lake sediments in mesotrophic to eutrophic systems is more labile (Killops and Killops, 2005), potentially providing carbon and nutrients for brGDGT-producing heterotrophic bacteria (Weijers et al., 2006, 2010).

Many previous studies that have examined water-saturated sediments in rivers, lakes, and surrounding soils have found increases in brGDGT concentrations, methylation and/or cyclization in water-saturated environments similar to what we observe at Lower King Pond (Peterse et al., 2011).
comparisons in North America (Schoon et al., 2013) and
and pH within Lower King Pond differs from inter-lake
disconnect between the fractional abundances of brGDGTs
inter-lake comparisons (Loomis et al., 2014); however, the
surface waters than in the bottom waters (Fig. 5a; Supple-
tmental Material, Fig. S2b–c). Temperature, in the bottom waters during the period of thermal stratifica-
tion (Aksan et al., 2009), and increased cyclization of GDGTs
leads to decreased permeability of water through the cellu-
lar membrane (Gliozi et al., 2002 and references therein).
Along with changing osmotic pressure, water saturation
affects the concentration and mobility of compounds and
ions – including O2, CO2, and protons – that can permeate
cell membranes (Becker et al., 1996), which may also affect
the structural composition of the lipid membrane.

There are also significant differences between the frac-
tional abundances of brGDGTs in riverine and lacustrine
brGDGTs (Table 2), indicating that water saturation is
not the only factor affecting the variability in the fractional
abundances of brGDGTs. These differences may be caused
by differences in temperature and/or pH between these
environments, or it may be that different microbial popula-
tions live in the different aquatic and sedimentary environ-
ments and produce different fractional abundances of
brGDGTs which help to best regulate permeability and flu-
idity in each specific habitat.

In addition to the changes in brGDGT distribution
between adjacent depositional environments in Lower King
Pond, the fractional abundances of brGDGTs in SPM vary
spatially and temporally over the course of our sampling
period (Figs. 4b–e and 6b). Overall, both surface and bot-
tom water SPM show similar direction and timing of
changes in the fractional abundances of individual
brGDGTs (e.g., IIIa; Fig. 4b); however, the magnitude of
these changes is larger in the surface water than the bottom
water. Variability in DO and pH do not have the same spa-
tial and temporal patterns as fractional abundances of
brGDGTs in SPM (Fig. 5b), as DO and pH both increase
throughout the summer in the surface waters but decrease
in the bottom waters during the period of thermal stratifica-
tion (Supplementary Material, Fig. S2b–c). Temperature,
however, varies in a similar manner to the fractional abun-
dances of brGDGTs, such that surface and bottom water
temperatures increase throughout spring and summer,
and the magnitude of temperature change is larger in the
surface waters than in the bottom waters (Fig. 5a; Supple-
mental Material, Fig. S2a). Fractional abundances of
brGDGTs in SPM are not correlated to nutrient, cation,
or anion concentrations in the water column, aside from
nitrate, which is significantly correlated to water tempera-
ture ($r = -0.44, p = 0.005$). The lack of correlation to nu-
trients and redox sensitive elements has also been observed in
inter-lake comparisons (Loomis et al., 2014); however, the
disconnect between the fractional abundances of brGDGTs
and pH within Lower King Pond differs from inter-lake
comparisons in North America (Schoon et al., 2013) and
East Africa (Tierney et al., 2010; Loomis et al., 2014), which
noted a significant negative correlation between CBT and
pH.

Interestingly, while brGDGT distributions in SPM gen-
erally do not correlate to water column chemistry, the
anomalously warm reconstructed temperatures from SPM
in February of 2011 (Fig. 5c) occurred during a period of
abnormally high nutrient concentrations in both the surface
and bottom waters (Supplementary Material, Fig. S3).
Thus, while small changes in water column chemistry have
little to no effect on the fractional abundances of
brGDGTs, it is possible that large changes in nutrient con-
centrations can affect their distribution. Nutrients do not
diffuse directly through cellular membranes and thus likely
do not directly cause the change in fractional abundances
of brGDGTs; however, there are several factors that could
cause this association. Firstly, increased nutrient concen-
trations may be associated with a change in concentrations of
other molecules not measured in our study, such as CO2,
that can permeate the cell membrane. Secondly, aquatic
heterotrophic bacteria may utilize different protein path-
ways to transport nutrients across the membrane depending
on the concentration of nutrients in the environment
(Kjelleberg et al., 1987). Because the structure and function
of membrane proteins are strongly coupled to the fluidity
and structure of the membrane lipids (Lee, 2004), it is
possible that drastic changes in nutrient availability alter
the structure of the membrane lipid by modifying the type
and/or structure of nutrient transport proteins through
the membrane. Finally, just as with changes in depositional
environment, it may be that different brGDGT-producing
microbial populations thrive under vastly different nutrient
concentrations. More work needs to be done to identify the
bacteria that produce brGDGTs in order to quantify the
conditions that drive these changes in cyclization and
methylation.

4.3. Effect of seasonal temperature variability on brGDGTs
in the water column

Overall, reconstructed MAAT is higher in surface water
SPM than bottom water SPM (Figs. 4d and 6c; Table 3),
similar to measured water temperatures (Fig. 5a). The
amplitude of variability in reconstructed MAAT is different
at different depths within the water column, but recon-
structed MAAT from brGDGTs in SPM follows the same
temporal pattern at different depths (Fig. 5c). Aside from
outlying values measured in under ice samples in February
2011 (see Section 4.2 for discussion), reconstructed tempera-
tures vary in concert with water column temperatures,
with cooler temperatures at the beginning of spring, gradu-
ally warming through summer, and cooling at the start of
autumnal lake mixing. Furthermore, the largest recon-
structed temperature changes occur in the surface waters
and smallest changes in the bottom waters, consistent with
measured temperature variability within the water column,
strongly implicating temperature as the dominant control
on water column brGDGT distributions (Fig. 5a–c).

Although distributional changes in brGDGT composi-
tion of SPM reflect changes in water column temperatures,
there is a 1–2 month lag between maxima and minima in measured water column temperatures and reconstructed MAAT from brGDGTs in SPM (Fig. 5a, c). Even after measured surface water temperatures begin to decrease in late July, reconstructed MAAT continues to increase until mid-September. This temporal lag in reconstructed vs. observed temperature maxima was also observed in settling particles at Lake Challa (Buckles et al., 2014), indicating that this pattern is likely prevalent in a broad range of lacustrine environments. Furthermore, both the means and the ranges of reconstructed MAAT from SPM (mean = 16.3 °C, range = 14.2 °C) differ from measured water column temperatures (mean = 11.6 °C, range = 28.3 °C for surface water; mean = 7.9 °C, range = 14.2 °C for bottom water). The differences in reconstructed MAAT compared to measured water temperatures could result from our choice of calibration, as we have used the Sun et al. (2011) MBT/ CBT calibration to reconstruct temperatures instead of one of the other published calibrations (Tierney et al., 2010; Pearson et al., 2011; Loomis et al., 2012). While application of these different calibrations results in different mean reconstructed MAAT values, all brGDGT temperature calibrations result in similar reconstructed temperature ranges (Supplementary Material, Table S7), indicating that the muted reconstructed temperature variability in SPM is not solely a result of calibration choice.

We speculate that both the temporal lag and muted range of reconstructed MAAT relative to observed temperatures results from a residual pool of brGDGTs that were produced earlier in the season but have not settled through the water column. The accumulation of the residual pool of brGDGTs during thermal stratification would result in a gradual accumulation of a higher reconstructed MAAT through the summer, lagging measured temperature variability. Following the period of stratification, isothermal mixing helps to deliver brGDGTs to the sediments, removing much of this residual pool (see Section 4.4). The rapid production of new brGDGTs during the mixing period – likely associated with increased organic matter production – accelerates the turnover of brGDGTs in the water column, causing the rapid cooling in reconstructed temperatures associated with mixing. While the differences in mean reconstructed and observed temperature could easily result from inherent inaccuracy in temperature calibrations, the muted range could also derive from the hypothesized residual pool of brGDGTs in the water column. Residual brGDGTs produced during periods when the water column was cooler would result in lower than observed peak reconstructed MAAT estimates, decreasing the observed range of reconstructed MAAT in the water column.

### 4.4. Seasonality of brGDGT production and sedimentation

Despite the variation in brGDGTs in SPM (Fig. 5b), the fractional abundances of brGDGTs in our sediment trap samples show little temporal variability and are not correlated to air or water temperature variations during the period of trap deployment (Fig. 6a). This may reflect limited input of brGDGTs from the upper water column, where fractional abundances change the most, perhaps due to
ineffective sedimentation and/or efficient grazing and recycling of brGDGTs in the surface mixed layer. ANOVA tests confirm that the fractional abundances of brGDGTs in bottom water SPM are not significantly different from particles settling to the sediment trap (Table 3), suggesting that the traps most strongly reflect bottom water brGDGT distributions during periods of thermal stratification.

As noted above, brGDGT fluxes to the sediment vary over the course of the year, with the largest fluxes during periods of isothermal lake mixing (Fig. 6b). Increased brGDGT fluxes are positively correlated to total particle, organic matter, and biogenic opal fluxes, and brGDGT concentrations are significantly correlated to fluxes of organic matter even after normalization to sedimentary TOC ($r = 0.46, p = 0.02$). This is consistent with results from sediment traps in Lake Challa (Sinninghe Damsté et al., 2009), Lake Lucerne (Blaga et al., 2011), and Lake Superior (Woltering et al., 2012), where brGDGT fluxes increased during periods of increased total sediment flux.

Various hypotheses have been used to explain these increases in brGDGT fluxes, including increased seasonal precipitation leading to increased soil erosion (Sinninghe Damsté et al., 2009) or redistribution of marginal sediments during storms in a large lake (Woltering et al., 2012). As described above (Section 4.1), brGDGT fluxes are not correlated to precipitation events at Lower King Pond. The small size of Lower King Pond (1.6 ha), the forested catchment, and the abundance of macrophytes in the littoral zones all prevent substantial redistribution of sediment within the lake, and the fractional abundances of brGDGTs in littoral sediments, which should be prone to reworking, are significantly different from lake surface sediments (Table 2).

Increased fluxes of brGDGTs in settling particles during the mixing season compared to the period of summer stratification could result from increased production of brGDGTs and/or increased export of brGDGTs from the water column to the sediments. Supersaturation of DO at the base of the epilimnion (Supplementary Material, Fig. S2) indicates the presence of a mid-summer (June–Aug) phytoplankton bloom at Lower King Pond, and variability in reconstructed MAAT from SPM (Fig. 5c) indicates that brGDGTs are produced during the summer months as well. However, both total sediment and brGDGT fluxes are relatively low during summer stratification (Fig. 6b–c), indicating that inefficient sedimentation and/or grazing and remineralization of organic matter in the water column inhibit export of this material to the sediments. In contrast to the low summer sedimentation rates, fluxes of total particulates, organic matter, biogenic silica, and brGDGTs all peak during spring and fall mixing (Fig. 6b–c), similar to sedimentation patterns seen in other dimictic lake systems (e.g., Poister and DeGuelle, 2005; Kulbe et al., 2006). Increases in particle fluxes during mixing are associated with increased diatom production, as the increased particle size and density of the diatom tests facilitates export from the water column to the sediments (Poister and DeGuelle, 2005). Particle-bound export of brGDGTs is likely responsible in part for increased fall and spring brGDGT fluxes, as brGDGT and sediment fluxes are correlated with biogenic silica fluxes (Fig. 6b–c). However, brGDGT concentrations normalized to TOC are highly correlated to particle fluxes and brGDGT fluxes, indicating that increased brGDGT production, potentially due to availability of organic carbon, is an added cause of increased brGDGT fluxes during isothermal lake mixing. Thus, while production of brGDGTs occurs throughout the water column during all times of year, increased production and export of brGDGTs occurs during isothermal lake mixing.

4.5. Spatial variability in the fractional abundances of brGDGTs within lake surface sediments

Although variations in fractional abundances of brGDGTs across the lake are small, reconstructed MAAT is correlated with depth at Lower King Pond. Given the strong, significant correlation between temperature, pH, and DO with depth, correlations alone cannot distinguish between the environmental controls on brGDGT variability in the surface sediments. Nevertheless, the strong link between SPM and temperature leads us to conclude that the spatial variability in reconstructed MAAT in surface sediments at Lower King Pond is likely driven by temperature gradients within the lake. The gradient of reconstructed MAAT (1.2 $^\circ$C; Fig. 7a), however, is lower than the gradient of mean water column temperatures (3.9 $^\circ$C; Fig. 7b). Increased production of brGDGTs during isothermal mixing (Section 4.4) could potentially account for this discrepancy, as biases in reconstructed temperatures towards mixing season temperatures, when the lake is homothermal, would decrease the range of reconstructed MAAT along the depth gradient.

Sedimentary production of brGDGTs could also influence the reconstructed temperatures we observe in sediment and possibly reduce the range of temperatures observed across the lake, as sedimentary production would be biased towards bottom water temperatures. However, fluxes of brGDGTs in settling particles (3.6 mg/m$^2$/yr) are higher than, but comparable to, accumulation rates of brGDGTs in the surface sediments (2.7 ± 0.4 mg/m$^2$/yr), inconsistent with substantial sedimentary production of brGDGTs. The difference between the annual flux of brGDGTs through the water column and accumulation rates of brGDGTs in the surface sediments could be a result of inter-annual variability in brGDGT fluxes, degradation of brGDGTs within the surface sediments, or unknown errors in our estimates of brGDGT accumulation rates. Regardless, the MBT, CBT, and reconstructed MAAT of settling particles is comparable to that of the lake surface sediments and the gravity core (Table 3), so any degradation and/or production of brGDGTs that may take place in Lower King Pond sediments does not alter reconstructed MAAT captured in the water column.

5. CONCLUSIONS AND IMPLICATIONS FOR PALEOTEMPERATURE RECONSTRUCTIONS

We examined spatial and temporal variability of brGDGTs within a temperate lake and catchment.
Differences in concentrations and fractional abundances of brGDGTs between lake sediment, soil, and river samples, combined with spatial and seasonal variability in the fractional abundances of brGDGTs in lake sediment and SPM, indicate that lacustrine brGDGTs are primarily derived from production within the lake itself. It is therefore critical that we understand the factors controlling brGDGT distributions and reconstructed temperatures within lakes.

Reconstructed MAAT from lake surface sediments vary spatially with depth, which likely results from spatial gradients in the integrated water column temperature with depth. Reconstructed MAAT from SPM also reflects seasonal changes in water column temperature; however, the lack of variability in settling particles relative to observed temperatures indicates that brGDGTs produced in the surface waters do not efficiently settle to the sediments when the lake is thermally stratified, and thus, particles settling during thermal stratification primarily reflect bottom water temperatures. Fluxes of brGDGTs are highest during isothermal mixing in the spring and fall, likely due to a combination of increased production and increased export to the sediments associated with particulate sedimentation. Annual fluxes of brGDGTs in settling particles are similar to estimated brGDGT accumulation rates in surface sediments, and the fractional abundances of settling particles are similar to those of the surface sediments. This indicates neither substantial production of brGDGTs in the surface sediments nor alteration of temperature signals captured by brGDGTs produced in the water column. Thus, reconstructed MAAT from brGDGTs likely reflects water column temperature, but is also likely biased towards bottom water and mixing season temperatures.

Although sedimentary brGDGTs derive largely from production in lakes, fractional abundances of brGDGTs are commonly calibrated to air temperature due to the strong spatial and temporal correlation of lake and air temperature (Livingstone et al., 1999). If brGDGTs were produced at constant rates throughout the year, it would be appropriate to calibrate brGDGTs to MAAT; however, if brGDGTs are produced within lakes during a particular season, brGDGTs should be calibrated to mean seasonal temperatures to account for variations in air/water temperature offsets. Multiple studies have suggested that summer calibrations are appropriate for brGDGTs (Pearson et al., 2011; Sun et al., 2011) because bacterial activity often increases in lakes during the summer months (Wetzel, 2001). Our data from Lower King Pond instead suggest that brGDGTs are primarily deposited during periods of lake mixing, and thus, brGDGTs in sediments likely reflect a mixing season, not summer season, temperature. If brGDGTs are primarily produced during the mixing season for all lakes, this would differentially affect reconstructed temperatures under different mixing regimes. Further sediment trap and SPM studies are needed to see if brGDGT fluxes do indeed increase during isothermal mixing in lakes under different mixing regimes, ideally examining variations at multiple depths to examine the possibility of biases towards bottom water temperatures, which is especially crucial in larger lakes where a majority of the water volume is contained below the thermocline. Given these findings, brGDGT temperature reconstructions from lake sediments should be based on lacustrine calibrations, and lacustrine brGDGT temperature calibrations should be regional and/or separated by lake mixing regime to account for potential temperature biases introduced by increased brGDGT production and accumulation during seasonal mixing.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2014.08.027.
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